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Abstracts submitted to the virtual 45th FEBS Congress from 3rd to 8th July 2021 (originally planned to be held in Ljubljana, Slovenia) and accepted by the Congress Organizing Committee are published in this Supplement of *FEBS Open Bio*. Late-breaking abstracts are not included in this issue.

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* Each poster has been given a unique number beginning with the letter P; the next part relates to the session in which the poster will be presented.

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POSTERS – RESEARCH

Genome structure and regulation

P-01.1-01
Gene desert area in the 1q21.1 makes the frequent contacts with nucleoli in different human cell lines

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There are large human genome regions where no genes were detected. These regions are called gene deserts (Ovcharenko et al., Genome Res. 2005. 15:137–145). There are some indications suggesting that nevertheless these regions could play an important role in regulation of expression of remote genes (Harimendy et al., Nature. 2011. 470:264–268). There is about 800-kb long gene desert area in chr1 with coordinates from 142,800 to 143,600 kb (hg19). We analyzed the whole-genome contacts of nucleoli in HEK293T, K562, and hESM01 human cell lines using 4C (circular chromosome conformation capture) approach. The same patterns of very frequent contacts of nucleoli were detected inside this gene desert in all three lines analyzed. No genes in the area are indicated in IGB Browser (hg19). Genome-wide annotations from ENCODE genome segmentations show the presence of repressed chromatin, CTCF sites, small isles of transcribed regions, and week enhancers in the desert. Surprisingly, we detected strong PARP1 binding sites in the region. No promoters or TSS are present in the desert. Our previous data strongly demonstrated that the contacts of rDNA clusters with different chromosomes are involved in development (Tchurikov et al., Cells. 2019). Nucleoli are the largest membrane-less organelles in nuclei. Potentially they could directly spread active or repressed chromatin states at the contacts sites via phase separation mechanisms. The conservation of nucleoli contacts in this gene desert in different cell types strongly suggests the importance of the region in 3D organization of chromosome 1. The study was supported by the grant from Russian Science Foundation No. 21-14-00035.

P-01.1-02
CRISPR/Cas9 mediated knockout of GLI1, GLI2 and GLI3 genes in melanoma cell lines

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GLI transcription factors are the main mediators of Hedgehog-GLI (HH-GLI) signaling pathway. They activate the transcription of many target genes which are involved in various aspects of tumorigenesis. Targeting the HH-GLI signaling pathway is one of the recent approaches in cancer therapy. Our preliminary data suggest that melanoma cells harboring the BRAF mutation show a better response to GLI inhibition than cells with the NRAS mutation, suggesting a differential role for the HH signaling pathway in melanoma cells with different genetic background. In order to elucidate the role of GLI proteins in melanoma with these genetic backgrounds (BRAF mutation, NRAS mutation, no mutation), we are now in phase of constructing GLI1/2/3 knockout melanoma cell lines using CRISPR/Cas9 system. For this purpose, for each GLI protein, we designed two sgRNAs which guide the Cas9 protein to the specific sequences in the genome where they create a double stranded break. The designated sequence was the region near the ATG of GLI1/2/3 and the region near the end of the genes. These two breaks were repaired via homology-directed repair (HDR) with a help of HDR cassette that was transfected along with CRISPR/Cas9. So far, we have managed to construct GLI2 knock-outs in two melanoma cell lines. In parallel, we have also over-expressed GLI1/2/3 proteins in the same melanoma cell lines. Each maternal cell line and its over-expressed cell line for GLI1, GLI2 and GLI3 will be analyzed by RNA-seq to determine the changes in transcriptomes. This analysis, in combination with knock-out cell line analysis results, should provide us with the information of which genes are specifically regulated by each of the GLI proteins in each of the genetic background. That may provide insight into the observed differences between the cell lines with different genetic background.

P-01.1-03
GLI transcription targets in melanoma cell lines

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Hedgehog-GLI signaling pathway is one of the key regulators of normal development. Its aberrant signaling activity has been implicated in the initiation, progression and relapse of various types of cancer, including melanoma. Previous studies in our laboratory demonstrated that BRAF and NRAS melanomas have different response to HH-GLI signaling pathway inhibition. Identifying GLI protein functions in melanoma cell lines with different mutational background represents a distinct potential for the development of combined therapy with HH-GLI signaling pathway and BRAF/NRAS inhibitors. For that purpose, chromatin immunoprecipitation sequencing (ChIP-seq) was performed. The mutational status of 14 collected melanoma cell lines was confirmed by Sanger sequencing and the cell lines were divided into three categories based on their mutational background: BRAF-mutated, NRAS-mutated, and wild-type for BRAF and NRAS. Three cell lines were selected for ChIP analysis based on protein expression of HH-GLI signaling pathway components: CHL-1 for the wild-type cell group, A375 for the cell group with BRAF gene mutation and MEL224 for the cell group with NRAS gene mutation. Purified chromatin samples were used for library preparation and proceeded for next-generation sequencing (NGS). Target genes were analyzed *in silico* and validated by quantitative polymerase chain reaction (qPCR). qPCR validation included selected 23 protein coding genes, 9 miRNAs and 3 lncRNAs involved in regulation of MAPK signaling pathway. Further analysis can bring new insights into HH-GLI and MAPK signaling interplay and development of combined therapy.

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P-01.1-04
miR-27b modulates insulin resistance in hepatocytes by targeting insulin receptor and repressing insulin signaling pathway

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Obesity is a global epidemic that has nearly tripled since 1975 and, with more than 1.9 billion overweight adults and 650 million obese people in 2016, constitutes the main risk of cardiovascular disease and type 2 diabetes mellitus (T2DM). Insulin resistance (IR) is one of the key factors in the development of T2DM but the molecular mechanism leading to disease is still unclear. The implication of microRNAs (miR) in the pathophysiology of multiple cardiometabolic pathologies, including obesity, atherosclerosis heart failure and IR, has emerged as a major focus of interest in recent years. Indeed, upregulation of several miRNAs has been associated to obesity and IR, among them, it has been shown that miR-27b is overexpressed in liver of obese people, but its role in IR has not been deeply explored. The main objective of the present work has been to investigate the possible role of miR-27b in insulin signaling pathway regulation in hepatocytes. Results: The results of the present study demonstrate that mir-27b is able to regulate hepatic insulin sensitivity by directly interacting with INSR and IRS1. Conclusion: This work emphasizes the importance of miRNA modulation studies to determine their functional effects. In fact, our study demonstrates the direct effect of miR-27b on INSR and IRS1 expression and its potential role as insulin signalling regulator.

P-01.1-05
The effect of the transcriptional repressor StpA on CRISPR-Cas activity in *E. coli* cells lacking H-NS

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Clustered regularly interspaced short palindromic repeats – CRISPR associated genes (CRISPR-Cas) is a prokaryotic defence system that protects against phage infection and foreign DNA, such as plasmids. It is comprised of cas protein genes and the CRISPR locus which consists of repeat arrays interspaced with sequences originating from invading DNA, that are transcribed and processed into CRISPR RNA (crRNA). *E. coli* has a type I-E system, in which foreign DNA targets are recognized by Cascade, a crRNA-guided complex comprised of five proteins (CasA, CasB, CasC, CasD, CasE) and degraded by Cas3. In *E. coli* the CRISPR-Cas type I-E system is repressed by the histone-like nucleoid-structuring protein H-NS, a global transcriptional repressor. In wt cells H-NS repression can be relieved by elevated levels of LeuO transcription factor which induce higher transcript levels of cas genes than was observed for Δhns cells. This suggested that derepression in Δhns cells is incomplete and that an additional repressor could be involved in silencing. We wanted to test if StpA, a paralogue of H-NS with similar DNA binding preferences as H-NS, is another repressor of cas genes. By overexpressing stpA from the plasmid we have managed to abolish resistance to phage in naturally resistant Δhns cells. To confirm the exact mechanism of this phenomenon, we determined cas3 and casA transcript levels in different mutant cells (wt, ΔhnsΔstpA, Δhns, and Δhns cells overexpressing stpA). Our results show that in the absence of H-NS, the StpA protein is another repressor of cas genes.

P-01.1-06
Characterization of sponge homolog of human metastasis suppressor DRG1

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Cancer is known as a disease of multicellular animals caused by the errors within the multicellular system, leading to the proliferation of "selfish" cell lines. Research of ancestral homologs of cancer-related genes in humans has gained more popularity in recent years since comparative genomic studies have confirmed that many homologs of human genes were already present in simple metazoans. From an evolutionary point of view, the development of cancer is most likely related to the development of multicellularity and the appearance of true tissues and organs. Despite their simple morphology, with only a few cell types and without true tissues and organs, sponges possess complex genomes harboring many genes highly similar to their vertebrate homologs. Therefore, they provide an excellent model for studying the evolution of different genes that were most possibly present in the genome of the animal ancestor. Our research focuses on metastasis suppressor genes. Metastasis suppressors inhibit metastasis formation without affecting primary tumor growth. Bioinformatics analyses have shown that homologs of metastasis suppressors were probably already present in the last common ancestor of all animals. To better understand the basic role of ancestral metastasis suppressor homolog, we analyzed the sponge homolog of the main metastasis suppressor gene: developmentally-regulated GTP-binding protein 1 (DRG1). Our bioinformatics and phylogenetic analyses showed that these proteins are conserved across animals. Transfection of sponge and human cells revealed the intercellular localization of DRG1 proteins. The proteins were then overexpressed in *E. coli* and confirmed by Western blot and the protein GTP-binding properties by a GTPase activity assay. Further biochemical and biological characterization is in progress. These results will provide a better understanding of the intracellular processes related to the metastasis suppression and pathology of cancer and metastasis.

P-01.1-07
Investigation of the gene expression pattern and the regulation of stearoyl-CoA desaturase 5 (SCD5)

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Elevation of free fatty acid (FA) levels is a key component in the development of severe diseases. The cellular stress caused by saturated FA overload can be reduced by local desaturation. Thus, the stearoyl-CoA desaturase (SCD1) enzyme is an important member in the cellular defense mechanism against lipotoxicity. The

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function and the regulation of Scd1 are well characterized, but SCD5, the other human isoform is barely been studied yet. The present work aimed to reveal, whether both SCD5 transcriptional variants (A and B) described in NCBI database were transcribed in human tissues and, if so, in what extent. We also aimed to identify the promoter region of SCD5 gene. Total SCD5 gene expression of hepatic and renal cell lines and eight different human tissues was assessed by RT-PCR. Specific primer pairs were designed to quantify the two transcriptional variants separately by qPCR. To analyze SCD5 promoter region, four fragments of different length were amplified from human genomic DNA and cloned into the pGL3-Basic luciferase reporter vector. Promoter activities were measured by luciferase assays from transiently transfected HEK293T or HepG2 cells. The SCD5 mRNA was detected in HEK293T cell line, whereas it is not present in HepG2 cells. The 1000 bp length region 5' upstream from start codon has been shown to be the most transcriptionally active in luciferase reporter system, however in cell line specific manner. The transcriptional variant A of SCD5 turned out to be the most abundant in the brain, while the highest expression level of variant B was measured in the pancreas. Variant A was present 10–100 times higher than B in all tissues. Although both transcriptional variants are expressed, the significantly lower expression of the B isoform cannot be explained by the common promoter. Further research is needed to elucidate the mechanism of the observed cell type specificity of SCD5 promoter activity, as well as its potential contribution in human diseases.

P-01.1-08
Evidence for the origin of CCR5 Delta32 mutation: detection of hot spot of DSBs in blood cells in the region of the gene where the deletion occurs

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CCR5 gene specifies CCR5 chemokine receptor that is used by HIV-1 and some other viruses to enter CD4+ T cells especially during initial infection. It was found that in Europe and western Asia average frequencies of the deletion of precisely the same 32 nucleotides (delta 32) in the gene changing the open reading frame are about 10% (see Novembre et al., PLoS Biol., 2005 and the references therein). The mechanism that is responsible for the origin of the deletion is not known yet. We hypothesized that physiological hot spots of DSBs in this particular site of the gene could serve as a natural genome edition tool. To test the supposition, we used quantitative PCR across a putative region possessing hot spots of DSBs (Tchurikov et al., JMCB, 2015). Two short adjacent DNA fragments of the CCR5 gene, one of which spans the delta 32 region were amplified. A pair of DNA samples was used for amplification with two sets of DNA primers. One template was DNA isolated from blood and another, PCR amplified DNA from the same cells covering the whole region selected for analysis. The DNA does not possess the delta mutation. In these experiments we detected that both regions are amplified at the same rate only if PCR-synthesized DNA was used. When the DNA from blood cells was used as a template, we reproducibly observed about 20–30% lower rate of amplification of the fragment spanning the region of delta 32 region. The data suggest the presence of hot spots of DSBs in the region of CCR5 gene where delta 32 resides. The data are consistent with the idea that the hot spots of DSBs and subsequent non homologous recombination could be responsible for the origin of delta 32 deletion. The study was supported by the grant from Russian Science Foundation No. 21-14-00035.

P-01.1-09
Development of a novel method to discover DNA: protein interaction partners

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Regulatory functions of certain DNA elements, be it linear or adopting higher-order structures like DNA-quadruplexes, are often achieved in concert with proteins that bind to them, either via stable or transient interactions. Conventional methods for detection of protein interaction partners of known DNA are in general suitable for the detection of strong and stable interactions, however, weak and transient ones are often overlooked. To be able to detect all types of interactions, we set on to develop a novel approach for DNA:protein interaction partners discovery based on a three-component system that enables enzyme-catalyzed proximity labeling of proteins with biotin. It can be used either in vivo or in vitro, with an appropriate source of proteins added. The three components are (1) a synthetic nucleotide fragment F-DNA, (2) chimeric protein Tus–TurboID, which contains a mutant biotin ligase, and (3) chimeric protein GAL4(1–147)–sfGFP, which serves as the internal control of the system. F-DNA is the core element of the system and consists of a DNA sequence of interest fused to two other protein binding sites, one for each of the chimeric proteins; the components of the system and target interaction partners are therefore colocalized. Upon biotin addition biotin ligase Tus–TurboID biotinylates GAL4(1–147)–sfGFP and target interaction partners of the DNA of interest. Biotinylated proteins can be easily isolated and analyzed. To characterize the system and optimize the method, we first constructed an in vitro system involving a known DNA: protein interaction pair, namely GADD(DNA):p53(protein). After preparation of purified Tus–TurboID, GAL4(1–147)–sfGFP, and F-DNA we confirmed that chimeric proteins colocalize with F-DNA, using electrophoretic mobility shift assay. We also tested some of the reaction conditions during the biotinylation step, however, to make the method applicable, there is still a need for further optimization.

P-01.1-10
Characterization, classification and localization of repetitive DNA sequences constituting the genome of the Pacific oyster (*Crassostrea gigas*)

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Bivalve molluscs are a large group of economically and ecologically important invertebrates, which made them particularly

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interesting as model organisms for genomic research. So far, 31 bivalve genomes have been sequenced, the first one corresponding to the Pacific oyster (*Crassostrea gigas*). Segments of the genome enriched in repetitive sequences still present a challenge in the genome assemblies, leaving the composition of DNA sequences underlying the heterochromatic regions and the centromeres still unexplored in many organisms. The typical landmark of centromere chromatin is a variant of the H3 histone, CenH3, while DNA sequences building constitutive heterochromatin are associated with H3K9me3-histone modification. In order to investigate the DNA composition of genomic regions enriched in repetitive sequences, we employed chromatin immunoprecipitation followed by high-throughput next-generation sequencing of the whole genome and CenH3- and H3K9me3-associated sequences. The obtained sequences were analysed using RepeatExplorer pipeline. Fifty-two satellite DNAs constitute the satellitome. CenH3-associated sequences resulted in diverse repetitive DNA content and were assigned to six different groups of repetitive sequences. Several sequences enriched in CenH3-ChIP were localized on metaphase chromosomes using fluorescence in situ hybridization, each encompassing centromeres of only a few chromosomes of *C. gigas*. The same sequences were also colocalized with CenH3 on male gonadal cells in different stages of spermatogenesis, revealing stage-specific distribution of the CenH3. The heterochromatin of C. gigas exhibited low abundance and localization limited on two chromosomal pairs, with H3K9me3-associated sequences being dominantly composed of DNA transposons. To conclude, we identified, characterized and localized the most abundant repetitive sequences present in the genome of the Pacific oyster.

P-01.1-11
Changes in expression patterns of anthocyanin biosynthesis genes influence fruit colour in purple-fruited peppers during ripening

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The purple fruit colour of several Capsicum cultivars is due to the accumulation of anthocyanin delphinidine derivatives in the peel. Except some pepper cultivars, during fruit ripening, the exocarp changes colour from purple to red due to the anthocyanin content decrease and carotenoid accumulation. In the present study, dynamics of the anthocyanin content was determined in fruit peel during ripening in purple-fruited pepper cultivars - *C. annuum* (cv. Sirenevii kub), *C. chinense* (cv. Pimenta Da Neyde), and *C. frutescens* (cv. Samocvet). In cv. Pimenta Da Neyde fruit peel, which was purple coloured at all developmental stages, the anthocyanin content gradually increased, reaching a maximum in the mature fruit (4.7 mg/g FW). In the other two studied pepper cultivars, only immature fruit had a purple colour, the anthocyanin content in the peel decreased as the fruit ripened, and mature fruit turned red. The obtained data were compared with the expression pattern of key anthocyanin biosynthesis structural genes CHS, F3'5'H, DFR, ANS, and UFGT, as well as regulatory genes of transcription factors MYB113 and MYC. It was found that the expression pattern of the studied genes correlated with the anthocyanin content in the peel of pepper fruits. Also, a positive relationship between the transcription levels of MYB113 and MYC, and the anthocyanin biosynthesis structural genes was revealed. The study was supported by the Russian Science Foundation grant #19-16-00016.

P-01.1-12
Molecular mechanisms underlying the regulation of HAVCR2 expression in myeloid cells

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TIM-3 (T-cell immunoglobulin and mucin-domain containing-3) is a membrane protein in humans encoded by the HAVCR2 gene. It is a member of the immunoglobulin superfamily that is expressed on differentiated T-cells and innate immunocytes. High TIM-3 expression correlates with suppression of T-cell responses during chronic viral infections and tumor development. Joint blockade of TIM-3 together with other immunological checkpoint molecules appears to be an attractive cancer treatment strategy. Besides T-cells, TIM-3 is also expressed by the cells of myeloid origin such as monocytes, macrophages, and dendritic cells where its biological role has not been studied sufficiently. In the current study, we identified the promoter and enhancer elements in the locus of human HAVCR2 gene using bioinformatics approaches and analyzed their functional activity in activated U937 monocyte cell line using luciferase reporter assay. Potential enhancer region located in the 3 intron provided a 10-fold increase in HAVCR2 promoter activity. We also characterized several disease-associated single nucleotide polymorphisms in HAVCR2 gene promoter and in the enhancer region. Thus, we defined the enhancer of HAVCR2 gene and performed functional annotation of several single nucleotide polymorphisms of its promoter. This work is supported by grant 19-14-00341 from Russian Science Foundation.

P-01.1-13
Prophages and phage related sequences in chromosomes of *Sinorhizobium meliloti* isolates native to Aral Sea region

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The abundance of phage related sequences (PRS) was evaluated in *Sinorhizobium meliloti,* agriculturally valuable nitrogen fixing symbionts of alfalfa. Sequences related to prophages (pPhs) and genomic islands (GIs) were searched by PHASTER and Islander algorithm, correspondingly. Strains were originally recovered from nodules of wild growing plants from the genus *Medicago* native to Aral Sea region (ICA2-CT-2000-10001). Genomes of AK170 and AK555 were sequenced by NGS and NNGS and assembled by using Unicycler. Sequences data of AK21 and AK83 are from GenBank. In total, the 19 PRS with the length varied from 8.0 to 53.0 kb were detected on chromosomes of the 4 strains. The 5 pPhs and 6 GIs site-specifically integrated in tRNA genes and 8 Phs were revealed. The phylogenetic analysis (Mafft UPGMA) done for PRS revealed two clusters. The cluster-I united sequences the 7 pPhs and the 4 of them were intact and native to Sinorhizobium and Loktanella. The cluster-II

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united the 4 groups of PRS sequences: i) GIs and sequence related to incomplete pPh from Siphoviridae family identified in distinct strains; ii) GI and pPhs sequences homologues to Ackermannviridae and Ackermannviridae phage families; iii) homologous sequence related to intact Myoviridae phage; iv) GIs and pPhs sequences homologues to phages from Myoviridae family. Sequences indentified in GIs were clustered as with each other as with pPhs related to Ackermannviridae, Ackermannviridae and Myoviridae. Function analysis of ORFs (BLASTn, BLASTp) of PRS showed that they are encoding hypothetical proteins and enzyme involved presumably in methylation processes. Thus, sequences of phylogenetically distant phages are abundant in genomes of *S. meliloti* strains. That fact is strongly evident at an important role of PRS in horizontal gene transfer that sharped rhizobia genome evolution. The work was supported by RSF 20-16-00105. *The authors marked with an asterisk equally contributed to the work.

P-01.1-14
The properties of a peptide encoded by a nested alternative open reading frame in the matryoshka gene determine the level of maternal mRNA accumulation

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Until recently, our understanding of protein-encoding genes was based on (a) the assumption that one open reading frame (ORF) encodes one protein and (b) the minimal length of the translated ORF. However, due to the discovery of translated ORFs upstream and downstream of the main ORF and of alternative ORFs within annotated ORFs, it became clear that these concepts were incorrect. By studying plant responses to stress, we identified the gene that encodes the Kunitz peptidase inhibitor-like protein (KPILP) in plants of the Solanaceae family, the mRNA content of which dramatically increased after abiotic and biotic stress. To reveal the mechanism underlying the regulation of KPILP mRNA, we identified a nested alternative open reading frame (aORF) encoding a 53-aa-peptide (MGRPQVCLRFRNMKMSMRYSIVQELQGV LLFAPDCCVGILVFHLQLMDRGVWL), which is predicted to form a transmembrane domain (residues 27–45 are underlined). We previously found that aORF translation determines the efficiency of "maternal" KPILP mRNA accumulation in the leaf. Here, we identified the features of the 53-aa peptide amino acid sequence that are responsible for the observed effects. For this purpose, we generated a series of genetic constructs encoding KPILP with substitutions that affect the properties of the 53-aa peptide. We found that mutations leading to a loss of the transmembrane domain (TMD) of the 53-aa peptide and its ability to bind to membranes resulted in the enhanced accumulation of "maternal" mRNA. The replacement of cysteine residues with alanine in the 53-aa peptide leads to the same result. We concluded that the presence of the TMD as well as intermolecular -S-S- bonds in the 53-aa peptide are responsible for the ability of the translated aORF to control the expression of the "maternal" KPILP gene. This study was performed with the financial support of the Russian Foundation for Basic Research (project No. 17-29-08012).

P-01.1-15
Transcriptomic changes in endothelial cells triggered by Na,K-ATPase inhibition: a search for upstream Na/K-sensitive genes

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Gene expression is regulated by diverse stimuli to achieve tissue-specific functional responses. Recently, we found that 3-hr inhibition of Na,K-ATPase by ouabain or by K^+-free medium sharply affects transcriptomes in vascular smooth muscle cells from rat aorta, the human adenocarcinoma cell line HeLa, and human umbilical vein endothelial cells (HUVEC). Importantly, mRNA levels' changes in these cells were highly correlated between two stimuli thus indicating that transcriptomic changes are triggered by the Na^+_i/K^+_i-mediated signaling pathway. According to the generally accepted paradigm, the Na^+_i/K^+_i-sensitive mechanism of excitation-transcription coupling is driven by changes in $[Ca^{2+}]_i$ and activation of several Ca^{2+}-sensitive pathways. In contrast, we found that Ca^{2+}-depletion using extra-and intracellular Ca^{2+}-chelators elevated rather than decreased the number of Na^+_i/K^+_i-sensitive genes. Thus, we suggest that along with canonical Ca^{2+}_i-mediated signaling, sustained elevation of the Na^+_i/K^+_i-ratio affects gene transcription via unknown Ca^{2+}_i-independent mechanism(s). In this study, we utilized Affymetrix arrays and performed a comparative analysis of time-dependent modulation of the Na^+_i/K^+_i-ratio and transcriptomic changes in HUVEC triggered by incubation with ouabain and K^+-free medium in order to identify intermediates of the upstream signaling pathway. According to our data, microRNAs, transcription factors, and proteins involved in immune response and inflammation might be considered as key components of Na^+_i/K^+_i-mediated excitation-transcription coupling. We speculate that Na^+_i/K^+_i imbalance mediates transcriptomic changes directly, through the change of DNA conformation of G-quadruplexes. Verification of the Na^+_i/K^+_i-sensitive transcription regulation mechanism should be continued in forthcoming studies. This study was supported by a grant from Russian Science Foundation (№ 19-75-10009).

P-01.1-16
Regulation of expression of genes from the Grainyhead-like family (GRHL) by transcription factors

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Genes from the Grainyhead-like (GRHL) family are present in all animal and fungi species that were studied so far. In humans there are three genes belonging to the GRHL family, named Grainyhead-like 1 (GRHL1), Grainyhead-like 2 (GRHL2) and Grainyhead-like 3 (GRHL3), respectively. The expression of these genes is observed primarily in various types of the epithelial tissues. GRHL genes are important factors in protection

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against cancer. Their silencing in non-tumorigenic cell lines induces tumorigenic features in these cells and, conversely, increasing the expression of these genes in cancer cell lines reverses their tumorigenic phenotype. Changes in the levels of expression of the genes from the GRHL family often brings about the development of many types of cancer. Thus, GRHL genes directly influence the process of carcinogenesis. Consequently, changes in GRHL gene expression are important for the development and progression of various cancers. The aim of our project is to perform a systematic analysis of promoter regions of the GRHL genes in order to identify and characterize transcription factors binding to these promoters. Our results should thus provide novel and valuable insights into the molecular mechanisms of cancer development. This work is supported by the National Science Centre grant 2016/21/B/NZ1/00279.

P-01.1-17
Proteomic profiles of nucleoid-associated proteins isolated from *Mycoplasma gallisepticum* in different growth phases

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Mycoplasma gallisepticum (MG) belongs to the class Mollicutes. It is characterized by a lack of cell wall and reduced genome size. As a result of genome reduction, MG has a limited variety of DNA-binding proteins (DBPs) and transcription factors. The study of the dynamic changes of the proteomic profile of MG nucleoid may assist in revealing its mechanisms of functioning, regulation of chromosome organization, and stress adaptation. For the first time, we isolated the nucleoid of MG with synchronized cell cycles in the logarithmic phase (LP) and in the stationary phase (SP). The method includes soft lysis and fractionation by centrifugation in sucrose gradient. 2D-DIGE reveals enrichment of DBPs against overall MG proteome. Proteomic profiling was performed by LC-MS/MS (Thermo Scientific Q Exactive Plus) in DDA mode followed by LFQ data analysis. First, the resulting proteomic profiles of LP and SP and corresponding whole cell lysates were analyzed. Proteins considered to be nucleoid-associated (NAPs) were enriched in the nucleoid samples in comparison to the samples of cell lysate (Log2FC > 1, P-value < 0.05). 61 NAPs were identified in both growth phases, along with 18 unique to LP and 12 unique to SP. Among all identified NAPs are DBPs HU1, HU2, and Dps, transcription factors MraZ and Fur, DNA-directed RNA polymerase subunits RpoA, RpoB, RpoE, and sigma factor RpoD. 22 and 18 enriched proteins with unknown function (UFP) were identified in LP and SP, respectively. A quantitative comparison of LP and SP proteomic profiles was also performed. In SP in comparison to LP were enriched 40 proteins, including some of the DBPs (HU2, Dps, Tuf) but only 2 UFPs, whereas in LP in comparison to SP 29 proteins were enriched including 7 UFPs. As a result of this study, we obtained detailed proteomic profiles and determined two sets of unique proteins of *M. gallisepticum* nucleoid in different growth phases. This work was supported by Russian Science Foundation №19-74-10105.

P-01.1-18
Bidirectional promoters with asymmetric expression profiles of the target genes induce differential transcription levels in different human cell lines

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Coordinated gene expression and it's spatiotemporal changes to both individual genes and groups of genes in response to different stimuli, comprises the basis of the life. The primary step of this multistage process is the initiation of transcription on the gene promoter. In the past decades, using different RNA-seq approaches, it has been revealed, that the vast majority of the gene promoters are capable to initiate transcription in both directions, producing mostly short and unstable antisense transcripts. However, there is a special group of bidirectional promoters, driving the processive and stable expression of protein-coding gene pairs. In human genome, they comprise about 10% of the all protein-coding genes, and often include genes involved in the same cellular processes. Generally, these gene pairs show similar expression levels, but of particular interest are the pairs with significant asymmetry of expression. To date, the mechanisms, that provide such an imbalance in transcription of gene pairs remains mostly unclear. To test the ability of such bidirectional promoter regions to initiate differential expression levels of the reporter gene in different human cell lines, we cloned 5 different promoter regions in both orientations into a vector, containing the firefly luciferase gene and transfected these constructs in HEK293T, HeLa, and K562 human cell lines. After measuring the activity of the reporter gene, we determined the expression levels in both directions. Interestingly, some constructions in different cell lines exhibited a different expression levels. The obtained data suggest that, outside of the preformed native chromatin context, bidirectional promoters are able to induce asymmetric expression by themselves, and the expression levels depend, apparently, on the influence of cell-specific transcription factors and different distal regulatory elements. The study was supported by the Russian Science Foundation (grant no. 21-14-00035).

P-01.1-19
CRISPRa-mediated targeting of FOXP3 gene regulatory regions

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Forkhead box P3+ (FOXP3+) regulatory T cells (Tregs) are a subset of lymphocytes, critical for the maintenance of immune homeostasis. Loss-of-function mutations of the FOXP3 gene in animal models and humans results in loss of differentiation potential into Treg cells and are responsible for several immune-mediated inflammatory diseases. Strategies of increasing FOXP3 expression represent a potential approach to increase the pool of Tregs within the lymphocyte population and may be employed in therapies of diverse autoimmune conditions. In the present study, a dCas9 CRISPR-based method was systematically employed to achieve upregulation and sustained high expression of endogenous FOXP3 in mammalian cell lines through targeting of the core promotor and several regulatory regions. Using an activator-domain fusion based dCas9 transcription activator, robust upregulation of

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FOXP3 was achieved, and an optimal combination of single guide RNAs was selected, which exerted an additive effect on FOXP3 gene upregulation. Simultaneous targeting of FOXP3 and EOS, a transcription factor known to act in concert with FOXP3 in initiating a Treg phenotype, resulted in upregulation of FOXP3 downstream genes CD25 and TNFR2. dCas9-based systems provide great promise in DNA footprint-free phenotype perturbations (perturbation without the risk of DNA damage) to drive development of transcription modulation-based therapies.

P-01.1-20
Natural ligands of tetraplex DNA structures as one of the important elements of gene regulation

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Over the past decade, many studies have confirmed that non-canonical DNA (ncNA) structures can play an important role in the regulation of critical cellular processes and can be promising targets for treating oncological and infectious diseases. An actively developing field of modern pharmacology is connected with the search for new synthetic drugs targeted to non-canonical DNA G-quadruplexes (G4s) and I-motifs (IMs) structures. Of particular fundamental interest is the potential gene-regulatory role of natural G4 / IM ligands — metabolites and components of nutrients. Although G4 stabilizing properties of a number of flavonoids and porphyrin derivatives are known today, this area has not been sufficiently studied. Here, we first characterized DNA structural selectivity of a set of metabolites of porphyrins and chlorins, abundantly present in the human body. Our approach is based on a set of common methods: spectrofluorometry, Förster resonance energy transfer (FRET) melting and fluorescent intercalator displacement (FID). The original panel of oligonucleotide targets included fragments of the human genome: G4 oligomers of various topologies, imperfect G4 and a set of IMs. Several natural ligands exhibited high affinity for the above targets, and specific interactions were of particular interest. For instance, we revealed a special selectivity of tripyromethane to the promoter site of oncogene cKit (Kd = 0.7 ± 0.2 mkM) and chlorine derivative Ce6 (Kd = 3.6 ± 0.2 mkM) to the telomeric G4 site 22AG. Collectively, the data obtained indicate the possibility of the participation of natural ligands in genomic regulation and can be useful for understanding the mechanisms of development of pathologies associated with impaired porphyrin metabolism (for example, with multiple sclerosis, lupus, etc.). This work was supported by RSF [20-15-00017].

P-01.1-21
Analysis of the chromatin spatial organization in the human keratin type II gene locus identified potential locus control regions

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Keratins are encoded by 54 genes clustered in two loci at chromosomes 12 and 17. Keratins expression patterns are highly specific for different epithelial cell types and differentiation stages, and are regulated by local microenvironment signals that influence epigenetic state of the keratin loci and activity of individual keratin promoters. Previously, it has been shown that, in mammals, switching of expression pattern within multigene tissue-specific loci are regulated by complex enhancer elements (locus control regions, LCR) characterized by the presence of domains of H3K27ac and H3K4me1 epigenetic marks, binding of numerous transcription factors and chromatin architectural proteins. Here, we applied high-resolution chromosome conformation capture method, C-TALE (Chromatin TArget Ligation Enrichment) to build the map of spatial chromatin organization of 12q13.13 locus in the human epidermal skin keratinocytes at two distinct stages of differentiation. We found that expression switching between keratin 5 (specific for basal epidermal keratinocytes) and keratin 1 genes (actively transcribed in spinous K1/K10-positive keratinocytes) is accompanied by drastic changes in chromatin loop profile inside the locus. Both genes in active state spatially interact with two regions located at 5'- and 3'-flanks of the locus, and loose these contacts upon inactivation. Comparison with publicly available datasets showed that both regions identified possess the features characteristic for LCRs: high level of histone H3 acetylation at K27 position, presence of numerous DNAse I hypersensitivity sites, binding of CTCF and transcription factors involved into keratin transcription regulation. These data potentially denote that transcription switching within keratin gene domain is controlled by two locus control regions forming chromatin loops with active keratin promoters. The reported study was funded by RFBR, project number 20-04-00778. *The authors marked with an asterisk equally contributed to the work.

P-01.1-22
Phosphorylation of PBAF subunit PHF10 is upregulated in the cell cycle

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Remodeling complexes play an important role in the regulation of gene expression during development and in the adult organism. The PBAF remodeling complex is the subfamily of SWI/SNF complexes in mammals that change the structure of chromatin, providing access for transcription factors to regulatory sequences of genes. One of the functions of the ATP-dependent chromatin-remodeling complexes is the regulation of the expression of genes involved in the cell cycle. Several studies have shown that phosphorylation can regulate the function of ATP-dependent remodeling complexes during the cell cycle. PHF10 is the part of the signature module of PBAF, which determines the interaction of the PBAF complex with chromatin. In mammalian cells, PHF10 is represented by four isoforms, that are alternatively incorporated in the PBAF complex and have different effects on the genes remodeled by the complex. We have shown that two isoforms of PHF10 contain N-terminal domain that interacts and can be phosphorylated by Akt kinase. Phosphorylation of the N-terminal domain of PHF10 is triggered by two key serines, that are the part of the motif recognized by the Akt kinase. The phosphorylation status of PHF10 is upregulated in G1/S transition during the cell cycle and correlates with the activation of Akt kinase, which is known to regulate the function of various proteins at the G1/S and G2/M transitions. Moreover, PBAF complexes that incorporate PHF10

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isoforms with the Akt kinase recognition site and those that can't be phosphorylated are differently distributed on the promoters of the genes related to the cell cycle. Thus, we can conclude that phosphorylation of PHF10 can modulate the functions of the PBAF complex, mediating chromatin remodeling and gene activation in the cell cycle. This study was supported by the Russian Foundation for Basic Research [grant number 180400885].

P-01.1-23
Revisiting the *Staphylococcus aureus* SarA regulon by high-throughput screening

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Staphylococcus aureus is a commensal bacterium and an opportunist pathogen causing a variety of human and animal infections, from benign to harsh. It is also the major cause of community-associated and nosocomial infections. Its pathogenicity involves virulence and metabolic factors whose expression can be regulated by regulatory RNAs (sRNAs) or transcriptional factors (TF). Among *S. aureus* TF there is SarA, a major virulence factor involved in biofilms formation and antibiotic resistance. Our laboratory has recently shown that SarA represses the expression of two RNAs, srn_3610_sprC and srn_9340. My project is to expand the SarA regulon especially to regulatory RNAs. Using RNA-Seq, we compared the transcriptome of the isogenic ΔsarA mutant to the wild-type strain and we found that, in absence of SarA, the transcription of 299 genes was repressed while the transcription of 142 genes was actived. To discriminate the direct and indirect targets, ChIP-Seq experiment was realized to localize the SarA binding sites in the whole genome. Around 340 SarA binding sites were identified with a *P*-value under 1.10^{-250}. Combining RNA-Seq and ChIP-Seq results, we highlighted one hundred genes for which the level of transcription appears to be directly correlated to the SarA binding on their promoter region. We chose to validate 14 potential targets by northern blot and gel shift experiments. Among the sRNA targets validated *in vitro*, we can cite the $SprA2_{AS}$ antitoxin sRNA which is part of the type I toxin-antitoxin (TA) system SprA2/$SprA2_{AS}$ and the SprG2 sRNA encoding the toxin of the SprG2/SprF2 type I TA system. In the type I TA system, a drop of sRNA antitoxin level induces toxic peptide translation that results to bacterial cell death or bacteriostasis. Our results reveal a new aspect of SarA which appears, for the first time, as a novel regulator of type I TA systems strengthening its involvement in *S. aureus* virulence. *The authors marked with an asterisk equally contributed to the work.

P-01.1-24
Transcription-facilitating histone chaperones interact with genomic and synthetic G4 structures

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The heterodimeric complex FACT (FAcilitates Chromatin Transcription) is a histone chaperone (HC) that promotes nucleosome reassembly to relieve the nucleosomal barrier for Pol II. In our recent human protein microarray (protoarray)-based analysis of the G-quadruplex (G4) interactome, FACT and its functional analog BRD3 efficiently bound several model G4 DNA structures [1]. Other structural and functional FACT-analogs, nucleolin and ATRX, were reported to interact with G4 DNA earlier [2,3]. So we assumed that affinity to G4 structures is a common feature of FACT-like HCs. First, we made a preliminary set of G4 motifs and verified their G4 folding in pseudointercellular and pseudoextracellular conditions by circular dichroism spectroscopy. Second, we checked HC-G4 binding *in vitro* by microscale thermophoresis, fluorescence assay, and polyacrylamide gel electrophoresis. As a result, we detected affinity in the low micromolar to nanomolar range. Then, we studied intercellular localization and toxicity of G4s on cancer cell lines to check if exogenous G4s can be used as anti-cancer agents. We also found out that genomic G4 motifs are often colocalized with occupancy sites of chosen HCs, so genomic G4s might be involved in transcription regulation too. We conducted molecular modeling experiments to discover whether HC-binding G4s could interfere with the HC function. To sum up, our findings encourage future investigations of genomic G4 contribution to nucleosome remodeling and transcription. This work was supported by RFBR [19-04-00050 A]. References: [1] Vlasenok M. et al. (2018) Data in Brief,18:348-359. [2] Bates P. J. et al. (2009) Experimental and Molecular Pathology, 86(3):151-64. [3] Law M. J. et al. (2010) Cell,143(3):367-78

P-01.1-25
Role of amino-terminal region of MSL1 in recruiting of dosage compensation complex of *Drosophila melanogaster*

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The dosage compensation complex (DCC) provides equivalent level of X chromosome transcription between females and males by approximately doubling upregulation of the transcription level of single male X chromosome. DCC specifically recognizes X chromosome in males but this mechanism remains unclear. DCC consists of five proteins, MSL1, MSL2, MSL3, MOF, and MLE,

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and includes two noncoding RNAs, roX1 (3.7 kb) and roX2 (0.6 kb), which perform partially redundant functions. Proteins MSL1, MSL3, MOF, and MLE are also present in females and are involved in the regulation of gene expression independent of dosage compensation. As the MSL2 protein is specific for males, it is believed that the MSL2 protein has a key role in the specific binding of DCC to the X chromosome of males. The MSL1 protein functions as a flexible framework for the assembly of DCC. The N-terminal coil-coiled domain provides homodimerization of the MSL1 protein and interaction with the N-terminal RING domain of the MSL2 protein. The C-terminal end of MSL1 is responsible for recruiting of MSL3 and MOF in DCC. Here we have tested role of the N-terminal region of MSL1 in specific recruitment of DCC to the X chromosome in males. Using yeast two-hybrid assay, we mapped two regions, 1–15 aa and 41–65 aa, in MSL1 that interact with many zinc-finger transcription factors. We expressed several MSL1 variants carrying different deletions in the N-terminal region. As a result, deletion of any of the regions (1–15 aa or 41–65 aa) led to male lethality suggesting critical role of these regions of MSL1 in activity of DCC. In accordance, we did not observe binding of DCC to polytene chromosomes in mutant males or females expressed MSL2 protein ectopically. These results suggest that specific interaction of zinc-finger proteins with MSL1 is critical for recruitment of DCC to the X chromosome in males. The work was supported by RSF grant № 21-14-00211.

P-01.1-26
Stochastic choice of expression between IL-2 and HIV-1 in T helper cells as a result of chromosomal interactions between IL-2 promoter and HIV-1 LTR

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When HIV-1 infects T helper (Th) cells, it intercepts the transcriptional regulatory mechanism of IL-2 for its own expression and survival. Transcription of both HIV-1 and IL-2 are stochastic events. The IL-2 promoter and HIV-1- LTR share high sequence homology to which common cellular transcription factors (TFs) bind to either activate or repress their expression. The Ets-2 TF acts as a transcriptional repressor for both IL-2 and HIV-1 in naive Th effector cells. The aim of our study was to investigate whether chromosomal interactions between IL-2 promoter and HIV-1-LTR may be responsible for a stochastic choice of transcriptional expression between IL-2 and HIV-1 in Th cells and whether Ets-2 is involved in these interactions. To this end, Jurkat T cell lines carrying a non-infectious copy of HIV-1 (Jurkat-Lat) or HIV-1-LTR region (Jurkat-LTRG) or none (Jurkat) were cultured for 6h ± mitogens (P/I). IL-2, Ets-2, HIV1-Tat and LTR-GFP reporter gene mRNA was determined by qPCR. In P/I-stimulated cells, IL-2 mRNA levels were increased in all cell lines, whereas HIV-1-LTR mRNA levels were increased in Jurkat-Lat and Jurkat-LTRG cells; Ets-2 mRNA levels were decreased in Jurkat and Jurkat-Lat cells. CoIP assays showed a strong protein-protein interaction between Ets-2 and Tat mainly in non-stimulated cells. ChIP assays verified the involvement of Ets-2 and Tat in IL-2 and HIV-1 transcriptional regulation by their simultaneous presence on the ARRE-1/TATA and ARRE-2 sequences of the IL-2 promoter and the RATS element of HIV-1-LTR. The presence of Tat on these elements was more pronounced in stimulated cells. Finally, 3C experiments showed that the IL-2 promoter and the HIV-1 LTR were localized in close proximity in the nucleus of unstimulated cells. Our results suggest that a physical protein-protein interaction between Ets-2 and Tat mediates the interaction between the IL-2 promoter and HIV-1- LTR. This mechanism may be responsible for HIV-1 latency in resting Th cells.

P-01.1-27
ppGpp binding to RNA polymerase accounts for its role in transcription-coupled DNA repair

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The small molecule alarmone guanosine-3',5'-(bis)pyrophosphate (ppGpp) serves in bacteria to adapt their physiology in response to environmental changes. It has been known for years that acting synergistically with its cofactor DksA, ppGpp globally reprogram gene expression in response to nutrient deprivation by altering the initiation properties of RNA polymerase. Besides RNA polymerase, variety of diverse cellular targets of ppGpp have been described, which are involved in a wide range of cellular processes. Evidence also suggests an independent role of ppGpp in preserving genomic integrity. It was shown previously that ppGpp is induced in response to DNA damage and couples transcription to DNA repair, suggesting RNA polymerase elongation complex as a target for ppGpp. Here, we prepared a series of *E. coli* strains carrying new chromosomal mutations in RNA polymerase affecting ppGpp binding to elongation complex that lead to markedly decreased cellular survival under different DNA damaging conditions. In contrast to previously described RNA polymerase holoenzyme mutants that eliminated ppGpp function in stringent response, new mutants are tolerable to amino acids starvation. Data suggest different mode of action of ppGpp under various stresses. This work is supported by the Russian Science Foundation grant 17-74-30030.

P-01.1-28
Genome sequencing of differently pathogenic *Fusarium oxysporum* f. sp. lini strains

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Fusarium oxysporum f. sp. lini causes flax wilt which is one of the most devastating diseases of flax grown for the production of numerous consumer and industrial goods. At the same time, *Fusarium oxysporum* demonstrates considerable diversity. For instance, representatives of the species may vary in the number of chromosomes and the presence and homology of the SIX genes associated with virulence, whereas strains can be hardly classified by morphology and differ in severity of caused wilt symptoms within the same forma specialis. Consequently, whole-genome sequencing is needed to establish the differences between genome structures of the flax pathogen. This work aimed at sequencing the genomes of 5 strains

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of *F. oxysporum* f. sp. lini of high (strain #483), medium (#476, #525), and low (#456, #482) virulence. The DNA was extracted according to the developed protocol and sequenced on the Oxford Nanopore Technologies and Illumina platforms (300+300 bp), and the collected Nanopore data were used to assemble draft genomes of the strains. In addition, we reassembled the genome of the highly pathogenic isolate #39 using our previously obtained data. Illumina reads of the sequenced strains were mapped against the resulting assemblies and the genome of the endophyte Fo47 (GenBank, GCA_013085055.1). Nearly 99% of the isolate #39 genome were covered by its Illumina reads, whereas the Illumina reads of other strains mapped against a larger fraction of the isolate #39 assembly in comparison with the Fo47 genome, but the percentages were similar for all but strain #482. The received data will be useful for further investigation of *F. oxysporum* virulence mechanisms and the structure of its population for the development of methods to prevent the disease and economic losses. This work was funded by RFBR according to the research project 19-34-90055. *The authors marked with an asterisk equally contributed to the work.

P-01.1-29
Differential expression of genes involved in the lignan synthesis in flax varieties with different content of secoisolariciresinol diglucoside

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Lignans prevent the onset and development of diabetes, cardiovascular and oncological diseases. Linseed is the richest source of lignans, with secoisolariciresinol diglucoside (SDG) being the main flax lignin; however, flax varieties significantly differ in the SDG content. On the Illumina platform, we performed transcriptome sequencing of capsules of five flax genotypes that vary in SDG content. As a result, we obtained about 10 thousand reads for each sample. For evaluation of gene expression, reads were trimmed with Trimmomatic, mapped to the Linum usitatissimum reference genome (GenBank assembly: GCA_000224295.2) using STAR, quantified using BEDTools, and analyzed using edgeR. Expression levels for a particular gene were quantified as read counts per million (CPM). It is known that PLR family genes encoding pinoresinol-lariciresinol reductases, DIR genes encoding dirigent proteins, and UGT genes, the products of which are UGT-glycosyltransferases, are involved in SDG synthesis, therefore we compared the expression levels of these genes in capsules of flax varieties with diverse content of SDG. Genotype-specific differences in expression were identified. Besides, for the identification of polymorphisms in the studied genes, variant calling was performed using FreeBayes for genome regions corresponding to the genes of PLR, DIR, and UGT families, and single-nucleotide polymorphisms (SNPs) were revealed. The obtained results contribute to the understanding of the role of PLR, DIR, and UGT genes in the determination of lignan content in flaxseed. This work was financially supported by the Russian Science Foundation, grant 21-16-00111.

P-01.1-30
Effect of radioprotective damage suppressor protein (Dsup) on non-irradiated and exposed to various types of ionizing radiation *D. melanogaster* at transcriptome and physiological levels

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High dose of ionizing radiation is one of the most harmful exposome factors, in that view, recently discovered tardigrade damage suppressor protein (Dsup) is promising for the development of new genetically engineering way of innate radioprotection that does not require targeted drug delivery. Dsup is a nuclear localized protein, which binds to DNA and nucleosomes and as suggested increases protection of chromatin by direct shielding from ROS. Real mechanism of radioresistance, structural properties of Dsup protein, possibility to induce hindrances to chromatin compaction, translation, reparation are remaining unexplored. The objective of this work was to reveal how Dsup protein affects gene expression and lifespan in normal conditions and after irradiation in well-studied model object *D. melanogaster*. We generated several lines of *D. melanogaster* expressing Dsup under control of Act5C gene promoter. Transcriptome analysis of Dsup expressing flies established alterations in expression of genes related to transcription, chromatin silencing, chromosome organization, mitotic spindle elongation etc. that indicates some level of disorder in DNA machinery and chromatin compaction. Currently ongoing lifespan test for *D. melanogaster* expressing Dsup will allow to elucidate the impact of this disorder on physiological parameters. To estimate radioresistance of Dsup expressing flies, we treated them with γ-radiation dose of 1000 Gy, which is close to LD_{50}, and plan to carry on irradiation with heavy ions. Three days survival rate after irradiation was higher in Dsup-expressing line and this is the first confirmation of *D. melanogaster* radioresistance enhancement by Dsup. Difference in response to ionizing radiation between Dsup-expressing and control biological groups will be estimated by transcriptome analysis. For further step we pretend to reveal Dsup spatial structure and parameters of Dsup-DNA conjugate by SANS and SAXS techniques. *The authors marked with an asterisk equally contributed to the work.

P-01.1-31
CP60 and BEAF-32 proteins are sumoylated *in vivo*

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Post-translational modifications of substrate proteins by proteins of the SUMO family are widespread among eukaryotes. Using a model organism *D. melanogaster*, we study the effect of sumoylation on the properties of transcription factors CP60 and BEAF-32. Using the yeast two-hybrid system (Y2H), we showed that the CP60 and BEAF proteins directly interact with the Ubc9 protein, which is a SUMO E2 ligase. The BEAF protein interacts with Ubc9 through the C-terminal sequence of 200–280 aa. The CP60 protein interacts with Ubc9 through the C-terminal sequence 420–440 aa. Using bioinformatics, canonical sumoylation sites of CP60 and BEAF were predicted. We made point substitutions at the CP60 and BEAF predicted sites. The Ubc9 protein did not interact with either mutant forms of CP60 or

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BEAF in Y2H assay. Therefore, the tested CP60 and BEAF sumoylation sites are involved in sumoylation. In addition, sumoylation of CP60 and BEAF was tested in IP experiments on S2 cells. As a result of immunostaining with antibodies to the tested proteins, we observed 2 bands. The lower one corresponded to the unmodified forms of CP60 and BEAF proteins, and the upper one corresponded to SUMO modifications. The upper band was detected by antibodies to the dSmt3 protein. The BiFC method was used to study the interaction between CP60, BEAF, and dSmt3 proteins. Constructs expressing the full-length CP60 and BEAF proteins, labeled with the fluorescent half of Venus, and the dSmt3 protein, labeled with the fluorescent half of the CFP, were created. On S2 cell culture, it was demonstrated that the CP60 and BEAF proteins are sumoylated in vivo. We have found that CP60 and BEAF form discrete speckles within the nucleus. Such speckles are partially colocalized with the CP190 protein, marking "insulator bodies". It is possible that, like the proteins of the Su(Hw) insulator, CP60 and BEAF are involved in regulatory complexes by sumoylation. The research was funded by RFBR, project number 19-04-00257.

P-01.1-32
Regulation of key lymphotropic factors by the transcription factor Ets-2 in T cell lines modeling uninfected and virus-infected T cells

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Ets-2 is a transcriptional regulator associated with cell differentiation. Our recent work showed that Ets-2 downregulates the expression of cytokine genes and HIV-1 in resting but not in activated T cells. In this work, we investigated Ets-2 role in regulating the expression of NFAT2, NF-κB/p65, c-Jun and c-Fos lymphotropic factors, which play critical role in T cell activation and differentiation, and CDK10 kinase, which controls Ets-2 degradation. *In-silico* analysis revealed putative Ets-2 binding sites at the NFAT2, c-Jun and c-Fos promoters. T cell lines Jurkat (modeling T cell signaling/activation) and H938 (containing full HIV-1 LTR) were transfected with increasing amounts of an Ets-2 overexpressing vector in the presence/absence of mitogens. mRNA levels were determined by qPCR and protein levels by Western immunoblotting. In unstimulated Jurkat cells, Ets-2 overexpression resulted in upregulation of NFAT2 and c-Jun mRNA and protein, increase in c-Fos mRNA and NF-κB/p65 protein, and downregulation of CDK10 mRNA and protein. In unstimulated H938 cells, Ets-2 increased NFAT2, c-Jun, and CDK10 mRNA and protein and increased NF-κB/p65 protein. In stimulated Jurkat cells, Ets-2 increased NFAT2, c-Jun, and c-Fos mRNA and protein and decreased CDK10 mRNA and protein. In stimulated H938 cells, Ets-2 increased NFAT2, c-Jun, and c-Fos protein and decreased CDK10 protein. In summary, Ets-2 upregulates key lymphotropic factors expression, either through its physical interaction with gene promoters or through its involvement in signaling pathways that have a direct effect on their expression. In Jurkat cells, Ets-2 downregulates CDK10 expression to its advantage. Although Ets-2 downregulates CDK10 expression in stimulated H938 cells, CDK10 is upregulated in unstimulated cells, accelerating its degradation; this may lead to disruption of HIV latency in resting virus-infected T cells. Part of this work is published in: Davoulou P et al (2020) Mol Biol Rep 47, 7871–7881.

P-01.1-33
Molecular mechanisms responsible for stress hyper resistance of Bacilli strains isolated from the International Space Station

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Microbes accompany humans everywhere, including orbiting space stations. The rather harsh living conditions inside the International Space Station (ISS) facilitate the emergence of bacterial strains that are hyper resistant to the action of various stress factors, including antibiotics, DNA damaging factors, and oxidative stress. The pathogenic microorganisms and biodestructors are found among these strains and represent a potential biological threat to long-term space missions. To date, the mechanisms of hyper resistance of ISS strains to the action of various stress factors are unknown. Strains *Bacillus subtilis*-20 and *Bacillus licheniformis*-24 were isolated from the Russian segment of the ISS's interior volumes. The isolated strains are highly resistant to stress factors that cause DNA damage and oxidative stress, such as 4-nitroquinoline 1-oxide (4-NQO), methyl methanesulfonate, and ultraviolet radiation. Compared with terrestrial strains, the ISS strains display overexpression of genes encoding components of DNA damage repair systems by homologous recombination (HR) and non-homologous end-joining (NHEJ). Overexpression of DNA repair genes is associated with overexpression of the corresponding transcriptional regulators. Using the CRISPR / dCas9 repressor system, we showed that both HR and NHEJ systems are required for the terrestrial *B. licheniformis* strain resistance to 4-NQO and zeocin. In contrast, in *B. licheniformis*-24 an essential role in resistance to these stress agents is played only by the NHEJ system. The data obtained indicate the greater significance of the NHEJ repair system in the hyper resistance of Bacilli strains to stress factors. This work was supported by the Russian Science Foundation (project no. 17-74-30030). The study was implemented within the framework of the Russian Academy of Sciences areas of research # 65.5.

P-01.1-34
Comparison between synthetic and natural G4-ligands and the effects of their binding to DNA

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Among local and non-canonical DNA structures are G-rich sequences that can form G-quadruplex (G4) sequences; these are widespread among prokaryotic and eukaryotic kingdoms and are

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an increasingly used target for regulatory proteins. There is evidence from more than three decades of research that confirms their function in important cell processes such as transcription and replication. G-quadruplexes have become targets for anticancer drugs because of their ligands' high specificity and affinity. We focused on the differences between binding of G4-ligands to DNA and their subsequent effect on cells. In the present study, the effects of G4-ligands on tumor (MCF-7) and non-tumor cell lines (HEK293ft) were studied in relation to binding to the DNA and connected mechanisms. Concentration and time-dependent studies were performed to study different effects of these compounds. Mentioned cell lines were used to study cell viability after exposure to G4-ligands. Morphological alterations were observed by fluorescent-based studies as confocal microscopy or ThT assay (Thioflavin T) supplemented by electromobility shift assay (EMSA). Apoptotic and anti-apoptotic genes were studied, and a strong effect derived from the binding of our tested molecules was observed. Our study proposes that natural G4-ligands were used preferentially due to their significant lower toxicity to non-cancer cell lines, and with the same specificity as designed synthetic ligands, that even at low concentrations cause significant cell damage. In conclusion, treatment with natural G4-ligands leads to comparable effects to what we obtained with synthetic ligands and holds a great potential as future cardio-oncological therapeutics. Another possible approach could be to use G4-ligands to protect cell from subsequent chemotherapy. *The authors marked with an asterisk equally contributed to the work.

P-01.1-35
Structural dynamics of human histone chaperone FACT

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Multifunctional histone chaperone FACT is involved in nucleosome dis- and reassembly during transcription, replication and repair of DNA, and has a relatively conserved structure. Human FACT (hFACT) is a heterodimer of SPT16 and SSRP1 subunits that moderately stabilizes nucleosomes. Here we studied human FACT structure using single particle electron microscopy after negative staining. We evaluated a set of conformational states and proposed a hypothesis describing the structural dynamics of human FACT. It has been shown that nucleosome-free hFACT is a dynamic structure: several states reflect its conformational flexibility. The "closed" complex is characterized by four compact domains; "intermediate" state represented by three domains having compact structure and more disordered fourth domain, and the "open" complex, represented by three domains forming almost linear structure. Based on results a mechanism of conformational flexibility of human FACT has been proposed. It has been shown that hFACT domains are connected to each other through flexible linkers and SPT16 and SSRP1 dimerization domains (DDs) form the "joint"-like connection between the two subunits. In the "closed" conformation the DNA-binding surface of FACT is covered by its two C-terminal and middle domains (MDs). We propose that during conversion to the "open" complexes SPT16 N-terminal domain (NTD) is moving away from the other subunits leading to formation of the first intermediate state with the NTD domain poorly resolved or not resolved, while less mobile DDs and MDs maintain more compact structure and the DNA-binding site is still protected by the CTDs. In the "open" state SPT16/SSRP1 visible MDs and DDs form almost linear structure, unmasking the DNA-binding sites and making them accessible for the interaction with a nucleosome. Work was supported by the Russian Science Foundation (#19-74-30003). Electron microscopy was performed on the Unique equipment setup "3D-EMC" of Moscow State University. *The authors marked with an asterisk equally contributed to the work.

P-01.1-36
Drosophila zinc finger protein CG9890 is localized on the promoters of active genes and involved in the regulation of both basal and inducible transcription

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In previous studies, we showed that Drosophila zinc finger protein CG9890 is localized in the nucleus and interacts with chromatin modifying and remodeling complexes SAGA and dSWI/SNF as well as with ORC complex, which is necessary for the positioning of the replication origins. ChIP-Seq of CG9890 protein revealed that the protein is localized mostly on the promoters of active genes. In this work we decided to investigate the role of CG9890 in transcription regulation, given that CG9890 interacts with main transcriptional complexes and was found predominantly on gene promoters including promoters of ecdysone-dependent genes. To this end we decreased the level of CG9890 in Drosophila S2 cells by RNA interference and analyzed the changes in the level of mRNA 21 of the CG9890-associated gene compared to the control samples. After knockdown of the CG9890 protein, the amount of mRNA of ten of these genes changed, including five ecdysone-dependent genes ftz-f1, hr39, CG15279, Eip78C and Eip75B. To investigate the role of CG9890 in the regulation of inducible transcription we have performed analysis of activation of ecdysone-dependent genes hr4 and dhr3 after 20-hydroxyecdysone treatment in cell upon RNA interference of CG9890 protein compared to the control cells. As expected, the mRNA level of both genes significantly increased after ecdysone induction (882 times for dhr3 and 148 times for hr4) in control cells. After knockdown of the CG9890 protein activation of hr4 and dhr3 genes was significantly lower (369 and 45 respectively). Thus, the CG9890 protein is a new member of the cell transcriptional network which is localized on active promoters, interacts with the main transcription and replication complexes, and is involved in the regulation of both basal and inducible transcription. This work was supported by the Russian Science Foundation (Grant No. 20-14-00269).

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P-01.1-37
Investigation of the mechanism of adaptation to mutations in the translation termination factor genes in yeast

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In the yeast *Saccharomyces cerevisiae*, there are two translation termination factors eRF1 and eRF3. These factors are encoded by the SUP35 and SUP45 genes and deletion of any of them leads to the death of yeast cells. However, viable strains with nonsense mutations in both the SUP35 (Chabelskaya et al., (2004) Mol. Genet. Genomics 272, 297–307) and SUP45 genes (Moskalenko et al., (2003) BMC Molecular Biology, 4, 2) were previously obtained in our laboratory. To invesigate the genetic factors supporting the viability of these SUP35 and SUP45 nonsense mutants, we performed whole genome sequencing of strains carrying mutant alleles sup45-n and sup35-n using Illumina technology. While no common SNPs or indels were found in these genomes, we discovered a systematic increase in the copy number of the plasmids carrying mutant sup35-n and sup45-n alleles. To validate these findings, we used qPCR method which confirmed the differences in the relative number of SUP35 and SUP45 gene copies between strains carrying wild-type or mutant alleles of SUP35 and SUP45 genes. Moreover, we used qPCR to compare the number of copies of the SUP45 and SUP35 genes in strains carrying different nonsense mutant variants of these genes in chromosomal location. qPCR results indicate that the number of mutant gene copies (in particular, sup35-218, sup35-222, sup45-104, sup45-105, sup45-107) is increased compared to the wild-type control, possibly due to tandem duplications or other chromosomal abnormalities. The results obtained support the hypothesis that an increase in the copy number of a mutant gene may be a universal mechanism of yeast adaptation to mutations of essential genes encoding translation termination factors. This work was supported by the RSF grant "Genetic and Epigenetic Control of Translation Termination" 18-14-00050. Equipment of the Resource Centers "Development of Molecular and Cellular Technologies" and "Biobank" of SPBU was used in this study.

P-01.1-38
Transcriptome responses in human skeletal muscle to acute and regular aerobic exercise: a meta-analysis

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Inactivity is strongly associated with the development of type 2 diabetes mellitus, cardiovascular diseases, depression, cachexia, and sarcopenia. Regular aerobic exercise is broadly used as a key intervention to prevent and treat these diseases. In our study we aimed to identify and compare (i) the transcriptome responses to acute and regular aerobic exercise in human skeletal muscle and (ii) the biological effects and transcription factors (TFs) associated with these transcriptome changes. We used the robust rank aggregation method to analyze 27 transcriptome datasets for the m. vastus lateralis of healthy humans subjected to acute and/or regular aerobic exercise. We found 493 and 815 differentially expressed genes (DEGs) (mainly up-regulated) for the acute and regular exercise, respectively. Gene ontology analysis found that acute exercise was associated with regulation of transcription, angiogenesis and response to various stimuli, while regular exercises – with extracellular matrix (ECM) organization and angiogenesis. Surprisingly, only a few genes encoding mitochondrial proteins were up-regulated after acute and regular exercise (18 and 27 out of 1097, respectively). Promoters of DEGs induced by acute exercise were strongly enriched by the TFs belonging to the ATF/CREB/AP-1 superfamily. In turn, promoters of DEGs induced by regular exercise were strongly enriched by the TFs RELB, JUND, ETV4. Summing up, acute exercise mainly induces the expression of genes encoding TFs and other regulators of gene expression. By contrast, regular aerobic exercise up-regulates genes encoding ECM proteins and many ECM regulators, as well as angiogenesis-related genes. Exercise-induced up-regulation of the mitochondrial proteins is not regulated at the mRNA level. This work was supported by the RFBR (no. 19-315-90135).

P-01.1-39
Chromosome-level genome assembly using both long-read and short-read sequencing and structural variant analysis of two yeast strains from the Peterhof genetic collection

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The yeast *Saccharomyces cerevisiae* is a model eukaryotic organism, which has been thoroughly studied for decades. Thousands of yeast strains have been described both on phenotypic and genomic level; however, the majority of investigations utilizes limited number of laboratory strains, which are closely related to the reference S288C strain. The Peterhof genetic collection (PGC) was established independently from S288C, though the lineages have been crossed several times during strain evolution. Several PGC strains are extensively used for studies in the fields of prion biology and translation termination; however, the genomic data is scarce and limited to short-read technologies (Drozdova PB et al. (2016) PLoS ONE, 11, e0154722). We analysed the genomes of two widely-used PGC strains, 74-D694 and U-1A-D1628, using both long-read sequencing with Oxford Nanopore (ONT) and short-read Illumina techniques. Reference-quality assemblies were obtained by constructing draft assemblies from ONT reads using canu, followed by polishing with Nanopore raw signal and Illumina short reads. Hybrid assembly also allowed us to reconstruct sequences of circular molecules,

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i.e., mitochondrial DNA and 2-micron yeast plasmid. Structural variant (SV) analysis showed multiple mid-length insertions and deletions within coding sequences, e.g., in the NUP100 and SCH9 genes. High contiguity of the assemblies allowed us to deduce possible routes of reciprocal unbalanced translocations between chromosomes I, VIII, IX, XI, and XVI of the PGC strains. We also showed that SV-driven formation of hybrid flocculin alleles is likely responsible for the lack of invasive growth of the strains studied (Barbitoff YA et al. (2021) G3, in press). This work was supported by the RSF grant 18-14-00050 "Genetic and epigenetic regulation of translation termination", RFBR grant 20-34-70156, State Research Program 0112-2016-0015, and by RCs "Development of Molecular and Cellular Technologies" and "Biobank" of SPbSU. *The authors marked with an asterisk equally contributed to the work.

P-01.1-40
Adaptation of CRISPR/Cas9 system for directed elimination of mitochondrial DNA copies with mutations

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Most pathogenic mitochondrial DNA (mtDNA) mutations are commonly heteroplasmic, whereby wild-type and mutant genomes co-exist in the same organelle. An increase in the number of mutant molecules can lead to the reaching of a certain threshold of heteroplasmy and the manifestation of mitochondrial disease. We propose a strategy for modifying the components of the CRISPR/Cas9 system to manipulate the mtDNA heteroplasmy level in a cell. Using the cybrid cell line of heteroplasmic mitochondrial disease, bearing the point mutation m.8993T>G in mitochondrial ATP6 gene we initially confirmed intramitochondrial localization of modified Cas9 nuclease, expressed from the cell nucleus by immunocytochemistry, western blot and electron microscopy. Stable and uniform expression of Cas9 nuclease in the cells was obtained by integrating the Cas9 gene, which contains the mitochondrial localization signal, into the genome of cybrid cells by Sleeping Beauty transposon system. To deliver the second component of the system, we made several modifications of the guide RNA (gRNA) using described determinants of nucleic acids import into mitochondria. In vitro cleavage analysis showed that such modifications do not affect the assembly and functional activity of the CRISPR/Cas9 system. During subsequent immunocytochemical analysis of the localization of fluorescent-labeled variants of modified gRNA we did not detect their import into the mitochondria. Now we aim to solve this problem by adapting self-complementary adeno-associated viruses type 2 (scAAV2) to deliver the gene encoding the gRNA as part of the viral genome into the mitochondria. The results of our work will contribute to the further development of the technology for treatment of mitochondrial diseases.

P-01.1-41
Association of vitamin D receptor gene FokI polymorphism with temporomandibular disc degeneration

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Background: Genetic variations might play a role in susceptibility to temporomandibular joint internal derangement TMJ-ID) with osteoarthritis (TMJOA). Vitamin D receptor (VDR) polymorphisms have been shown to be associated with disc degeneration-linked pathologies, particularly osteoarthritis (OA). Objective: The aim of this study was to evaluate the association of VDR FokI polymorphism (rs2228570, C>T) with TMJOA by gender. Experimental details: The study included 58 (46 women, 12 men) unrelated TMJOA patients (32.07 ± 8.1) and 71 (34 women, 37 men) healthy controls (28.22 ± 5.9). DNA was extracted from blood samples using the standard proteinase K/phenol–chloroform method. FokI polymorphism was investigated using a polymerase chain reaction-based restriction fragment length polymorphism assay. Results: FokI genotypes were different between overall TMJOA group and the healthy controls ($P = 0.026$, $\chi^2 = 7.2$). Heterozygote Ff genotype was statistically different compared to FF homozygote genotype between overall TMJOA patients and the healthy controls (OR = 0.43, 95% Cl:0.2–0.92, $P = 0.028$). In overall TMJOA patients ff genotype brought a risk factor of 1.88 although statistically not significant (OR = 1.88, 95% Cl: 0.51–6.86, $P = 0.33$). Similarly, in TMJOA women heterozygote Ff genotype was different (borderline significance) compared to FF genotype (OR = 0.43, 95% CI:0.16–1.10, $P = 0.07$). In TMJOA women although ff genotype was not different compared to FF genotype, carrying the ff genotype was 2.77 times more risk factor (OR = 2.77, 95% Cl: 0.29–26.03, $P = 0.37$) for temporomandibular joint degeneration. Conclusion: VDR FokI receptor polymorphism may present susceptibility to TMJOA and ff genotype may be associated with joint degeneration in TMJOA patients and TMJOA women. This relation needs to be further evaluated in a large cohort study.

P-01.1-42
Molecular mechanisms driving MYC-mediated cell competition in a colon cancer model

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Cell competition is a mechanism through which weaker cells are recognized by their fitter neighbors and eliminated through apoptosis. It is important to detect and eliminate these cells, to maintain healthy tissues and prevent the development of diseases. However, cells can acquire alterations, such as upregulation of MYC, increasing the relative fitness and inducing the elimination of neighbor wild-type cells. Here, we optimized a cell competition assay to screen for genes involved in this mechanism in winner and loser cells using two colon cancer cell lines expressing different levels of MYC. We observed that LoVo and LS174 engage in cell competition, with LoVo behaving as loser and LS174 behaving as winner, as previously reported. In

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this scenario, loser cells are eliminated by apoptosis in a caspase-dependent manner, arrest cell cycle at G0/G1 phase and migrate less. On the other hand, winner cells are even less eliminated when in presence of loser cells, increase the number of cells entering S phase and start to migrate more. Also, results from conditioned media and caspase-inhibition experiments suggest that improved survival and increase proliferation of winner cells is based on the presence of a survival factor in culture media, whereas cell elimination of the loser cells is dependent on cell-cell contact with winner cells. In addition, treatment with a chemotherapy agent abolish competition and loser cell elimination suggesting potential therapeutically translation of results. To identify new genes involved in this cell competition scenario we have performed RNA-sequencing of winner and loser cells. We found three genes upregulated in winner cells, AQP3, MYT1 and NRIP1, which were functional validated to be required for the elimination of the loser cells. Additionally, members of the PI3K/AKT/mTOR and HER2/EGFR pathways were found to be altered. These data improve our knowledge on cell competition and its role in cancer development.

P-01.1-43
Prediction of transcription factors regulating contractile activity-induced gene expression in skeletal muscle

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Skeletal muscle tissue makes up more than a third of the human body mass, playing a key role in fat and carbohydrate metabolism; maintenance of normal/increased level of diurnal physical activity provides effective protection against various metabolic disorders. Therefore, investigation of the molecular mechanisms underlying skeletal muscle gene expression to physical exercise is of fundamental importance. We aimed to predict transcription factors (TFs) regulating transcriptomic response to an exercise in human skeletal muscle using the position of transcription start sites (TSSs) and individual promoter regions surrounding each TSS. Biopsies from the vastus lateralis muscle were taken prior to, and 1 h, 3 h, and 6 h after an aerobic exercise in 10 males. The exact TSSs were identified using the cap analysis of gene expression. The individual promoters were identified using the open chromatin position (obtained in myotubes) and the density of various TFs binding sites (15982 human ChIP-seq experiments; the GTRD database). The position of TSSs and individual promoters (with length from hundred to 2000 b.p.) were identified for ~12000 genes. The position weight matrix method showed that the individual promoters work better for prediction of TFs than promoters with "standard" length. Finally, using unsupervised clustering we identified 20 groups of co-expressed exercise-induced genes and predict TFs for each cluster: several well-investigated TFs (Ca^{2+}-dependent and early response TFs, regulators of fat and carbohydrate metabolism, etc.), as well as TFs with unknown role in the regulation of skeletal muscle gene expression (zinc fingers, etc.). A robust approach to prediction of TFs using the position of TSSs and individual promoters was developed. The method can be used to study the dynamics of TFs activation in co-expressed genes in human skeletal muscle subjected to various physiological and pharmacological stressors. This work was supported by the RFBS grant #20-015-00415.

P-01.1-44
Effect of oropharyngeal swab quality on the success of NGS library preparation

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SARS-CoV-2 genomes rapidly change due to mutations, and thus the fast evolution of this virus has been observed worldwide. As is known, the majority of the identified mutations do not influence a significant effect on the spread. While some mutations or combinations can provide the virus advantages because some strains spread quickly around local populations. We had tested 477 oropharyngeal swabs from patients from different regions of Russia with diagnosis COVID-19 to perform a whole-genome sequencing to detect new variants of SARS-CoV-2. We were faced with the problem of low-quality samples. It had led to the deterioration of amplification. It's a significant moment to perform screening tests. Methods: Previously designed primers panel [1] was used for SARS-CoV-2 whole genome amplification. The PCR products ranging from 1757 to 2054 bp were mixed, purified. Libraries were constructed using Nextera XT DNA Library Preparation Kit (Illumina, FC-131-1096). Sequencing was performed as described in [1]. The consensus sequence was submitted to GISAID database (hCoV-19/Russia/CRIE). Results: We completely amplified and whole-genome sequenced the 173 of SARS-CoV-2 genomes from 477 samples. The success of genome fragments amplification varied from 76% to zero when we used samples obtained from different sources (from different clinics). We suppose that this dramatic difference could be explained by the composition of transportation buffers that are purchased by different clinics for routine diagnostics of COVID-19 by RT-PCR methods. Our results demonstrated that the success of amplification does not depend on the storage and transportation time. Acknowledgements: The study was funded by RFBR, project number 20-04-60561. References: [1]. AS Speranskaya, VV Kaptelova et al. SCV-2000bp: a primer panel for SARS-CoV-2 full-genome sequencing. bioRxiv 2020.08.04.234880; doi: https://doi.org/10.1101/2020.08.04.234880. Keywords: SARS-CoV-2, mutations, high-throughput sequencing.

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P-01.1-45
Metagenomic analysis of viruses in bat fecal samples from Moscow region reveals the whole genome sequences of Mastadenovirus and Alphacoronavirus

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Due to the current epidemiological situation caused by the pandemic of COVID-19 the study of virus diversity in bats as a potential source of zoonotic diseases is crucially important. Our previous study based on genus specific PCR-using Coronaviridae-specific primers and following high throughput sequencing revealed the presence of several types of coronaviruses. In this study we performed the whole genome sequencing for the samples which were positive for Coronaviridae in order to get more information about viral communities in bat fecal samples. Fecal samples from bat species (*Pippistrellus nathusii*, *Nyctalus noctula*, *Myotis brandtii*, *Myotis daubentonii*) were collected in 2015 in the Moscow region and stored in RNA later. RNA extraction using Viral RNA Mini Kit (Qiaqen), library preparation using NEBNext Ultra II Directional RNA Library Prep Kit for Illumina (NEB) and HTS on Illumina Hiseq platform was performed. Metagenomic data was obtained using FastQC, trimmomatic 0.38 and SPAdes 3.13 for genome assembly, taxonomy of resulting contigs was identified by the BLASTn.For 8 bat fecal samples positive for Coronaviridae we performed the whole genome sequencing and metagenomic analysis. In one sample we detected the complete genome (37915 bp) with 74,66% nucleic identity to Mastadenovirus sp. isolate WA3301 from a *Nyctalus noctula*, in the other sample from *Pippistrellus nathusii* we found the complete genome sequence with 82% nucleotide identity to Bat alphacoronavirus isolate BtCoV/020_16/M.dau/FIN/2016 (28.245 bp).Whereas, as we've reported the presence of Alphacoronavirus before, this is the first time when we detect the whole genome of Mastadenovirus. The fact that the material was obtained from bats which nest next to human and domestic animals our findings might give better understanding the potential risk of the cross-species transmission and virus migration in bats from Moscow Region. This work was funded by RFBR, project number № 20-04-60561\20.

P-01.1-46
Regulation of ferritin and globin genes in cold-water sea sponges *Halisarca dujardini* and *Halichondria panicea* and its role during reaggregation

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Sponges (Porifera) are the oldest multicellular organisms with the unique ability to restore the functional organization of the body after mechanical dissociation in the form of multicellular aggregates. The cold-water sponges of the White Sea *Halisarca dujardini* (H.d.) and *Halichondria panicea* (H.p.) (cl. Demospongia) are extremely resistant to daily and seasonal changes in water temperature, salinity and oxygenation. We studied sponge ferritins (the main iron-storage proteins) and globins (heme-containing oxygen transporters) along with their contribution to cellular plasticity during the reaggregation experiment. In the transcriptomes of H.d. and H.p. (previously published in: Finoshin AD, Adameyko KI, et al. (2020) PLoS ONE 15(2): e0228722) and in the draft genome of H.d. we identified genes of interest and confirmed their amino acid sequences by mass spectrometry. Three ferritin genes were identified in H.d. and one in H.p. A TATA box, Inr and DPE promoter elements were found in H.d. ferritin genes. The 5'UTRs of H.d. and H.p. ferritin mRNAs contain iron-responsive elements (IREs). In both sponge species, we identified two globin genes: androglobin and neuroglobin. Expression of neuroglobin of H.d. unlike human Ngb is regulated by a TATA-containing and not CG-rich promoter (previously published in: Adameyko KI et al. (2020) Mol Biol 54, 416–420). RNA-Seq samples of H.d. collected at different life cycle stages and subjected to the reaggregation experiment revealed differential expression: in the samples collected at the end of body growth (autumn) and the beginning of spermatogenesis (winter) expression of ferritin and neuroglobin decreased during dissociation and then restored during reaggregation; in the samples collected at the beginning of body growth (summer) expression of both genes decreased during both stages. Our results suggest the evolutionary importance of globins and ferritin genes in the morphogenetic processes of multicellular animals. *The authors marked with an asterisk equally contributed to the work.

RNA function

P-01.2-01
Small noncoding RNAs as a tool to modulate gene expression

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Pseudomonas putida is a highly attractive production system for industrial needs. However, for its improvement as a biocatalyst at the industrial level, modulation of its gene expression is urgently needed. We report the construction of a plasmid expressing a small RNA-based system with the potential to be used for different purposes. Due to the small RNAs modular composition, the design facilities and ability to tune gene expression, they constitute a powerful tool in genetic and metabolic engineering. In the tool presented here, customized sRNAs are expressed from a plasmid and specifically directed to any region of a chosen target. Expression of these customized sRNAs is shown to differentially modulate the level of endogenous and heterologous reporter genes. The antisense interaction of the sRNA with the mRNA produces different outcomes. Depending on the particularity of each sRNA-target mRNA pair, we demonstrate the duality of this system, which is able either to decrease or increase the expression of the same given gene. This system combines high specificity with the potential to be widely applied, due to its predicted ability to modulate the expression of

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virtually any given gene. This plasmid can be used to redesign *P. putida* metabolism, fulfilling an important industrial gap.

P-01.2-02
Large scale screening system for protein–protein interactions based on mRNA-display and Psoralen Analysis of RNA-Interactions and Structures (PARIS)

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Most proteins in eukaryotic cells do not exhibit their function individually, but mediate their action through interaction with other proteins, forming a complex protein–protein network interactome. Detecting and analyzing protein–protein interactions (PPI) is still a relatively cumbersome process, a big issue being that many methods rely on detecting PPI of previously known interacting proteins, failing to detect yet unknown interaction partners. On the other hand, methods to detect the interactions of DNA or RNA *in vivo* have been previously described. One such method is Psoralen Analysis of RNA-Interactions and Structures (PARIS), which locks the interacting RNA sequences inside of cells with the help of a crosslinking reagent AMT and UV light. The interacting RNAs are then ligated and sequenced with NGS to reveal all RNA—RNA interactions. We aim to develop a method for analyzing RNARNA interactions, similar to that utilized in PARIS, but instead of native RNA I will use mRNAs bound to their coded protein. The covalent binding of proteins to their coding mRNA will be achieved by using the mRNA-display, in which 3' modified RNA molecules are covalently linked to their coded polypeptide on the ribosome during protein translation in vitro. Interaction between the two polypeptides also brings into proximity their adjacent coding mRNAs. We expect that due to the proximity of the RNAs (which is achieved through PPI of their coded proteins) they will be more likely to interact with the mRNA coding for the interacting polypeptide, than with mRNAs of polypeptides, which do not form PPI. The initial work will be done for in vitro transcribed and translated polypeptides, which have been previously described to form strong PPI. The successful implementation of the method on proof of principle peptides will have strong implications for future development of modular bionanostructures.

P-01.2-03
Transcriptional analysis of the human apoptosis-related BOK gene reveals novel, alternatively spliced messenger RNAs, a previously unknown 5′untranslated region (5′ UTR), and two new, shorter 3′ UTRs

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The BCL2 family includes pro- and anti-apoptotic members, basically differing in the combination of BCL2-homology (BH) domains. BOK is an apoptosis facilitator, yet an anti-apoptotic behavior has been described as well. This ambiguity could be attributed to the existence of multiple, alternatively spliced transcripts encoding for distinct BOK protein isoforms. In this study, we describe the discovery of novel transcripts of the human BOK gene, most of which comprise new open reading frames (ORFs) and probably encode for new BOK protein isoforms. Moreover, we determined a new 5′untranslated region (5′ UTR) and two shorter 3′ UTRs. In brief, we started by performing bioinformatical analysis of publicly available expressed sequence tags (ESTs). Next, total RNA was isolated from 23 cancer cell lines originating from different human tissues and first-strand complementary DNA (cDNA) was synthesized starting from 5 μg of total RNA. PCR primers were designed to amplify only BOK cDNA-specific sequences and were used in two successive PCRs. Rapid amplification of cDNA ends (RACE) was used to study the 5′ and 3′ UTRs. Nested PCR and RACE products were electrophoresed on an agarose gel; bands of unexpected size were gel-extracted, purified, and sequenced using Sanger sequencing. We also performed next-generation sequencing (NGS) to unravel rare BOK transcripts. Our results led to the discovery of 21 novel BOK transcripts, 13 of which have distinct ORFs. in silico translational analysis revealed the putative existence of 7 novel BOK protein isoforms lacking internal peptides and possessing distinct C-termini. Moreover, we identified a previously unknown 5′ UTR, probably preceded by its own promoter, as well as two novel, shorter 3′ UTRs, with fewer post-transcriptional regulatory regions. Overall, the prospect of novel BOK protein isoforms and alternative UTRs raises questions about the role of this gene in both normal and pathological states and necessitates additional research.

P-01.2-04
Study of milk exosome nucleic acids

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Exosomes are nanovesicles contained in various biological fluids and participating in intercellular communication. The biochemical compound of exosomes demonstrates the presence of various nucleic acids, in particular, microRNA and mRNA. In this regard, exosomes are promising candidates for developing new drug delivery vehicles in personalized medicine. Among all biological fluids containing exosomes, milk is the only available on an industrial scale; therefore, the analysis of nucleic acids in milk exosomes is an urgent task. A modified protocol was applied to isolate milk exosomes, including several stages of centrifugation, ultrafiltration, ultracentrifugation, and gel chromatography. Simultaneously, the absolute amount of 20 different types of microRNA was determined in the fat fraction of bovine, goat, and horse milk. For these microRNAs, specific patterns of relative expression were determined in each type of milk. Further studies of the miRNA composition of milk fractions will reveal specific miRNA markers for each of the studied species. This research was supported by the Russian Science Foundation grant 18-74-10055. *The authors marked with an asterisk equally contributed to the work.

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P-01.2-05
The role of post-transcriptional editing of herpes simplex virus 1 miR-H2 during latency

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Herpes simplex virus 1 (HSV-1), an important human pathogen, has been shown to encode 29 microRNAs (miRNAs), the function of which is yet to be revealed. The functions of miRNAs are defined by their sequences, as only one nucleotide difference can dramatically change the specificity of miRNA for its target. Using advanced bioinformatics tools, we recently found that miR-H2, an HSV-1 miRNA targeting important virus gene ICP0, is extensively edited by the function of the adenosine deaminase acting on RNA (ADAR) in latently infected human ganglia. The ADAR proteins deaminate adenosines to inosine (recognized as guanosine), they have a critical role in homeostasis, and it is possible that expression of ADAR proteins in neuronal tissue specifically regulates the establishment and maintenance of latency. This editing function could indicate that the virus is using cellular processes to broaden the scope of possible miRNA targets, including viral and host targets, or to affect their stability. Furthermore, to comprehensively investigate this phenomenon and biological relevance, we used biological approaches to test the relevance of a few most important bioinformatically predicted targets of edited HSV-1 miR-H2. In addition, to extensively analyze this phenomenon during infection with other viruses, we analyzed many publicly available small-RNA deep-sequencing data sets including samples infected with Epstein–Barr virus, Kaposi's Sarcoma-associated herpesvirus, cytomegalovirus, human herpesvirus 6 and human papillomavirus and our preliminary results show that many viruses employ this phenomenon to specifically change the crucial part of the miRNA sequence important for binding to its target or have effect on host miRNAome during the course of the infection. Understanding this novel aspect of non-coding RNA biology will not only shed light on an incredibly complex life cycle of HSV-1 but also might reveal important cellular pathways.

P-01.2-06
Novel approach to the delivery into the cells and light-activation of the guide RNA for the genome editing CRISPR/Cas9 system

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Use of the CRISPR/Cas9 system for molecular biological and gene engineering purposes is the issue of the day. Effective delivery of the components of system in cells is obligatory for their successful application. The main goal of our study is the development of novel approach for the delivery of guide RNA (crRNA) in the cells. The proposed approach is based on the usage of additional photocleavable oligodeoxyribonucleotide complementary to guide RNA and bearing ligands facilitating the penetration of the whole construction through the cell membrane. The presence of photocleavable linkers in oligodeoxyribonucleotide conjugate permits to destroy them by UV-irradiation after cells' penetration, to liberate guide RNA and to initiate the CRISPR/Cas9 system functionalizing in the cells. This approach is proposed for the first time in our study. Oligodeoxyribonucleotides containing three photocleavable linkers and their 3'-functionalized analogs were synthesized by solid-phase phosphoramidate method using phosphoramidite on the base of 1-(2-nitrophenyl)-1,2-ethanediol. Non-modified stable oligodeoxyribonucleotides and their 3'-functionalized analogs were also prepared as controls. The conjugates of 3'-amino-, 3'-alkyne and 3'-phosphate containing oligonucleotides with cholesterol, pyrene, peptide and GalNac were prepared using different methods of conjugation. The kinetics of modified oligonucleotide photocleavage and thermal stability of the duplexes of the additional oligodeoxyribonucleotides and their conjugates with guide RNA were investigated. The possibility of activation of designed CRISPR/Cas9 system by UV-irradiation was demonstrated using model DNA plasmid. Proposed approach to the delivery into the cells and light-activation of the genome editing CRISPR/Cas9 system can be applied in future for photocontrollable gene editing in cells. The reported study was funded by RFBR, project number 19-34-51026.

P-01.2-07
Antibodies from human milk hydrolyze microRNAs

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Milk is a unique biological fluid; it contains all components necessary for the development and protection of newborn. Milk is not just a mixture of such substances, as proteins, lipids, carbohydrates and nucleic acids. Of particular interest are milk immunoglobulins, possessing various catalytic activities, such as protease, phosphatase, DNase, RNase and others. In this work, the ability of milk IgG and sIgA to hydrolyze various RNA substrates was investigated. MicroRNAs that regulate the expression of many genes are found in many biological fluids, including milk. It was shown that miRNAs regulate the expression of genes associated with the development of the newborn's immune system. Milk immunoglobulins possess RNase activity in the hydrolysis of various miRNA substrates, both highly expressed in milk and unrepresented in milk, as well as homooligoribonucleotides and cellular ribosomal RNA. In addition, microRNA isolated from human skimmed milk and various fractions of milk: cell sediment, lipid fraction, and milk plasma were studied. Analysis of the isolated RNAs was performed using an Agilent 2100 Bioanalyzer on RNA 6000 Pico and Small RNA chips. Using reverse transcription with stem-loop primers and subsequent quantitative real-time PCR, the expression of 25 miRNAs in different fractions of milk was evaluated. This work was supported by a grant from the Russian Science Foundation 18-74-10055.

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P-01.2-08
In vitro analysis of Hfq chaperone involvement in binding of small RNAs from the LhrC family to the fri gene transcript of *Listeria monocytogenes*

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Listeria monocytogenes (Lm) is a Gram-positive human pathogen. The ferritin protein (Fri), encoded by the fri gene, is responsible for the storage of iron in Lm cells and plays an important role in the stress adaptation and pathogenesis process. The transcription of gene fri originates from three distinct promoters, a σB-dependent and two σA-dependent ones, leading to 3 types of transcripts with different 5'-end sequences. Recently, we found that fri is the first gene of a five-gene operon in which the last one is lhrC5 encoding a small non-coding RNA. The LhrC5 belongs to a multicopy sRNA family comprising seven sibling sRNAs, from which LhrC1–5 have been identified as sRNAs interacting with the Hfq chaperone, however so far, the interaction between LhrCs and mRNAs was shown to be Hfq-independent. The aim of the study was to determine the binding capacity of small RNAs from the LhrC family with the 5'-end of fri gene transcripts, and a potential involvement of the Hfq chaperone in the formation of sRNA-mRNA complexes. The results of RNA EMSA assays showed that LhrC5 possess weak ability to interact with the mRNA of the fri gene originating from the σA2 promoter. This binding was efficiently strengthened in the presence of Hfq chaperone. An analogical analysis performed for LhrC4 also showed its ability to bind to the fri transcript, but the Hfq chaperone did not enhance the LhrC4-mRNA fri interaction. Therefore, the obtained results indicate that Hfq protein mediates the formation of LhrC5-mRNA fri complexes, but not the formation of LhrC4-mRNA fri complexes. These results imply the specific role of LhrC5 in the regulation of the expression of fri gene, however further research is needed to pinpoint the biological relevance of the observed in vitro involvement of Hfq in the formation of LhrC5-mRNA fri complexes. Acknowledgements: This work was supported by a grant no. 2015/18/E/NZ6/0064 from the National Science Center, Poland.

P-01.2-09
The role of RNA interference in the formation of protective systems of wheat against the pathogen Septoria *Stagonospora nodorum* Berk

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RNA interference (RNAi) can control the activity of host genes or partner genes in an interorganismal interaction system. It is well known that RNAi is important in the formation of an immune response against viral pathogens, but its role in infection with fungal pathogens is poorly studied. Dicer-like (DCL) proteins and Argonaute (AGO) proteins are the objects of interest in our research. DCL proteins are RNases that initiate the processing of double-stranded RNAs. AGO proteins bind siRNA and use it as a guide for the recognition of mRNA of target genes or pathogen RNA. Both proteins are involved in the formation of an RNA-induced gene silencing complex. There is evidence that the accumulation of DCL1 protein in wheat upon infection can cause structural defects in pathogens. The importance of AGO1 proteins in the formation of compatible relationships is confirmed by the fact that the suppression of their synthesis increased resistance to fungal infections. RNAi was never studied in local varieties of wheat, so we measured expression level of the genes of these two protein families in variety Tulaykovskaya 108, that was bred to be resistant to *Stagonospora nodorum*, and variety Salavat Yulaev, which is susceptible to septoria. Tulaykovskaya 108 responded to infection with an aggressive *Stagonospora nodorum* strain by both accumulation of transcripts of TaAGO1 gene and a decrease in TaDCL4 gene activity. Changes in the expression level of these genes in the variety Salavat Yulaev were precisely the opposite. Our results suggest that TaDCL4 and TaAGO1 genes play an important role in the formation of a protective response of the Tulaykovskaya 108 variety. Conversely, the accumulation of TaDCL4 transcripts in a susceptible variety of wheat Salavat Yulaev, as well as a decrease in the transcriptional activity of TaAGO1, may be a substantial condition for the successful formation of compatible relationships and the development of the infection.

P-01.2-10
Localization of functional elements in 3′ untranslated region of orb mRNA required for Drosophila oogenesis

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Post-transcriptional regulation of gene expression includes transport and localization, translation and turnover of mRNA molecules. mRNA 3' untranslated region (3' UTR) participates in all these processes interacting with RNA-binding proteins in cells. Orb protein is required for formation of the egg chamber and establishment of polarity during Drosophila oogenesis. When the orb 3'UTR is deleted, the process of oocyte specification is disrupted and the homozygous mutant flies are sterile. The aim of the study was to localize in orb 3'UTR functionally important elements required for Drosophila oogenesis. To determine evolutionarily conserved elements of the orb 3' UTR, we mapped phastCons score calculated in a multiple alignment of twelve Drosophila species, mosquito, honeybee and red flour beetle, on the sequence. Then we used CRISPR/Cas 9 system to introduce a deletion of almost complete 3'UTR of the orb gene and inserted three different sequences in the orbΔ3'UTR deletion mutant using the phiC31 integrase system to compensate the deletion. We obtained two fly lines with insertions which partially overlap each other, one of them included a complete region with conservative elements of orb 3' UTR (line hpn1), while another one included a part of this conservative region (line XN). As a control we used flies with an insertion of complete orb 3'UTR sequence. Females of line hpn1 laid eggs with normal morphology (92.6–98.7%) and hatching rate was the same as in control line (74–75.5%). Eggs of XN females had polarity defects (in

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96–99% of eggs analyzed) and low hatching rate (0–2.9%). We showed that insertion of a conservative sequence with a total length less than 50% of orb 3'UTR rescues female fertility. This might suggest about redundancy of functional elements in orb 3'UTR or that the rest of the sequence is required for other processes in Drosophila. The work is supported by the Russian Science Foundation (18-74-10051).

P-01.2-11
Identification of five novel circular RNAs deriving from BCL2L12 apoptosis-related gene in colon cancer cell lines

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Circular RNAs (circRNAs) result from back-splicing of pre-mRNA. Recent advances in high-throughput sequencing uncovered the widespread expression of circRNAs in cancer cells, arousing scientific interest. However, current knowledge regarding circRNAs deriving from human apoptosis-related genes is poor. BCL2L12 is a member of the BCL2 family, which – despite not bearing a typical anti-apoptotic protein structure – has been reported to exert an anti-apoptotic activity. Moreover, BCL2L12 is widely expressed in colon cancer. The purpose of this study was to identify novel BCL2L12 circRNAs in colon cancer cells. Total RNA extracts from seven colon cancer cell lines (Caco-2, HCT 116, HT-29, COLO 205, SW 620, DLD-1, and RKO) were reverse-transcribed, using random hexamer primers. First-round PCR was conducted using divergent primers, to amplify BCL2L12 cDNAs having derived from circRNAs. Nested PCR was then performed, followed by Sanger sequencing, to determine the sequence of each circRNA. Subsequently, bioinformatical tools were used to predict the interactions of these circRNAs with microRNAs (miRNAs) and RNA-binding proteins (RBPs), and to predict internal ribosome entry sites (IRES) and open reading frames (ORFs). Thus, we discovered five circRNAs, all consisting of both complete and truncated exons of BCL2L12 gene. Expression analysis revealed differential expression patterns of these circRNAs in the seven colon cancer cell lines. Next, in silico analysis revealed that these circRNAs are likely to interact with miRNAs and RBPs; for instance, circ-BCL2L12-1 is predicted to bind miR-1915-5p, while circ-BCL2L12-1 and circ-BCL2L12-2 are predicted to sequester splicing factors such as MNBL1, SRFS1, SRFS2, SRFS3, and SRFS5. Moreover, circ-BCL2L12-2 is predicted to possess a functional ORF, but no IRES. In conclusion, this study uncovered, for the first time, the complete sequence of circRNAs deriving from BCL2L12 and suggested some putative interactions of these molecules.

P-01.2-12
Functional studies of RNA-binding properties of the *Escherichia coli* RNA chaperone ProQ

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Structural and functional research of interactions between small RNAs and RNA-binding proteins takes an important place in studies of gene expression regulation processes in all living organisms. For over two decades only two proteins, CsrA and Hfq, have been known as RNA chaperones in bacteria. Recent studies have shown that FinO domain-containing proteins seems to be another important class of bacterial RNA chaperones. ProQ protein, a FinO family member, was originally identified as an osmoregulatory factor required for optimal expression of the proline channel protein ProP. However, ProQ has now emerged to function as a sRNA binding protein in Salmonella and *Escherichia coli*. A similar role is predicted for proteins containing ProQ/FinO domain from various bacteria. ProQ from *Escherichia coli* is a monomeric protein. It adopts an elongated structure with two domains separated by a disordered linker region. The N-terminal domain of ProQ (NTD), spanning residues 1–121, is composed of a ProQ/FinO domain and shares 35% sequence identity with its paralogs. C-terminal domain (CTD) is structurally related to Tudor-like domains commonly found in eukaryotic chromatin regulators. N- and C-terminal domains may interact with target RNA independently or cooperatively, depending on context. The aim of our investigation is to study interactions of *E. coli* ProQ as well as its isolated domains with potential sRNA targets in the cell. We have identified a number of sRNAs that bind to ProQ and have measured the affinity of the protein to the RNAs. Based on these data significant differences in the RNA binding properties of the N-terminal and C-terminal domains of ProQ have been revealed. Using hydroxyl radical footprinting we have also identified protein-binding sites in the sRNAs. This work was supported by Russian Scientific Foundation 21-74-00086.

P-01.2-13
Novel long non-coding RNA is dysregulated in different liver and pancreatic cancers

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Long non-coding RNAs (lncRNAs) can be dysregulated in many diseases including various cancers. They could serve as perspective biomarkers for primary diagnostics of malignancies, discrimination of different tumors or as prognostic criteria for patients. In our previous work we discovered a novel lncRNA HELIS (aka "healthy liver specific") that is significantly down-regulated in hepatocellular carcinoma (HCC), but is not expressed in liver cholangiocarcinoma (CCA) and hepatoblastoma (HBL) neither

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in other normal nor malignant human tissues including pancreas [1]. During our search for additional biomarkers, we have found a novel transcript that we have called CHOL. We showed that its expression is upregulated mainly in CCA samples (but not in HCC) as well as in case of pancreatic duct adenocarcinoma (PDAC) in comparison to adjacent normal tissues. Despite of its extremely short length (about 200 nt), CHOL RNA has two splicing variants consisting of two or three exons. We demonstrated that long and short isoforms are differentially expressed in hepatic (Huh7, HepG2, HepaRG) and pancreatic (MIA PaCa2, Capan-2, AsPC-1, PANC-1) cell lines as well as in a number of liver and pancreatic cancer tissues. We suggest these RNA isoforms might have different functions in carcinogenesis and increased shorter isoform can be associated with more malignant phenotype. In sum, obtained data allows us to consider CHOL lncRNA as a potential oncomarker of liver and pancreatic cancers that could be used together with HELIS for development of a lncRNA-based diagnostic panel. This work is supported by Russian Science Foundation grant No. 20-74-00141. Reference: [1] Burenina O. Y. et al. Panel of potential lncRNA biomarkers can distinguish various types of liver malignant and benign tumors. J Cancer Res Clin Oncol. 2021; 147 (1):49–59.

P-01.2-14
Characterizing PNPase activity, an exoribonuclease relevant for the virulence of the human pathogen *Campylobacter jejuni*

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Bacteria need to promptly respond to environmental changes. Ribonucleases (RNases) are key factors in the adaptation to new environments, by enabling a rapid adjustment in RNA levels. The exoribonuclease polynucleotide phosphorylase (PNPase) is essential for low-temperature cell survival, affects the synthesis of proteins involved in virulence and has an important role in swimming, cell adhesion/invasion ability, and chick colonization in *C. jejuni*. However, the mechanism of action of this ribonuclease is not yet known. In this work we have characterized the biochemical activity of *C. jejuni* PNPase (Cj-PNP). Our results demonstrate that Cj-PNP is a processive 3' to 5' exoribonuclease that degrades single-stranded RNAs. Its activity is regulated according to the temperature and divalent ions. We have also shown that the KH and S1 domains are important for trimerization, RNA binding, and, consequently, for the activity of Cj-PNP. Moreover, our results show the activity of PNPase is modulated by several cellular metabolites, which suggests a link between the cellular metabolic status and RNA metabolism. We have solved the 3D structure of Cj-PNP by X-ray crystallography. The structural information helped to understand the mechanistic details of the degradative process. These findings will be helpful to develop new strategies for fighting against *C. jejuni* and may be extrapolated to other foodborne pathogens.

P-01.2-15
How ribonucleases control characteristic traits of *Pseudomonas putida* lifestyle

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Ribonucleases (RNases) are important effectors on post-transcriptional regulation and coordinators of bacterial adaptation to stress. The aim of this work is to shed light on the importance of ribonucleases function for the versatile metabolism of *Pseudomonas putida*. This Gram-negative saprophytic bacterium is generally recognized as a laboratory work-model of environmental bacteria and is endowed with a diversity of metabolic and stress-endurance traits that make it an ideal chassis for biotechnological needs. Bacterial RNases have been most extensively studied in the model organism *Escherichia coli*, however, in *P. putida* that information is still scarce. Following this line, we have constructed mutants for five different *P. putida* ribonucleases, two exoribonucleases (PNPase and RNase R) and three endoribonucleases (RNase E, RNase III and RNase G). We globally analyse the physiological and metabolic costs of the absence of each of these enzymes. The impact of these mutants is also tested in terms of growth, motility and morphology, as well as the effects of different oxidative chemicals that could act as a proxy of the stressors present on the natural environment of this microorganism. We conclude that each ribonuclease seems to be specifically related with different traits of the metabolism of this microorganism. Moreover, the physiological response of *P. putida* in the absence of each enzyme differs, in some cases, from the one previously observed in *E. coli* revealing evident differences in the metabolism of these two bacteria but also different enzymatic functions of the ribonucleases in each bacterial landscape.

P-01.2-16
The importance of ribonucleases in human pathogens

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Bacteria needs to rapidly adjust to different environmental conditions and ribonucleases (RNases) are key factors in the adaptation process. These enzymes ensure maturation, degradation and quality control of RNA thus, contributing to the maintenance of the optimal amount of each transcript in the cells. Moreover, there is growing evidence of the direct involvement of these enzymes in bacterial pathogenicity. In this investigation we aim to understand the role of two different ribonucleases in the cell metabolism of the human pathogens *Campylobacter jejuni* and *Streptococcus pneumoniae*. Our results demonstrate that PNPase from *C. jejuni* is a processive 3' to 5' exoribonuclease that degrades single-stranded RNAs. We have also shown that the KH and S1 domains are important for trimerization, RNA binding, and, consequently, for the activity of PNPase. Mutational analysis allowed us to detail the knowledge about the mechanism of action of this ribonuclease. We also demonstrated that RNase R from *S. pneumoniae* directly controls the expression levels of frr, fusA and infC mRNAs, the corresponding transcripts of RRF, EF-G and IF3, respectively. We present evidences showing

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that accumulation of these factors leads to a decreased amount of 70S active particles, compromising translation. This study provides insights into processes that are important in cell metabolism, highlighting the importance of ribonucleases in bacterial adaptation and survival.

P-01.2-17
Role of long non-coding RNAs in the formation of oncogenic chromosomal rearrangements associated with thyroid cancer

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Various types of cancer are characterized by certain chromosomal rearrangements, these mutations play a vital role in the oncogenesis often leading to the formation of fusion genes. However, the molecular mechanisms of oncogenic translocations have not yet been established. There is a hypothesis that this process is mediated by specific RNAs that have homology regions with two chromosomes areas near the junction site. Recently, an example of an RNA-mediated specific genomic rearrangement in mammalian cells has been described, with enhanced expression of AZI1 mRNA in prostate cancer cells acting as an "initiator" RNA to induce oncogenic TMPRSS2–ERG gene fusion. Based on this hypothesis and approach, we assumed that lncRNAs may have similar roles in various osncogenic chromosomal rearrangements. We have developed an in silico method for searching for candidate lncRNAs that can potentially trigger the formation of chromosomal mutations, and compiled a database of candidates that can drive the following translocations in thyroid cells: PAX8/PPARγ, CCDC6/RET, NCOA4/RET. According to our preliminary data, expression of some of the candidate RNAs causes significant effects on the frequency of the corresponding oncogenic translocations. This work is supported by grant 19-74-10083 from Russian Science Foundation. *The authors marked with an asterisk equally contributed to the work.

P-01.2-18
Thermodynamics of interaction of interferon α-2b with different forms of oligonucleotides

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Interactions of protein–nucleic acids play a decisive role in many biological processes. RNA-based drugs that can bind and affect the work of epigenetic regulators and transcriptional proteins through interaction, with regulatory domains, can be used as safe analogues. We studied the ability of yeast oligoribonucleotides (ORN), yeast ORNNa salt (ORNNa), and yeast ORNs-D-mannitol complex (ORNs-D-M) to affect fluorescence quenching and conformational changes of Interferon α2b – a vital protein of the antiviral cell defence mechanism. To determine the energy parameters of protein–ligand interactions, we used isothermal titration nanocalorimetry Nano ITC. The results of the study of enthalpy changes in the titration of interferon α-2b acid form of ORN and ORN: D-M was −63.28 kJ/mol and −96.61 kJ/mol, respectively, and for the ORNNa and ORNNa: D-M respectively 4,52 and 5,14 kJ/mol. The change in entropy when adding the ORN to interferon α-2b was −38.72 and in the case of the ORN: D-M −63.53 kJ/mol*K, respectively. The change in entropy when adding the ORNNa to interferon α-2b was 17.05 kJ/mol*K, and the ORNNa: D-M, respectively, 17.58 kJ/mol*K. A similar pattern demonstrated when studying the change in Gibbs energy during titration of interferon α-2b with ORNORN and ORN: D-M and it was −24.56 and −33.07 kJ/mol, respectively. And when titrated with ORNNa and its ORNNa: D-M, respectively −12.9 and −12.43 kJ/mol. The obtained curves of titration of INF with ORN indicate that the reaction of the interaction between the protein and acidic ligands occurs exothermally, and with salt endothermically. These results of studying the effects of thermodynamics of different forms of RNA and their complexes with D-mannitol in the titration of interferon α-2b may indicate different sites of binding of different forms of ORN to protein, as well as other modes of binding and various types of conformational changes in the protein.

P-01.2-19
Role of the RNA exosome complex and non-coding RNAs in regulation of the cell wall stress response in yeast *S. cerevisiae*

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The nuclear RNA exosome is a conserved complex involved in 3'-5' RNA degradation and processing in eukaryotic cells. In yeast, its 9-subunit structural core and catalytic subunit Dis3 are essential for viability, while the absence of the second catalytic subunit Rrp6 is inviable only at high temperature. In this work, we show that RNA exosome mutants undergo osmoremedial cell lysis at high temperature or upon treatment with cell wall stressors (published in Novačić A et al. (2021) Mol. Biol. Cell 32 (5), 363–375). The catalytic subunit Dis3 provides exoribonuclease catalytic activity needed for maintenance of cell wall stability in *S. cerevisiae* and is supported by a non-catalytic function of the Rrp6 subunit and the RNA exosome cofactors Rrp47 and the TRAMP complex. Moreover, we identify the mechanism leading to cell wall destabilization, by showing that genes encoding proteins involved in the early steps of protein glycosylation pathway are downregulated in RNA exosome mutants at high temperature, through mechanisms involving increased accumulation of non-coding RNAs transcribed at their gene loci. Consistent with this, overexpression of the essential enzyme Psa1, which catalyzes the synthesis of the mannosylation precursor, suppresses temperature sensitivity and aberrant morphology of these cells. Additionally, we show that the reason why the cell wall instability phenotype of RNA exosome mutants is most pronounced for the commonly used W303 laboratory strain is a mutation that inactivates the SSD1 gene, which encodes an RNA-binding protein involved in regulating the translation of cell wall-related transcripts.

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P-01.2-20
All components of mPSF subcomplex, but not symplekin, participate in polyadenylation of transcripts generated by RNA polymerase III

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Most RNAs synthesized by RNA polymerase II (Pol II) are subject to polyadenylation (PA). The main cis-signal directing PA is the AAUAAA hexamer near the 3'-end of mRNA. A few multiprotein complexes are involved in poly(A) tail synthesis by poly (A)-polymerase (PAP). The main of these complexes is CPSF consisting of two subcomplexes: mPSF directly binding the AAUAAA signal and mCF responsible for the cleavage of RNA downstream of this signal. MPSF consists of CPSF160, CPSF30, hFip1, and WDR33 subunits, whereas mCF includes CPSF73, CPSF100, and symplekin. Previously, we discovered that not only Pol II transcripts but also certain transcripts generated by RNA polymerase III (Pol III) could be polyadenylated in an AAUAAA-dependent manner. Namely, this unique feature is inherent to Pol III-synthesized transcripts of some mammalian SINEs, short mobile genetic elements. Here we established a group of proteins responsible for the PA of Pol III transcripts of SINEs. We performed individual knockdowns (KDs) of proteins of interest by siRNA in HeLa cells, transfected cells by a SINE-containing plasmid, and estimated the PA of SINE RNA by northern blotting. We found that the KD of each of the four components of the mPSF subcomplex, as well as PAP, decreased the PA of SINE transcripts, whereas the KD of symplekin had no such effect. Since symplekin maintains the stability of the mCF subcomplex, it is very unlikely that mCF is involved in the PA of SINE transcripts. Thus, mPSF and canonical PAP but not mCF are involved in the PA of RNA transcribed by Pol III. The analysis of B2 (mouse) and Ves (bat) SINEs allowed us to identify the nucleotide sequences of two additional cis-signals (β and τ) contributing to the PA of their transcripts. Finally, the mutation analysis and KD showed that an auxiliary factor CFIm interacts with the τ signal of the B2 transcript and enhances its polyadenylation. The study was funded by the Russian Scientific Foundation (grant no. 19-14-00327).

P-01.2-21
Functional RNA-AuNP conjugates for gene expression regulation based on the GFP example

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Gene expression is precisely regulated in all cells. One of the mechanisms responsible for this process is RNA interference, which can suppress protein expression by directed recognition of mRNA with short interfering fragments. On the other hand, RNA can occur in tertiary form and many RNA structural motifs have been defined. This is a foundation for RNA architectonics, which focuses on rational design and synthesis of structural and functional RNA nanoparticles (tectoRNA). Owing to development of bioinformatics and bionanotechnology, RNA motifs can be turned into the functional tectoRNAs with regulatory sequences to target a gene of interest. Scientists are keen to use gold nanoparticles (AuNPs), due to their optical properties, biocompatibility and the fact that they can penetrate cells or be a carrier of other molecules, such as: drugs, peptides, antibodies or nucleic acids. The conjugates of nucleic acids and AuNP, referred as spherical nucleic acids, were described previously and applied as potential therapeutics and biosensors. In our research the merger of the RNA and AuNP nanotechnologies has led to creation of spherically organized, structural RNAs. Here, for the first time, we present a structural RNA conjugated with spherical AuNP. The main advantage of RNA-AuNP is the increased local concentration and controlled composition of regulatory fragments in tectoRNA, which cannot be achieved with dsRNAs. To study this system, we designed, synthesized and assembled a set of siRNAs and tectoRNA trimers targeting CopGFP gene in the model cell line. The RNA structures were hybridized with AuNPs and applied in the MDA-MB-231 cell line, stably expressing GFP. Our studies prove that the tectoRNA-AuNP nanoparticles penetrate cells, which was observed by TEM analysis; and can regulate gene expression indicated by reduction of cells' GFP fluorescence measured with plate reader, flow cytometry and under fluorescent microspore.

P-01.2-22
Clostridioides difficile CRISPR-Cas regulation by biofilm-related secondary messenger c-di-GMP

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Clostridioides difficile is an anaerobic spore-forming bacterium that is the major cause of nosocomial diarrhea associated with antibiotic therapy. Many aspects of *C. difficile* pathogenesis and its adaptation to changing conditions inside the host are poorly understood. Our deep-sequencing data previously published in: Soutourina et al. (2013) PLoS Genet. 9(5), e1003493 strongly suggests the importance of RNA-based mechanisms for the control of gene expression and infection cycle in *C. difficile*. More than 200 regulatory RNAs were identified, including abundant CRISPR RNAs for the prokaryotic adaptive immune system against foreign invaders. *C. difficile* possesses an unusual CRISPR-Cas system characterized by a large set of CRISPR arrays, multiple type I-B cas operons, and the toxin-antitoxin type I systems, linked to several arrays. In present study we investigated the role of biofilm-related secondary messenger c-di-GMP in *C. difficile* CRISPR-Cas system regulation. We explored the global effect of the c-di-GMP on the expression of CRISPR-Cas system components. Real-time PCR experiments showed expression induction of both cas operons and several CRISPR arrays in *C. difficile* 630Δerm strain by high c-di-GMP levels. Additionally, we found a c-di-GMP-dependent riboswitch associated with the CRISPR12 array in *C. difficile* 630Δerm strain, which can indicate the direct impact of c-di-GMP-dependent regulation on this array function. Plasmid conjugation efficiency experiments revealed a slight induction of interference in *C. difficile* 630Δerm CRISPR12 array by high levels of c-di-GMP. Obtained results demonstrate *C. difficile* CRISPR-Cas regulation in biofilm conditions and show the possible role of this system in *C. difficile*

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survival during its infection cycle. This work was supported by the Russian Science Foundation (project No. 20-74-00052).

P-01.2-23
Mycoplasma–host cell interaction mechanisms at the transcriptomic level

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Due to the small size of the genome, bacteria of the genus Mycoplasma can serve as a model for a minimal cell. The classic way to study mycoplasma as a minimal cell is to use various perturbation models. One of the most interesting models for studying mycoplasma is the model of intracellular parasitism, its study will make it possible to draw conclusions about the possibilities and principles of the minimal cell structure. The aim of our work was to study the adaptation and regulation mechanisms of *Mycoplasma gallisepticum* at the transcriptomic level during intracellular infection. Infection was initiated by co-culturing HD3 cells with *M. gallisepticum* S6 culture, and then getting rid of extracellular mycoplasmas by treatment with gentamicin. Then, intracellular mycoplasmas were released by lysis of eukaryotes and cultured for several passages, after which RNA was isolated and libraries were prepared for sequencing, which was performed on a HiSeq 2500 device (Illumina). There were no large-scale changes in gene expression relative to the control culture cultivated on a rich medium. Only the gene of the family of lipoproteins and hemagglutinins (VlhA) showed a 4-fold increase in expression. Previously, proteins of the VlhA family were described as important for mycoplasmas in pathogenesis and escape from the immune response. Changes in the expression of other genes indicate that invasion is stressful for the pathogen – the expression of other variable genes (10) is suppressed. Previously, we studied the change in the proteomic profile of *M. gallisepticum* in the same model and found significant changes in the proteome of mycoplasma. The observed differences in the adaptation of mycoplasma to the intracellular environment at the level of mRNA and proteins indicate an alternative way of regulating the response to stress in the minimal cell. This work was supported by the RSF grant No. 19-15-00427. *The authors marked with an asterisk equally contributed to the work.

P-01.2-24

This poster has been moved to a Speed Talk

P-01.2-25
Substrate specificity of the MBP-SelU fusion protein (*Escherichia coli* tRNA 2-selenouridine synthase)

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Transfer RNAs (tRNAs) constitute a unique group of RNA oligonucleotides, which is characterized by the highest content of modified nucleosides. The 5-substituted nucleosides, like 2-thiouridines (R5S2U), S-geranyl-2-thiouridines (R5geS2U), and 2-selenouridines (R5Se2U), found in bacterial tRNAs specific for Lys, Glu, and Gln at the first position of their anticodons (a position 34, the wobble position), play a pivotal role in the decoding of genetic information. Bacterial tRNA 2-selenouridine synthase (SelU, MnmH) exerts a two-step post-transcriptional transformation of (c)mnm5S2U-tRNA to (c)mnm5Se2U-tRNA via a (c)mnm5geS2U-tRNA intermediate. [1] In the current studies we used an MBP-SelU fusion protein, with a maltose binding protein (MBP) tag attached to the N-terminus of the wild type SelU synthase. MBP-SelU (84.4 kDa) shows high activity in the S-geranylation (> 90%) and selenation (~100%) of model 17-mer oligonucleotides mimicking the anticodon-stem-loop (ASL) of $tRNA^{Lys}$. These models contained at the position corresponding to the wobble position in tRNA anticodon either an S2U or geS2U unit, respectively. The MBP-SelU activity and the minimum length of the RNA oligonucleotide substrate, as well as the influence of the position of modified nucleoside and flanking sequences on the enzyme/substrate recognition were determined. We confirmed that the MBP-SelU protein binds the bacterial $tRNA^{Lys}$, $tRNA^{Glu}$ and $tRNA^{Gln}$. Using Microscale Thermophoresis (MST) technique the constants for binding of Cy3-labeled 17-mer oligonucleotides (containing U, S2U, geS2U or Se2U) to MBP-SelU protein were determined. The geS2U-RNA exhibits the highest affinity for MBP-SelU (Kd = 3.946 ± 0.41 μM) in comparison with S2U-RNA (Kd = 18.54 ± 3.01 μM), Se2-RNA (Kd = 27.33 ± 4.33 μM). Acknowledgements: This research was financially supported by The National Science Centre in Poland, Grant number [UMO-2018/29/B/ST5/02509] for years 2019–2022. Reference: [1] Sierant M et al. (2018) FEBS Lett. 592, 2248–2258

P-01.2-26
Therapeutic targeting of long non-coding RNAs in cancer using synthetic small molecules

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Long non-coding RNAs (lncRNAs) are a recently identified class of regulatory RNAs greater than 200 nucleotides in length that constitute the largest portion of the mammalian non-coding transcriptome. They play an essential role in regulating the expression of target genes in normal biological contexts as well as pathologic processes including expression of oncogenes [1]. MALAT1 (metastasis-associated lung adenocarcinoma transcript 1) is a highly conserved lncRNA involved in metastasis and tumor proliferation in multiple cancer types. Upon folding, the 3'-terminal end assumes a triple-helical conformation that promotes the nuclear accumulation and persistent function of MALAT1 and can be targeted by synthetic small molecules[2]. The aim of our project is to develop new fluorescence-based screening assays suitable for medium-throughput analysis to identify potential selective interactions between the MALAT1 triple helix and compounds from various libraries including RNA binders [2,3]. A first screening of an in-house focused library of RNA ligands allowed for the identification of efficient MALAT1 binders. The mechanism of action at the molecular level of the identified hits is currently under study by using biochemical and biophysical assays as well as intracellular studies. The screening of larger libraries (50–10000 compounds) is also ongoing. Therapeutic targeting of tertiary structure of MALAT1 with selective small molecules represents a relevant model to explore the druggability of RNAs as well as a very original and promising anticancer approach. Once validated, we envisage to apply similar

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screening strategies to other lncRNA targets. References: [1] Morris, K., Mattick, J. The rise of regulatory RNA. Nat Rev Genet 15, 423–437 (2014). [2] ACS Chem. Biol. 2019, 14, 223−235. [3] Angew Chem Int Ed Engl. 2018 Oct 1; 57(40): 13242–13247

P-01.2-27
The conserved endoribonuclease RNase III affects formation of photosynthetic complexes in *Rhodobacter sphaeroides*

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Bacteria are frequently adjusting their transcriptome to adapt to changes in their environment. This does not only take place on level of transcription, but also involves ribonucleases (RNases) that control RNA processing and turn-over. RNase III (rnc) is a highly conserved endoribonuclease, which is present in all known eukaryotes (Dicer and Drosha are RNase III enzymes) and prokaryotes, and was shown to be an important regulator of gene expression in many organisms. The enzyme can contain one or two nucleolytic active RNase III domains (RIIID) that harbour a 9 amino acids RNase III signature motif. Bacterial RNase III enzyme typically contain a single RIIID, often followed by a C-terminal dsRNA binding domain. *Rhodobacter sphaeroides* is a facultative phototrophic alpha proteobacterium that can perform aerobic respiration in presence of oxygen, as well as anoxygenic photosynthesis, anaerobic respiration or fermentation in absence of oxygen. To better understand the role of RNase III in adjusting the *R. sphaeroides* transcriptome to changes in the environment, we constructed a mutant strain lacking RNase III activity by exchanging two highly conserved amino acids in the signature motif. An obvious phenotype of this mutant was its lighter red color indicating that formation of photosynthetic complexes differs to the wild type. Indeed, we could confirm lower amounts of bacteriochlorophyll and carotenoids and lower levels of photosynthetic complexes in the mutant. Quantification of several mRNAs encoding structural proteins of photosynthetic complexes or enzymes required for pigment synthesis, revealed that their levels are influenced by RNase III. These results demonstrate, how much a single RNase can influence important physiological processes.

Molecular evolution and phylogenetics

P-01.3-01
Mutagenesis on an ancestral background elucidates new determinants of the xanthine specificity in the NAT/NCS2 family of transporters

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NAT/NCS2 (Nucleobase-Ascorbate Transporter/ Nucleobase-Cation Symporter-2) is a family of transporters with wide evolutionary distribution and diversity in substrate preferences. To date, research on this family has been based on the study of present-day homologs. Herein, we present an evolutionary strategy, based on ancestral reconstruction, that is applied for the first time, to analyze structure-function and specificity relationships in this family. We performed phylogenetic analysis of the bacterial NAT/NCS2 homologs and reconstructed AncXanQ, the putative common ancestor of all homologs comprising a clade represented by the well-studied xanthine-specific permease XanQ of *E. coli*. In contrast to XanQ, AncXanQ transports both xanthine and guanine and recognizes a wide range of analogs. Homology modelling showed that AncXanQ conserves all key binding-site residues of XanQ. We subjected both homologs to rationally designed mutagenesis and found that 5 amino acid residues outside the predicted binding-site are involved in the specificity change. In particular, we reveal Ser377 of XanQ (Gly in AncXanQ) as a major determinant. Replacement of this Ser to Gly enlarges the specificity of XanQ towards an AncXanQ-phenotype. Orthologs from Neisseria meningitidis retaining Gly at this position are also xanthine/guanine transporters with an extended substrate profile like AncXanQ. The specificity effect of S377G is masked by G27S or other mutations through epistatic interactions. Acknowledgment: «This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning» in the context of the project "Strengthening Human Resources Research Potential via Doctorate Research" (MIS-5000432), implemented by the State Scholarships Foundation (IKY)»

P-01.3-02
Compensatory mutations in multidrug-resistant *Mycobacterium tuberculosis* complex isolates from Kazakhstan

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Kazakhstan is one of the 30 countries with the highest burden of multidrug-resistant tuberculosis. A recent expansion of drug-resistant isolates in the country is linked to the spread of Central Asian/Russian sublineage of *Mycobacterium tuberculosis* Beijing genotype. We studied a random sample of 11 multidrug-resistant Central Asian/Russian isolates of *M. tuberculosis* from the two most populated cities in Kazakhstan, namely, Nur-Sultan and Almaty. We utilized whole-genome sequencing to differentiate the isolates and to look for the adaptive compensatory mechanisms lowering the fitness-cost of mutations causing resistance to Rifampicin. According to genomic analysis, nine isolates belong to K-2 (Central Asia outbreak) clade and two belong to the K-1 (Central Asia) clade of Central Asian/Russian sublineage. All the studied isolates had sigE gene codon 98 CTG>CTA polymorphism that is specific for the Central Asian/Russian sublineage. The mutations previously associated with fitness-compensatory mechanisms were found in the rpoC gene (V431M, Q435H, L449V, V483G, V483A, D943N, and S1100A) of seven isolates. One compensatory SNP was found in the rpoA gene (T187A) in a single isolate. One SNP was found in the rpoB gene (Q980R) of another isolate. Two phenotypically resistant isolates have harbored neither compensatory SNPs nor resistance mutations to Rifampicin, despite positive phenotypic drug susceptibility testing. In conclusion, the acquisition of compensatory mechanisms, in addition to accumulated

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resistance-associated mutations, makes Central Asian/Russian sublineage a clear threat to global health. This work was funded by grant AP09058045 from the Ministry of Education and Science of the Republic of Kazakhstan.

P-01.3-03
Evolutionary relationships between the laccase genes of Polyporales

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Laccase (benzenediol:oxygen oxidoreductases, EC 1.10.3.2) is one of the oldest studied enzymes. Over the years, countless number of reviews and original articles were devoted to different structural, functional, biological, ecological and biotechnological aspects of laccases. Nevertheless, much remains to be discovered about this enzyme. As it was already pointed out: "laccases are a never-ending story". Here we present our recent discoveries regarding evolutionary history of laccases of Polyporales fungi. These discoveries were previously published in: Savinova OS et al. (2019) Front Microbiol 10, 152; and to some extent were previously published in: Moiseenko KV et al. (2019) Microorganisms 7(11), 527. Fungal genomes almost always contain several non-allelic copies of laccase genes laccase multigene families; however, evolutionary history of these families and their relationships among them are mostly unknown. In the presented work, a gene-tree/species-tree reconciliation analysis for the laccase multigene families from 28 wood-decaying fungal species from the Polyporales order was performed. The sample included representative fungi from all four main Polyporales clades: the Core Poliporoid, the Antrodia, the Phlebioid, and the Residual Polyporoid. The results of the analysis suggest that all Polyporales laccases derived from a single ancestral gene. Extensive duplications of this gene began almost immediately after the splitting of the Polyporales order into its four main clades, and continued with the evolution of the angiosperms, which may be a consequence of the conquest of new ecological niches by the fungi. This work was partially funded by the Russian Foundation for Basic Research, Grant No. 19-04-01183.

P-01.3-04
First microsatellite markers developed for population genetic studies of unique sponges from Lake Baikal

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Freshwater sponges play a significant role in lake and river ecosystems, such as filtering and bio-indicating the condition of water masses. Lake Baikal is a unique natural object and an explosive speciation point, which is confirmed by the number of endemic species of sponges in the lake. In recent years, massive diseases and the mortality of endemic sponges have been observed on Lake Baikal. This issue threatens the state of the lake, which contains 20% of the world's freshwater reserves. It has been a UNESCO World Heritage Site for 23 years. Sponge disease spread across the lake unevenly. There are spots where the sponges are damaged by 80%, and at the same time, there are untouched spots. Here we report about microsatellite genetic markers development for the first-ever population analysis of Baikal endemic sponges *Lubomirskia baikalensis*. Twelve microsatellite markers specific to the species of endemic Baikal sponge *Lubomirskia baikalensis* were developed based on genome data and tested on eight sponge samples from different points of the lake. PCR protocol was optimized for a more specific reaction. All out of twelve microsatellite markers showed to be polymorphic and are ready to use in population genetic studies. The analysis would take place at ten spots of Lake Baikal, which would cover all three basins. This data will provide not only the understanding of the differences between populations located in various basins of the Lake Baikal but also the first understanding of the differences in the structure of cosmopolitan and endemic freshwater sponges populations which differ by breeding strategies. The reported study was funded by RFBR and the Government of the Irkutsk Region, project number 20-44-383010. *The authors marked with an asterisk equally contributed to the work.

P-01.3-05
Which way to the centre? Structural and evolutionary basis of substrate transport in epoxide hydrolases

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Epoxide hydrolases belong to the wide-spread and multifunctional superfamily of α/β-hydrolases. They have eight-stranded antiparallel structure with the central β-strands surrounded by α-helices. Their active site is buried between the core and the cap domains and connected with the environment by tunnel(s). To investigate the basis of substrate transport pathways in epoxide hydrolases, we combined the structural and evolutionary analysis. We ran multiple classical molecular dynamics simulations and analysed the flow of water molecules within the protein structure and the catalytic pocket. We used AQUA-DUCT software to investigate the internal architecture of the epoxide hydrolases. Moreover, we compared the obtained data with the information about the size and shape of the substrates catalyzed by analysed proteins. To investigate the variability of each protein residue and functionally important regions of investigated enzymes, we used BALCONY (Better ALignment CONsensus analYsis), an R package dedicated for analysis of the evolution of amino acids dispersed in sequence. We identified multiple tunnels in all analysed epoxide hydrolases structures, however only one of them is conserved in most cases. The presented approach shed light into evolution of the access pathways leading to the buried active site and provided information useful for drug design and/or protein re-engineering.

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P-01.3-06
Comparative citogenomic analysis of tandemly repeated DNA elements in four *Allium* species from the section Cepa

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Allopolyploidy, which involves both polyploidy and inter-species hybridization, contributes to plant evolution and diversification. Most of the known allopolyploids are based on even-ploidy levels with very few odd-ploidy level taxa persisting in nature. One of such unusual cases is triploid onion, *Allium* × *cornutum* (Clementi ex Visiani, 1842), (2n=3x=24), an established minor garden crop, widespread in the northern hemisphere. By using molecular-phylogenetic approach we previously confirmed its unique triparental origin and successfully identified parental species. One of the ancestors was common onion, *Allium cepa* L., whereas the two other ancestral species were shown to originate from South-Himalayan regions, *Allium pskemense* B. Fedtsch and *Allium roylei* Stearn (Puizina et al. 1999, Fredotović et al. 2014). To better understand the genome evolution of allotriploid and its ancestral species, we performed de novo identification of repetitive DNA families by application of next generation sequencing utilizing low-pass genome sequence data (~ 2%) and using the RepeatExplorer2 and TAREAN pipelines (Novak et al., 2020). The analyses showed that the genomes of all four species have a quite similar compositions, and that they are dominated by LTR-retrotransposons, making up 40–50% of nuclear DNA, with Tekay and Retand from Ty3 lineage and SIRE from Ty1 lineage being the most abundant. In contrast, satellite DNA was represented by only 0.1–1.6% of nuclear DNA and ribosomal DNA on average by 0.3%. The results of comparative repeat analysis revealed shared and species specific repeats. By application of fluorescent *in situ* hybridization we mapped the two ribosomal and the three satellite DNA repeats from each parental species to the chromosomes of both diploid and allotriploid species. The mapped satellite and rDNA repeats provided useful chromosomal markers which allowed the individual identification of the majority of chromosomes within the analyzed karyotypes.

P-01.3-07
Genomic analysis suggests that moderate IGF signaling can be involved in the longevity of giant tortoises

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Giant tortoises constitute one of the longest-lived vertebrate animals, whose longevity, according to some estimates, can exceed 100 years. Multiple works have used comparative genomic analyses with the genomes of long-lived mammals to shed light on the signaling and metabolic networks that might play a role in regulating age-related conditions. Here, we describe a global analysis of the genomes of Lonesome George –the iconic last member of *Chelonoidis abingdonii* – and the Aldabra giant tortoise (*Aldabrachelys gigantea*). Unsupervised and supervised comparative analysis of these genomic sequences led us to add new genetic information on the evolution of turtles, and to detect lineage-specific variants affecting DNA repair genes, inflammatory mediators and genes related to cancer development. Our study has also provided novel candidate genes that might underlie the extraordinary lifespan of giant tortoises, and has expanded our understanding of the genomic determinants of ageing. In this context, we have annotated a specific variant in IGF1R, which is expected to affect the interaction between this receptor and the IGF1/2 growth factors. The IGF signaling pathway has been associated with longevity in different species, which suggests that this unique change in IGF1R may constitute an attractive candidate to study the cellular mechanisms underlying the exceptional lifespan of these animals. Previously published in: Quesada V et al. (2019). Nat Ecol Evol 3(1):87–95.

P-01.3-08
Analysis of genes from the sex-determining region of Populus x sibirica revealed sex-associated differences in their expression

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The genus *Populus* is presented by dioecious species, which are characterized by significant diversity in the structure and location of the sex-determining region (SDR). We performed transcriptome sequencing of flowers, catkin axes, and leaves of male and female *Populus* x *sibirica* plants and genome sequencing of the same individuals on the Illumina platform for the first time. Analysis of polymorphisms of three genes from the SDR – encoding T-complex protein 1 subunit gamma (TCP), chloride channel protein CLC-c (CLC-c), and DNA-methyltransferase 1 (MET1) – was performed based on genome and transcriptome

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sequencing data. *P.* x *sibirica* is an intersectional hybrid likely between species from sections Aigeiros and Tacamahaca, so a significant number of heterozygous polymorphisms were identified. We revealed that both allelic variants of the TCP1, CLC-c, and MET1 genes were expressed in females, while in males, both allelic variants were expressed for TCP1 and MET1, but only one – for the CLC-c gene. Targeted sequencing of TCP1, MET1, and CLC-c gene regions obtained from DNA and cDNA of 10 male and 10 female plants of *P.* x *sibirica* confirmed the predominant expression of only one allelic variant of the CLC-c gene in leaves, flowers, and catkin axes of *P.* x *sibirica* males. Thus, identified sex-associated allele-specific expression of the CLC-c gene in vegetative tissues of *P.* x *sibirica* suggested that differences between male and female poplars could take place in not only generative organs but whole plants. The reported study was funded by RFBR according to the research project № 20-34-90159.

P-01.3-09
Engineering protein dynamics for the understanding of divergent evolution of the *Renilla luciferase*

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Current protein engineering methods typically use amino acid substitutions to improve protein activity, stability or solubility. However, to alter the protein function profoundly or even develop a novel function, substitutions might not be sufficient. In the present study, we employed the methodology of insertion and deletion (InDel) mutagenesis (Previously published in: Emond S et al. (2019) BioRxiv) which allows more significant changes in a protein backbone conformation and dynamics, while it might also lead to the collapse of the structure. To compensate for the disruptive effect of the methodology, we applied the InDel mutagenesis on a recently reconstructed hyperstable ancestor of two structurally similar but functionally different enzymes, haloalkane dehalogenases and coelenterazine-dependent Renilla-type luciferases (Previously published in: Chaloupkova R et al. (2019) ACS Catalysis 9, 4810–4823). Screening of the generated libraries led to the identification of three distinct protein regions, whose alteration resulted in a markedly improved luciferase activity. Crystallographic analysis of the best InDel variant identified open and closed conformations, introduced by the mutagenesis. Transient kinetics revealed a two-step binding of the bulky substrate and enhanced substrate affinity, both linked to the altered dynamics. The hydrogen-deuterium exchange experiments and molecular dynamics simulations confirmed increased flexibility of the identified regions. Our multidisciplinary approach underlines the significance of dynamics for the evolution of the luciferase activity and proposes the putative evolutionary divergence pathway from hydrolytic dehalogenation to oxidative bioluminescence. The results also demonstrated the potential of InDel mutagenesis for a modification of protein dynamics and the development of novel catalytic activities. *The authors marked with an asterisk equally contributed to the work.

P-01.3-10
Towards the elucidation of the molecular mechanisms underlying the cold adaptation of low-temperature viruses specific for a mesophilic host

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Escherichia coli phage T4 and its relatives form the largest phage subfamily Tevenvirinae. Most tevenviruses, whose natural environment is the intestine of warm-blooded animals, infect *E. coli* or related bacteria. Such phages have an optimum temperature for development ~37–40°C, that corresponds to that of their mesophilic host. Since the growth rate of *E. coli* decreases at < 20°C and stops at < 7°C, most of the phages adapted to infect this particular host are incapable of development at < 12°C or in non-dividing cells. Tevenviruses VR, all unique in their ability to multiply at 4°C, represent the only known collection of low-temperature T4-related coliphages, and are uniquely suited for the investigation of molecular mechanisms of cold adaptation in viruses. To identify *E. coli* genes that are essential for the development of VR phages at low temperature, phage VR20, known to also grow on both stationary-phase and starving *E. coli*, was selected. VR20 was screened against all 3985 nonessential single-gene deletion mutants in *E. coli* K12 from the Keio collection. The plaque assay was used, and the plates were incubated at 8 and 22°C. At least 60 genes in *E. coli* K12 (involved in the cellular processes, such as nucleic acid and protein metabolism, cell transport, cell wall structure, etc.) were found to influence VR20 phage propagation at low temperature, including 50 genes not previously stated to have a role in phage infection. Notably, one mutation in E. coli (ΔymfR) known to negatively affect the growth of different *E. coli* phages under standard laboratory conditions, while detrimental at 22°C, had lesser impact on the development of VR20 at 8°C. Though the results of this study already give us a better insight into the cold adaptation of phages, a further analysis of the effect of the identified *E. coli* genes on the development of VR viruses is ongoing. This research was funded by a grant (No. S-MIP-19-58) from the Research Council of Lithuania. *The authors marked with an asterisk equally contributed to the work.

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P-01.3-11
High satellite repeat turnover in allopolyploids *Anemone multifida* (2n = 32) and *Anemone baldensis* (2n = 48) (Ranunculaceae)

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Hybridization and genome doubling is accompanied by rapid and dynamic genetic and epigenetic changes, as well as changes in gene expression and phenotypic variation. How the emerging plant balances two coevolved genomes remains largely unknown. Here we focus on satellite DNA, aiming to trace its appearance, amplification and loss during plant speciation and allopolyploidisation. As a model, we used allotetraploid *Anemone multifida* (BBDD, 2n = 4x = 32) and allohexaploid *A. baldensis* (AABBDD, 2n = 6x = 48) originating from the crosses of diploids *A. sylvestris* (donor of the A subgenome), *A. cylindrica* (donor of the B subgenome) and *A. parviflora* (donor of the D subgenome). Using next generation sequencing, TAREAN integrated in the RepeatExplorer2 pipeline, Southern blot and fluorescence *in situ* hybridisation (FISH) we characterize three highly repetitive DNA sequences (AparSAT3, AcylSAT1 and AcylSAT2). AparSAT3 is highly abundant in the D subgenome of *A. multifida* and *A. baldensis*, as well as in its parental species *A. parviflora*. We detected dispersal of AparSAT3 between subgenomes B and D after allopolydisation. AcylSAT1 and AcylSAT2 occur in different but multiple, discontinuous tandem arrays scattered over all chromosomes of *A. multifida*, *A. baldensis* and their putative progenitors. FISH pattern of AcylSAT2 in each subgenome differs in comparison to its parental species suggesting events of intergenomic homogenisation after allopolyploidization. Our results illustrate complex evolutionary pathways of satellite repeats through *Anemone* speciation and allopolyploidization. *The authors marked with an asterisk equally contributed to the work.

P-01.3-12
Are cryptic plasmids of *Sinorhizobium meliloti* attractive within phage infection?

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The genome of root nodule bacteria *Sinorhizobium meliloti* is enriched by non-symbiotic cryptic plasmids. The attractiveness of such plasmids to phages was evaluated in this work for the first time. The 20 cryptic plasmids with sizes varied from 17.2 to 453.8 kb were identified in whole sequenced genomes of the 5 strains from the origin of plant diversity at the NW of the Big Caucasus Ridge (NCG) and of the 7 strains from the center of the introgressive hybridization of alfalfa at the Kazakhstan NW, subjected to salinity (PAG). Phage sequences (PS) were detected in the 7 out of the 8 analyzed plasmids of NCG strains, and in the 7 out of the 12 plasmids in PAG strains according to the PHASTER (https://phaster.ca/). The number of PS was varied from 1 to 11 per plasmid replicon, and their sizes ranged from 5.1 to 33 kb. PS related to Siphoviridae family were significantly prevailed (frequency 0.64; $P < 0.05$), then those which were homologous to Podoviridae were extremely rare in plasmids from NCG strains. Prophages related to both above families were found with the same frequency (0.37) in cryptic plasmids of PAG strains. PS related to Myoviridae were found with similarly equal frequencies (0.24 in average) on plasmids of NCG and PAG strains. Remarkable, but PS from all three families were detected on the same cryptic plasmid with a frequency of 0.40. Significant differences were revealed between the occurrence of intact, questionable and incomplete PS on plasmids of NCG and PAG strains ($P < 0.05$). Thus, incomplete prophages prevailed (0.67) on the plasmids of PAG strains and their active participation in intragenomic rearrangements in rhizobia adapted to extreme salinization environment is proposed. A significant portion of intact prophages detected on cryptic plasmids of *S. meliloti* strains from NCG is indicated at a high infection activity of phages from Siphoviridae family in this gene center of cultivated plants. The work was supported by the RSF 20-16-00105. *The authors marked with an asterisk equally contributed to the work.

P-01.3-13
Recombinant form of *Sinorhizobium* phage AP16 isolated from mountain region of Caucasus

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Bacteriophages (Bph) are widespread in soils and are characterized by a high diversity. In the case of agriculturally valuable rhizobia species, only a small number of Bph were sequenced. The Bph AP16 was isolated from soil sample from Caucasus according to Barnet method. It's lytic activity tested on 50 *Sinorhizobium meliloti* isolates native to the same region revealed that a third part of strains was lysed. Pure phage culture was obtained by phage lysate passaging on *S. meliloti* L5-30 by Adams method. Bph DNA was isolated by GeneJet Viral DNA and RNA Purification kit (Thermo Fisher Scientific, USA). The AP16 genome was sequenced by NGS methods (MiSeq, Illumina), assembled (SPAdes, Flye, Racon and Medaka modules, Pilon), annotated by Prokka. The AP16 was characterized as dsDNA phage from Siphoviridae family which genome was 61.0 kb and the GC content was 59.22%. Genome of the AP16 encoded all typical virion elements portal complex according to PHASTER. Portal protein 26 (P26) of 430 aa was homologues of P26 (pdb4ZJN) of thermostable Bph G20C of *Thermus thermophilus*. The amino acid sequences analysis revealed ORF homologous to Escherichia phage ECD7 and 16 ORFs homologous to different Caudovirales phages. Besides those there are 15 ORFs homologous to bacteria from Rhizobiaceae family. Sequences homologous to the AP16 were searched in genomes of 12 *S. meliloti* isolates from the lab collection. One intact prophage phiK7-6 with a size of 40.2 kb harbored 3304 bp homologous to the AP16 was identified in the strain K7-6 (Identity = 85.65%, Cover = 12%, Evalue = 0.0). The phiK7-6 contained 43 ORFs encoding integrase, head, capsid and portal protein. Thus, intact prophage homologous to recombinant Bph AP16 harboring bacterial genes and sequences related to several phage families were revealed. This data strongly evident the significant role of phages in horizontal gene transfer within rhizobia population. This work was financially supported by RSF 20-16-00105. *The authors marked with an asterisk equally contributed to the work.

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P-01.3-14
Abundant of CRISPR/Cas elements in genomes of nitrogen-fixing bacteria from the genus *Sinorhizobium*

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Aputative CRISPR sequences and cas genes were searched in full genome sequenced reference strains among which were *S. meliloti* Rm1021, *S. medicae* WSM419, S. fredii NGR234 and *S. americanum* CCGM7. Genomes of all tested strains have a multireplicon organization and their sizes varied from 6.68 to 6.9 Mb. The 7 CRISPR sequences on the chromosome and the 6 CRISPR sequences on SMb were detected in *S. meliloti*, while in *S. medicae* the 2 and 1 corresponding sequences were identified, as well per the 4 cas genes were revealed in each strain by CRISPRCasFinder. In *S. fredii* and *S. americanum* the 2 and the 6 CRISPR sequences, as well 4 and 5 cas genes, were identified, respectively, all of which were located on corresponding chromosomes. Only one spacer was found in 84% tested CRISPR sequences of *Sinorhizobium* spp., while few others had 2 or 4 spacers. It was found that all identified cas genes encoded Cas proteins of the type I CRISPR/Cas system. The 4 non-homologous cas genes were detected in studied *Sinorhizobium* spp. strains. The homology between corresponding cas genes detected in strains related to different species was 80.5–90.3%. Since CRISPR sequences and cas genes were localized at a distance exceeded to 80 kb in all tested strains, therefore it was concluded that they are not clustered in S*inorhizobium* spp. No prophages sequences next to regions of CRISPR sequences and cas genes were found (according to web server PHASTER). Thus, putative CRISPR sequences and cas genes detected in *Sinorhizobium* spp. are abundant on chromosome and on megaplasmids as well. All studied CRISPR sequences were short and not clustered with cas genes and presumably encoding proteins of the type I CRISPR/Cas system. The work was supported by the RSF 20-16-00105. *The authors marked with an asterisk equally contributed to the work.

P-01.3-15
PIA3, a pipeline for mining assemblies for opsin protein sequences

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Vision is one of the most important senses in animals. The molecular system enabling vision in the absolute majority of animal eyes is based on the visual pigments consisting of opsin proteins and retinal derivatives. Multiple opsins in one species allow distinction of colors. The diversity of opsins in different extant species ranges from one to several dozens. Opsins belong to different subclasses of G-protein coupled receptors, and finding them in newly assembled genomes and transcriptomes by sequence similarity can be challenging due to numerous false positive hits. Approaches to solve this problem have already been proposed, but they are mostly delivered as a bunch of scripts requiring setup for each new user. To address this question, we re-wrote the PIA2 algorithm (https://github.com/xibalbanus/PIA2) as a Python-based pipeline, resulting in PIA3. PIA3 installation and parameter adjustment are straightforward and require minimal knowledge of bash and no knowledge of Python programming. The pipeline requires a genome or transcriptome assembly and a database of target opsins from other species and outputs putative opsin sequences as amino acids, open reading frames and full opsin-containing contigs, as well as a phylogenetic tree showing the relationship between the database and the newly found sequences. We tested the performance of PIA3 using the endemic Lake Baikal amphipods as an example and found that different species may contain up to five diverse opsin transcripts, but all of them belong to the long wavelength-sensitive opsin subfamily. PIA3 is not restricted to opsins and can be tuned to mine for any gene family. The current version of PIA3 is available from https://github.com/AlenaKizenko/pia3_amphipod_opsins. The work was supported by the Russian Science Foundation (grant #19-74-00045). Expected to be published in: Drozdova et al. (under consideration) BMC Ecology and Evolution. *The authors marked with an asterisk equally contributed to the work.

P-01.3-16
Investigating the possible evolution of isopentenyl diphosphate phosphohydrolase from isopentenyl diphosphate isomerase

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Isopentenyl diphosphate phosphohydrolase enzymatic activity was first identified in plants in proteins from the Nudix superfamily. The presence of this activity was postulated based on the presence of isopentenyl phosphate kinase in plants, previously published in Henry L et al. (2015) PNAS 112, 10050-10055, and was found in members of the Nudix hydrolase superfamily due to sequence similarity to isopentenyl diphosphate isomerase, previously published in: Karačić Z et al. (2017) Biol Chem 398, 101–112. This enzyme is presumed to be involved in the regulation of isoprenoid biosynthesis in plants. We found homologues of this protein in all land plants, but also in genomes of some eukaryotic and prokaryotic microorganisms. We solved the crystal structure of isopentenyl diphosphate phosphohydrolase from the amoeba *Dictyostelium discoideum* and found that the most similar structures are those of isopentenyl diphosphate isomerase type I, not other Nudix hydrolases. Additionally, we identified sequences from green algae that show characteristics of both isomerases and hydrolases – a conserved tryptophan residue that stabilizes the carbocation intermediate in isomerases, and the catalytic glutamate necessary for phosphohydrolase activity, however in an alternate position. We expressed one such protein from *Parachlorella kessleri* and confirmed that it is an isopentenyl diphosphate phosphohydrolase. Based on our results, we hypothesize that proteins with isopentenyl diphosphate phosphohydrolase activity evolved in green algae before the advent of Embyophyta, and spread to other microorganisms via horizontal gene transfer or endosymbiosis. *The authors marked with an asterisk equally contributed to the work.

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P-01.3-17
Signatures of selection and adaptation to subterranean lifestyle across the transcriptomes of Arvicolinae (*Rodentia, Cricetidae*)

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Subterranean rodents from the Arvicolinae subfamily constitute an ideal model to test hypotheses about adaptive evolution due to ecological shifts. We obtained raw RNA-seq reads for ten representatives of the subfamily to detect adaptive signatures of subterranean species. For seven species raw reads were available in the NCBI SRA. In addition, we used previously assembled *M. ochrogaster* and *C. griseus* transcriptomes. Data covered such specialized subterranean species as *P. schaposchnikowi*, *E. lutescens*, *L. mandarinus* and *T. subterraneus* as well as closely related terrestrial species. We revealed 112 single-copy orthologs, which were subsequently analyzed for signatures of natural selection. The median dN/dS value (free model) was higher for subterranean voles compared to terrestrial ones. However, when evaluating the selection independently for each line of subterranean rodents (branch model) genes with significant differences were not found. We found at least ten genes with parallel amino acid substitutions that were specific for subterranean rodents: Erp29, Rad23b, Hikeshi, Zadh2, Mrps14, Pycr2, Ccdc86, GTPBP2, Snapc2 and Ttll12. These genes can potentially be involved in the process of rodent adaptation to the subterranean lifestyle: Mrps14 regulates the mitochondrial translation process. The Rad23b and Pycr2 genes are associated with DNA repair processes and the response to oxidative stress, respectively. Genes Zadh2 (production of prostaglandins), CCdc86 (viral response), and Ttll12 (regulation of interferon) are involved in changing the immune response and adaptation of rodents to a new infectious background. It could occur, in particular, due to high humidity in burrows. The remaining genes we found are involved in general regulatory processes: protein folding - Erp29, stress response with HSP70 - Hikeshi, transcription (Snapc2), and translation (GTPBP2). This study was conducted with financial support from the Russian Science Foundation grant N19-74-20110.

P-01.3-18
Identification, biochemical characterization and intracellular localization of sponge homolog of RRAS2

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Cancer is one of the most extensively studied diseases that occurs in almost all multicellular organisms. It is most likely that cancer appeared in parallel with multicellularity and the development of true tissues and organs. Thus, investigation of proteins involved in processes of intercellular cooperation, cell division control and multicellularity is crucial for better understanding of the disease. Comparative genomic analyses have shown that most genes/proteins associated with human cancer emerged during the early evolution of Metazoa. Hence, the study of these proteins in simpler organisms, such as sponges, provides a new approach in understanding cancer. Sponges are important model organisms because of their simple morphology and a complex genome with many genes/proteins highly similar to their vertebrate homologs. Using bioinformatics tools, we identified a homolog of human RRAS2 (Ras-related protein R-Ras2), a cancer-related protein, in the freshwater cave sponge *Eunapius subterraneus*. RRAS2 (also known as TC21) belongs to the subfamily of RAS-related proteins. When constitutively expressed and activated, it has a regulatory role in cell proliferation and migration, and is often overexpressed in human cancers. Our aim is to understand the physiological functions of RRAS2 protein in humans using sponges as a model system and to gain a better insight into the evolution of this oncogene. Bioinformatics analysis showed high conservation of RRAS2 protein and its homologs among Metazoa. The sponge cDNA encoding RRAS2 protein was successfully cloned into the expression vector and overexpressed. We confirmed GTPase activity of sponge RRAS2 protein. Additionally, cDNA was cloned into vectors for biological characterization of RRAS2. Intracellular localization of sponge RRAS2 protein was determined in transfected human tumour cells using immunofluorescence and confocal microscopy.

P-01.3-19
The chymotrypsin-like S1 peptidase family of Tenebrionidae beetles

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The chymotrypsin S1 family is one of the most numerous families of peptidases. We studied and characterized in detail the set of predicted S1 serine peptidases (SP) in the transcriptomes and genome of two model Tenebrionidae insects, *Tenebrio molitor* and *Tribolium castaneum*. S1 SP provide up to 60% of the total digestive proteolytic activity in *T. molitor* and up to 27% in *T. castaneum* larvae. The search for sequences of SP was carried out in the gut and whole larvae RNA-Sec transcriptomes of *T. molitor* lacking sequenced genome, and in the genome and larval gut transcriptome of *T. castaneum*. *T. molitor* SP were manually annotated and GenBank annotation of *T. castaneum* was manually curated if needed. *T. molitor* larval gut transcriptome contained 196 predicted sequences of proteins homologous to S1 SP, and *T. castaneum* gut transcriptome – 157 sequences. Among them, there were 97 predicted active enzymes in *T. molitor* and 102 in *T. castaneum*, containing typical catalytic residues. The remaining sequences, 99 and 55 respectively, lacked conservation in the active site residues and were classified as inactive SP homologs. Additional 138 sequences were found in *T. molitor* whole larvae transcriptome. The sets of SP from both insects contained predicted sequences of trypsins, chymotrypsin-like peptidases, elastases, collagenases, and 22–26 non-annotated sequences contained unusual residues of the S1 substrate binding subsite. The most numerous group in both insects was trypsins (34–44 sequences), while collagenases were the smallest group (5–4). Only 8–10 of active SP had a high level of mRNA expression, lacked additional regulatory domains, and can presumably be related to digestive peptidases. At the same time,

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almost half of the trypsins with a low level of mRNA expression contained a regulatory clip domain and presumably belonged to the regulatory SP. A phylogenetic analysis of S1 SP was carried out. This research was funded by the RFBR, project # 20-54-56044 Iran_t. *The authors marked with an asterisk equally contributed to the work.

Epigenetics

P-01.4-01
The impact of tamoxifen on active DNA demethylation in breast cancer cell lines

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The active DNA demethylation process, which involves TET and AID family proteins, can affect DNA methylation pattern. TET dependent demethylation contributes to DNA hypomethylation by oxidation 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) and its derivatives, whereas AID is possibly implicated in deamination of 5–hmC to 5–hydroxymethyluracil (5–hmU). Moreover, epigenetic processes, including DNA methylation, may affect regulation of genes involved in drug responses and drug targets. Tamoxifen is a leading drug in breast cancer hormonal therapy, which belongs to selective estrogen receptor modulators (SERMs). It has the ability to act like estrogen or antiestrogen depending on the receptor type to which it is bond: G-protein coupled or estrogen nuclear receptor, respectively. Thus, tamoxifen's pharmacological activity may be more complex than just controlling the estrogen signaling. Therefore, we tried to verify if SERMs supplementation of breast cancer cells may evoke changes in DNA methylation pattern. The study involved three breast cancer cells lines with differently expressed hormone receptors, which were supplemented with estrogens and the active derivatives of tamoxifen. We applied qRT-PCR and western blot for analyses of gene expression and protein levels involved in active demethylation process, and 2-D HPLC with tandem mass spectrometry detection for 5-mC and its derivatives assessment. We found differences between dissimilarly treated cells. The expression levels of TET2 and TET3 were dependent on the doses of the drugs. Furthermore, the distinctness in 5-mC level was also observed. According to our study, epigenetic changes in DNA are closely linked to cancer treatment. Our initial research may pave the way for new diagnostic and therapeutic methods as well as innovations in personalized medicine approaches. The work was supported by the Polish National Science Center [2018/29/N/NZ3/02514].

P-01.4-02
Identification of Pipsqueak interacting proteins in *Drosophila melanogaster*

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Combinatorial expression of the genes in multicellular organisms leads to the development of different cell types. The important epigenetic regulators of higher eukaryotes are the Polycomb group (PcG) and Trithorax group (TrxG) proteins. PcG and TrxG proteins act antagonistically: PcG proteins repress, while TrxG proteins activate gene transcription. These factors control the transcription of a large number of genes involved in various cellular processes. Dysregulation of PcG and TrxG systems leads to developmental abnormalities and cancer. It was demonstrated that, in Drosophila, PcG and TrxG proteins communicate with specialized DNA elements termed PREs (Polycomb Response Elements). PREs are bifunctional elements that can act as repressors as well as activators of transcription. The number of DNA-binding proteins involved in recruitment of PcG/TrxG complexes to chromatin were identified. One of the is the Pipsqueak (Psq) protein that inderacts with (GA)n sequences. In current study, we have affinity purified Psq complex and analyzed it by a highly sensitive mass spectrometric analysis. As a result, we have identified a number of known PcG and Trx group proteins, including PRE DNA-binding factors and ATP-dependent chromatin remodelers. A number of previously unknown and not related to PcG/TrxG group Psq partners were identified suggesting that they together with Psq can participate in regulation of transcription. This work is supported by the Russian Science Foundation (project 18-74-10091).

P-01.4-03
Placental and cord blood DNA methylation of the serotonin receptor type 2A gene: modulation by fetal sex and genotype and maternal metabolic state

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Serotonin receptor type 2A (HTR2A), a widely distributed G-protein-coupled receptor for multifunctional signaling molecule serotonin, has been implicated in many physiological processes and its dysregulation has been associated with a number of mental health and metabolic conditions. Different lines of evidence indicate that epigenetic modifications of the HTR2A gene play a role in molecular mechanisms through which early life environment influences development and lifelong health outcomes. The promoter region of the HTR2A gene contains a number of partially methylated cytosines that have been shown to modulate the gene's transcriptional activity. Here we investigated placental and cord blood HTR2A methylation levels in relation to fetal sex and genotype as well as maternal obesity and gestational diabetes mellitus. The study was performed on mother-infant dyads enrolled at the Clinical Hospital Center Zagreb as a part of our ongoing birth cohort study PlaNS (Placental and Neonatal Serotonin). All newborns were healthy, of

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normal birth weight and born at term by planned C-section. Cord blood samples were obtained via umbilical venipuncture and placental tissue samples were isolated from the fetal part of the placenta. DNA methylation levels were quantified at four CpG loci within the HTR2A promoter region using bisulfite pyrosequencing. In addition, samples were genotyped for two polymorphisms (rs6311, rs6306) in the respective gene region. The four targeted CpG cytosines were methylated to different degree in the cord blood and placental tissue. rs6311 and rs6306 genotypes as well as fetal sex were found to be significant predictors of the methylation levels in both tissues. Maternal metabolic parameters modulated placental HTR2A methylation in a sex-dependent manner. Taken together, data point to a complex interaction of genetic and environmental factors influencing HTR2A methylation levels during human fetal development. Funded by HrZZ (IP-2018-01-6547). *The authors marked with an asterisk equally contributed to the work.

P-01.4-04
Looking for hypoxia fingerprint in malignant tumors

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Hypoxia, a decreased availability of oxygen, is a feature of most tumors, which increases patients' treatment resistance and favors tumor progression. Although several pathways have been identified to regulate hypoxic adaptation of cells, the primary mediators of this response are HIF-1 (hypoxia inducible factor 1) transcription factors. The aim of our research was to identify the hypoxia signature in malignant tumors. For our research we have chosen two types of tumors: malignant melanoma, developing in constantly hypoxic environment; and multiple myeloma, which develops in an environment where only hypoxic niches occurs. In the first step, cancer cell lines were cultured under normoxic ($21\%O_2$) and hypoxic ($1\%O_2$) conditions for 16h. The presence of hypoxia was confirmed by the stabilization of HIF-1α subunit and detection of protein-pimonidazole adducts. Next, the panel of HIF-1 target genes, selected based on the literature, was examined using RT-PCR. All the studied genes were confirmed as HIF-1 targets and contained hypoxia response elements (HRE) in their promoters. Next, we determined molecular hypoxia signature using the genes with low basal expression and significant induction in hypoxia. Subsequently, using binominal Bernouli distribution, the molecular signature was verified on patients' transcriptomic data to answer the question of whether a group of hypoxic patients is actually present in a given tumor. Our work has shown that hypoxia molecular signatures vary between different types of cancer, and that for each type of tumor the molecular signature must be determined individually. The approach we proposed may constitute a universal tool that will allow for searching for hypoxia fingerprint in transcriptomic data of cancer patients.

P-01.4-05
Epigenetic biomarkers in precision treatment of obesity: differential methylation levels between responders and non-responders to weight loss diets

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Epigenetic marks, particularly DNA methylation levels in CpG sites, may be useful in the personalized treatment of obesity, helping to predict a better response to specific weight loss diets. The purpose of the present research was to examine whether DNA methylation levels could be associated with the metabolic response to weight loss diets. For this purpose, DNA methylation was analyzed in responders and non-responders to two different hypocaloric diets (30% energy restriction), at the beginning of the dietary intervention. DNA from white cells of 100 volunteers who participated in the Obekit dietary intervention program for 6 months was used. Volunteers were randomized in two different diets: moderately high-protein (30% protein, 30% lipids and 40% carbohydrates) and hypolipidic (18% protein, 22% lipids and 60% carbohydrates). To analyze the CpGs whose methylation was associated with better response to each intervention, the population of each diet was divided into quartiles according to their weight loss response, and the methylation levels of quartiles 1 and 4 were compared for each diet. DNA methylation levels were quantified using Infinium Methylation EPIC Bead Chip kits (Illumina). This study identified 14 CpG sites with DNA methylation differences greater than 5% between responders and non-responders to hypocaloric diets. Seven genes were associated with a better response to the high-protein diet and five genes with better response to the hypolipidic diet ($P < 0.05$). In summary, epigenetic biomarkers could help to predict the response to a weight loss diet and be used in the personalization of obesity treatment.

P-01.4-06
Effect of co-treatment of rats with cisplatin and tannic acid on substances involved in poly (ADP-ribose) polymer turnover in rat liver nuclei

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Defective mechanisms of DNA repair are intrinsic to vast majority of tumor cells and are exploited as potent pharmacological target for therapeutic treatment of cancer patients. One of the key events involved in DNA damage repair is coordinated activation of poly(ADP-ribose)polymerase 1 (PARP 1) and poly(ADP-ribose)glicohydrolase (PARG). PARP 1 and PARG are responsible for poly(ADP-ribose) (pPARr) chains turnover involved in regulation of DNA repair. Cisplatin is a powerful antineoplastic drug. Currently, chemotherapeutic regimens are employed based on the use of antioxidant supplementation alongside with cisplatin. Tannic acid (TA) is a plant-derived polyphenolic substance employed in medicine exhibits anti-oxidant and anticancer activities and inhibits PARG. Here we study the effect of co-administration of TA and cisplatin on PARP 1, PARG and NAD+ content in rat liver. Our data come to show that in 48 hours after injection cisplatin inhibited PARP 1 activity in liver

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nuclei by 50%. In 48 hours after co-treatment of rats with TA and cisplatin PARP 1 was stimulated by 50% in liver nuclei. Intra-nuclear NAD+ content in liver of rats treated with cisplatin or TA was down-regulated. Co-treatment of rats with TA and cisplatin had no impact on basal level of NAD+ content in liver nuclei of rats. Our data indicate that co-treatment with TA and cisplatin can decrease bioavailability of drugs, which eventually modulated the content and activity of biochemical components involved in pPARr turnover. Source of support: This work was supported by the RA MES State Committee of Science, in the frames of the research project № 18T-1F011. *The authors marked with an asterisk equally contributed to the work.

P-01.4-07
Effect of cadmium exposure by maternal cigarette smoking on expression of candidate microRNAs in maternal and cord blood plasma

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Women of reproductive age are a vulnerable population group for harmful effects of toxic metals, especially cadmium. Besides diet, cigarette smoking is the main source of environmental Cd exposure. Cadmium interacts with essential elements, mimics estrogenic effects and may affect epigenetic mechanisms responsible for foetal programming during intrauterine life that could be reflected in altered expression of microRNAs (miRNAs) in mother–infant pairs. The aim of the study was to investigate the effect of increased Cd exposure by maternal cigarette smoking on the expression of circulating candidate miRNAs in healthy postpartum smoking (n=35) and non-smoking women (n = 30) who gave birth at term by vaginal delivery in clinical hospitals in Zagreb. The miRNAs were isolated from maternal and cord blood plasma using miRNeasy Serum/Plasma Kit and transcribed to cDNA by miScript II Reverse Transcription Kit (Qiagen, DE). The expression of miR-1537, miR-190b, miR-16, miR-21 and miR-146a were measured by qPCR after preamplification (by miScript PreAMP PCR Kit) using custom miScript miRNA PCR Array (Qiagen, DE). Levels of Cd were analysed in maternal and cord blood by ICP-MS. There was a higher expression of miR-16 in maternal and miR-146a in cord plasma of smokers vs. non-smokers ($P < 0.05$) and the expression of other miRNAs did not differ in either maternal or cord blood plasma. Expression of miR-16 in maternal plasma was positively correlated with the number of cigarettes smoked and Cd levels in maternal and cord blood, whereas expression of miR-1537 in maternal plasma and miR-190b in cord plasma negatively correlated with Cd levels in maternal blood. These are the first results in the literature on the expression of circulating candidate miRNAs in maternal and cord blood plasma related to Cd exposure by maternal smoking that may serve as early markers of developmental origins of health and disease. (The study was funded by Croatian Science Foundation grant HRZZ-IP-2016-06-1998, METALORIGINS).

P-01.4-08
Tracing histone posttranslational modifications in suicide victims

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According to World Health Organization is suicide, with around 800.000 suicide victims, one of the leading causes of death worldwide. Suicide is an important public health problem also in Slovenia. Although the number of suicide victims in Slovenia has been declining over the past years, we still rank among the counties which has the highest suicide rates. Numerous factors affect suicide, and besides social and economic factors, it has been shown that also biological factors importantly influence this behaviour. Among the latter, the genetic factors have been studied in suicidal behaviour, and in the past decade, the genetic studies have been extended towards the (epi)genetic factors. Epigenetic factors influence gene expression without changing the DNA sequence, and they can be modified due to the environmental factors. Among the epigenetic mechanisms, DNA methylation, histone modifications, and lncRNAs can be counted. Histone proteins play essential structural and functional roles in the transition between active and inactive chromatin states through histone's variable N-terminal tails, which can be modified. The most studied histone modifications are methylation and acetylation. There are few studies connecting depression and suicide to histone modifications and consequently gene expression in the human brain [1]. In our group, we are already studying epigenetic factor in suicidal behaviour, and we have determined new candidate genes for DNA methylation in suicide [2]. In order to deepen our understanding of suicidal behaviour, we are extending the epigenetic analysis also in the field of histone posttranslational modifications. Using chromatin immunoprecipitation and next-generation sequencing we will perform genome-wide study and determine the genes importantly affected by the histone modifications and associated with suicidal behaviour. References: [1] Cheung S et al. (2020) J of Affective Disorders 265, 423–438. [2] Kouter K et al. (2019) Psychiatria Danubina 31, 392–396.

P-01.4-09
Analysis of RNA–DNA interactome discloses the transcriptional dynamics of protein-coding genes

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The past few years have witnessed the development of a bunch of technologies for mapping genome-wide localization of chromatin-associated RNAs using proximity ligation. So far, most of the research in this area has been focused on non-coding RNAs and their functions in the nucleus, including transcriptional control, shaping of the 3D genome, and assembly/maintenance of functional nuclear compartments. In the present work, we used RNA–DNA interaction data from human K562 cells to study

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the transcriptional dynamics of protein-coding genes. Analysis of contact frequencies of different regions of mRNAs with the body of encoding genes allowed us to trace how mRNA is dragged behind the RNA polymerase during transcription and disengages from the gene after transcription termination. Our data support the model of co-transcriptional intron splicing, but not the hypothesis of the circularization of actively transcribed genes. In addition, we show that longer mRNAs are characterized by a higher proportion of cis to trans contacts, apparently due to a longer linkage with the parental chromosome in the course of transcription. Analysis of RNA–DNA interactome may become a useful tool in future studies of transcription mechanisms. This work was supported by the Russian Science Foundation (grant 18-14-00011).

P-01.4-10
DNA non-canonical interactions as one of the possible factors of genomic rearrangements

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The current view on chromatin plasticity suggests that protein factors orchestrate chromatin rearrangements, while DNA plays a more or less passive role. This paradigm may be a simplification: polymorphism of the DNA secondary structure and its impact on the higher-order structure are underestimated. We show that lengthy dsDNA harboring tetraplex motifs (G-quadruplexes, G4s, and i-motifs, IMs) can spontaneously form interduplex junctions. We visualized such complexes between model and genomic DNA fragments using high-resolution atomic force microscopy. Control duplexes lacking tetraplex motifs formed no junctions. We additionally confirmed the participation of G4s in DNA–DNA synapsis using tetraplex-DNA-specific antibodies and elucidated the topologies of the synaptic complexes using molecular modeling. The complexes are sensitive to microenvironment (pH, molecular crowding, monovalent cations, et cetera). They sustain pseudo-physiological conditions, but their existence *in vivo* awaits bona fide verification. The general principle of their formation resembles presumed G4-driven enhancer-promoter interactions, but we show that both G4s and IMs contribute to the DNA association [1,2]. We also provide in vitro evidence for G4/IM recognition by chromatin remodeling factors. For instance, we show that transcriptional repressor CTCF, which regulates the demarcation of topologically associated domains, is highly sensitive to IM folding. Secondary structures, rather than CpG methylation levels, may determine CTCF occupancy at IM-prone CpG islands. Collectively, our findings indicate that noncanonical (tetraplex) DNA folding may underlie engagement of chromatin remodelers, facilitate transient DNA-DNA contacts and contribute to shaping the long-standing chromatin organization and genomic rearrangements. This work was supported by RFBR [19-15-00024]. References: [1] A. D. Protopopova et al. PCCP, 2018, 20, 3543–3553. [2] A. M. Varizhuk et al. NAR 2018 (46) 8978–8992.

P-01.4-11
Sex-specific age-related changes in methylation of certain genes

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For many decades, the mechanisms that underpin the differences in life expectancy for the two genders remain largely undisclosed. In this regard, understanding epigenetic regulation of gene expression, in particular, with regard to ageing has a major importance. For that all genes with age-dependent gender-specific methylation were divided into 3 groups: genes that change methylation level with age only in men (143 genes), only in women (401 genes) and in both genders ("duplicate" group) – 322 genes. KEGG analysis of the "duplicate" group revealed 6 significant changes ($P < 0.05$) of metabolic cascades, including genes responsible for morphogenesis, age-dependent pathological processes and biological processes associated with functioning of the nervous system. Analysis of the biological functions of groups of genes whose age-dependent methylation is characteristic only for women revealed six important ($P < 0.05$) cascades, including calcium signaling pathway, synthesis of melanin, adhesive contacts. The unique changes in the female genome that is responsible for neuronal activity in the age aspect include vasopressin and oxytocin systems, synthesis of receptors for dopamine, glycine and opioid receptors. It is interesting to note that for men, changes in the methylation of genes of the serotonin system predominate, while for women, changes affect the dopaminergic system. Thus, it was found that most of the gender-dependent age-related changes in methylation belong to the genes responsible for the development and functioning of the nervous system. Importantly, the changes are found in various mechanisms, suggesting the development of features of gender-specific reactions of the body. Russian Federation mega grant DPM-AGEING (grant 2017-220-06-4741) on Digitalized and Personalized Medicine of Healthy Ageing. *The authors marked with an asterisk equally contributed to the work.

P-01.4-12
Eukaryotic DNA methyltransferase Dnmt3a as one of the cellular targets of novel anticancer DNA-intercalating drug curaxin CBL0137

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Various anticancer drugs exert antitumor activity by interacting with DNA via mechanisms such as intercalation into DNA, groove binding and covalent modifications of heterocyclic bases thus causing cytotoxicity. Recently characterized curaxin CBL0137 is a member of a new class of potent carbazole-derived anticancer compounds that combines two of these DNA binding modes: the planar carbazole core intercalates between the base pairs of DNA with symmetrical side chains protruding into the major groove, while the positively charged nitrogen-containing chain fills the minor groove. CBL0137 binding was

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shown to cause nucleosome disassembly and spatial genome organization changes in cancer cells. We investigated the impact of CBL0137 on the key epigenetic process of DNA methylation by de novo murine DNA methyltransferase Dnmt3a and compared it to that of the other DNA intercalator doxorubicin. CBL0137 binding to DNA saturated at ~one molecule/bp, which was demonstrated by fluorescence polarization using specially designed fluorescently labelled oligonucleotide substrates. Binding of CBL0137 to Dnmt3a-CD was not observed. CBL0137 significantly inhibited in vitro DNA methylation by Dnmt3a at low micromolar concentrations (IC_{50} 9 ± 3 to 3 ± 2 μM) and reduced the binding affinity of Dnmt3a to its DNA target, causing up to four-fold increase in the K_d of the DNA-enzyme complex. Doxorubicin acted as a significantly weaker inhibitor of DNA methylation. Therefore, decreased methylation efficiency in the presence of CBL0137 can be attributed mostly to blocking of the DNA minor groove by the side chain of curaxin. Given that many cancer cells exhibit hypermethylation of tumor suppressor genes by Dnmt3a and/or express mutated Dnmt3a variants, the obtained results allow one to consider Dnmt3a as one of the cellular targets of DNA-intercalating anticancer agents. This work was supported by RFBR grants 18-34-00364 and 19-04-00533.

P-01.4-13
Can urinary HNF1B expression be a biomarker for multicystic dysplastic kidney?

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Although HNF1β is known to be as a transcription factor in many stages of renal embryonic development, it is important to investigate the level of expression in renal-derived cells in urine to understand its effect on epigenetic control. Thirty nine patients (0–21 years) with multicystic dysplastic kidney followed up from Marmara University School of Medicine, Pediatric Nephrology were included. After isolation of DNAs from blood, DNA molecules were subjected to methylation-sensitive and methylation-dependent endonuclease cleavage, methylated and unmethylated DNA fractions in the promoter region of the HNF1β gene were detected by real-time PCR. In patients with HNF1β methylation, urinary HNF1β expression of isolated renal cells was analyzed. Of all patients, 10.26% of them had HNF1β methylation. Among them one patient had a disappearance/decrease of the cysts. In one patient, cysts did not show any size change. HNF1β gene expression analysis was performed from urine samples of patients with HNF1β methylation and the group with methylated HNF1β had lower expression level compared to control group. Herein, the analysis of urinary HNF1β gene expression using a non-invasive method was performed for the first time in patients with HNF1β promotor methylation with multicystic dysplastic kidney. The use of a more easily applicable, non-radical method instead of histopathological analysis of biopsy specimens will provide significant diagnostic advantage for these patients. Although no statistically significant relationship was found, this is the first study in this field, that gives opportunity to elucidate the pathogenesis of the disease. Our results are preliminary findings to evaluate the downstream effects of HNF1β methylation and in addition these results provide hope about repairing the mechanisms involved in the pathogenesis. *The authors marked with an asterisk equally contributed to the work.

P-01.4-14
Functional role of ZBTB33 in clear renal carcinoma

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DNA methylation is crucial for proper vertebrate's development. Proteins interacting with methylated DNA can both interfere with the binding of other transcription factors and affect the formation of a new chromatin landscape. Often methyl-DNA binding proteins possess non-methyl-DNA binding activity. ZBTB33 can bind methylated CpGs and sequence containing CTGCNA. ZBTB33 is not necessary for mouse development, but it can influence the formation and progression of colon tumors due to DNA-methylation depending regulation of tumor suppressor genes. Deficiency of ZBTB33 in spontaneous colon cancer model and in breast cancer cells results in delay of tumor growth, influences the tumor size, lifespan. The aim of this work was to create and characterized the model system of renal carcinoma cells deficient for ZBTB33. We generated 3 clones of ZBTB33 knockout clear renal carcinoma cell line Caki1 by frame shift via CRISPR/CAS9 genome editing technology. Frame shift was generated in the middle of N-terminal BTB/POZ domain. Clones were analyzed for tumorigenic potential in Nude mice. Deficiency of ZBTB33 led to misregulation of 1587 genes ($P_{adj} < 0.01$). We performed ChIP-seq analyses for ZBTB33 binding sites. For negative control we used ZBTB33 deficient cells. We detected that ZBTB33 binds within regulatory elements and genic regions of 200 and 180 genes that was upregulated or downregulated after ZBTB33 depletion respectively. DNA methylation status of ZBTB33 binding sites was determined by whole genome bisulfite sequencing analyses. KEGG pathway analyses revealed downregulated genes from p53 pathway. Also, we detected that deficiency of ZBTB33 results in upregulation of several tumor suppressor genes. This work was supported by the Russian Science Foundation (19-74-30026) and the Russian Foundation for Basic Research (19-29-04139).

P-01.4-15
NELF promotes RNA polymerase II pausing at ecdysone-dependent genes in *Drosophila melanogaster*

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Ecdysone response plays a crucial role in development of *Drosophila melanogaster*. A number of genes which are induced in Drosophila cells under the influence of an ecdysone hormone were described long time ago. But exact molecular mechanism causing transcription induction upon ecdysone treatment remains largely under-investigated. We have recently described some novel molecular partners interacting with ecdysone receptor (EcR) [1] One of them, subunit A of Negative Elongation Factor (NELF A), we have investigated in detail [2]. NELF A subunit was found to co-immunoprecipitate with ecdysone receptor both in S2 Schneider cells and pupal extract. The knockdown of NELF A by RNA interference in Drosophila S2 cells were shown to decrease an inducible transcription of several ecdysone-dependent genes. ChIP-Seq analysis of NELF A demonstrated

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presence of this protein on EcR-bound sites in Drosophila genome. It is known that ecdysone-dependent genes (at least in Drosophila S2 cells) is characterized with a high level of RNA polymerase II pausing: the level of promoter-bound RNA polymerase II several times exceeds the level detected in a gene body. Usually, genes regulated via RNA polymerase II pausing mechanism show a high level of abortive transcription which can be detected in promoter-proximal regions. Using previously published Gro-Seq data (Core et al, Cell reports (2012)), we have demonstrated a presence of abortive transcription at the promoter-proximal regions of the most of ecdysone-dependent genes. Moreover, the level of this abortive transcription was found to decrease upon knockdown of NELF subunits, demonstrating that NELF complex indeed regulates promoter-proximal pausing at the ecdysone-dependent genes. The work was supported by the Russian Science Foundation [grant 18-14-00219]. References: [1]. Mazina, M. Y., et al. Sci. Rep. 10, 4793 (2020). [2] Mazina, M.Y., et al. Sci. Rep. 11, 172 (2021).

P-01.4-16
Possible ways for manipulating the gene expression in *Ixodes ricinus* ticks

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Ticks (*Ixodes Ricinus*) are blood-feeding parasites that can transmit various viruses, bacteria, protozoa, and, thus, are vectors of various diseases, such as tick-borne encephalitis, Lyme disease, anaplasmosis, and others. Extensive knowledge of the genome and transcriptome of Ixodes ticks has opened up the possibility of studying these ticks at the level of molecular biology. Therefore, in our work, we aimed to find ways to manipulate the expression of the *I. ricinus* genes. One of them may be epigenetic regulation, in particular, DNA methylation. We identified DNA methyltransferases in the *I. ricinus* transcriptome, namely DNA methyltransferase 1 (DNMT1), DNMT3, and DNA adenine methyltransferase (DAMT) (Previously published in: Kotsarenko K et al. (2020) Ticks Tick Borne Dis 11, doi: 10.1016/j.ttbdis.2019.101348). We found the differences in the expression level and DNA methylation at the different life stages: egg, larvae, nymph, and an adult female. These results suggest that DNA methylation is essential for the physiology and transstadial development of tick. Tick cell lines might serve as a model system of tick *I. ricinus* for studying the genome manipulation. Thus, we analyzed the highly-passaged IRE/CTVM19 and IRE/CTVM20 cell lines (provided by Tick Cell Biobank) and found that, despite some changes in the karyotype, they still retain the similarity of the 16S rRNA sequence to the parental tick. We have also optimized the transfection procedure for these tick cell lines, and they successfully expressed some reporter genes. Thus, our findings opened up the possibility of editing the gene expression in *I. ricinus* cells using epigenetic regulation or CRISPR/Cas9 technology, as well as to obtain recombinant proteins in tick cell lines.

P-01.4-17
G-quadruplex sites at TAD boundaries may contribute to CTCF and cohesin recruitment

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G-quadruplexes (G4s) are often found within or nearby CTCF-bound sites that mark TAD boundaries [1]. We used bioinformatics analysis and in vitro binding assays to verify two possible pathways of G4 contribution to CTCF recruitment and TAD demarcation. The first pathway is related to G4s formed upon replication. They reportedly bind with DNA methyltransferase DNMT1, sequester it from the CpG sites, and thus protect hypomethylated CpGs. CTCF is recruited mainly to the hypomethylated sites. The converged CTCF-bound sites are then recognized by cohesin, and the TAD boundaries are established. To verify this pathway, we estimated CTCF frequency in CpG-rich G4-harbouring sites that overlap with DNMT1 occupancy sites. We observed substantial colocalization and, for a subset of sites, confirmed proximity to chromatin loop boundaries. The second pathway is related to G4s formed upon transcription. They may recruit modulators of chromatin remodeling that create favorable conditions for CTCF interactions with linker DNA. Previously, we have identified several such chromatin modulators, including the polycomb complex subunit ASXL1 and high mobility group proteins HMGN3 and HMGB2, among top G4 binders. HMGN3 promotes chromatin decondensation, which renders linker DNA accessible for CTCF. ASXL1 attracts cohesin. HMGB2 acts as a CTCF insulator (prevents its aggregation). To verify this pathway, we analyzed G4 frequencies in HMGN3/HMGB2/ASXL1 occupancy sites. The frequencies were significantly higher than those predicted by chance. For a set of representative G4s, we also verified interactions with the recombinant proteins by physicochemical methods. In line with previous hypotheses [2], our results indicate that G4s might contribute to chromatin organization at multiple levels, including TAD demarcation. This work was supported by RSF [19-15-00128]. [1] Hou Y et al. (2019) Epigenetics 14, 894–911 [2] Varizhuk A et al. (2019) BioEssays 41, e1900091

P-01.4-18
Inhibition of miR-155-5p reduces NADPH oxidase expression and oxidative stress in the aorta of hypercholesterolemic ApoE-deficient mice: potential implication in human atherosclerosis

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NADPH oxidase (Nox)-derived reactive oxygen species are important molecular effectors underlying oxidative stress and inflammation in atherosclerosis. miR-155-5p has been implicated in a number of human malignancies as key regulator of inflammatory and immune responses. Yet, the role of miR-155-5p in atherogenesis is poorly defined. The aim of this study was to

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determine the role of miR-155-5p in mediating Nox up-regulation and the ensuing oxidative stress in the aorta of hypercholesterolemic ApoE-/- mice. Non-atherosclerotic and atherosclerotic human arterial samples, and ApoE-/- mice were employed. After 10 weeks on normal or high-fat, cholesterol-rich diet, male ApoE-/- mice, were randomized to receive via intraperitoneal injection miR-155-5p inhibitor, or its negative control, once per week for 4 weeks. Human and mouse specific miRCURY LNA™ miRNome real-time PCR panels were employed to determine the expression of 752 miRNAs in arterial tissues. miRNA expression profiling revealed that miR-155-5p was significantly up-regulated both in human atherosclerotic tissue samples and in atherosclerotic aorta of ApoE-/- mice. TargetScanTM analysis predicted that miR-155-5p interacts with genes (e.g., SHIP-1, IKBKE, SOCS) that may control Nox expression. Treatment of atherosclerotic ApoE-/- mice with miR-155-5p inhibitor reduced significantly the progression of atherosclerosis, the aortic expression of Nox1, Nox2, and Nox4 protein levels, and the formation of 4-HNE-protein adducts (oxidative stress marker). Moreover, inhibition of miR-155-5p led to a marked reduction in CD45, CD68, and NOS2 protein levels (markers of immune cell infiltration and inflammation). The data of this study point to miR-155-5p as potential therapeutic target to reduce oxidative stress and inflammation in atherosclerosis-related cardiovascular disorders. Work supported by PN-III-P1-1.1-TE-2016-0851, PN-III-P2-2.1-PED-2019-2512, PN-III-P2-2.1-PED-2019-2497.

P-01.4-19
Identification of potential lysine-specific histone demethylase-dependent mechanisms underlying oxidative stress and inflammation in human and experimental atherosclerosis: a transcriptomics approach

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Complex epigenetic alterations converging to changes in gene function and cell phenotype have been increasingly implicated in atherosclerosis. Within the epigenetic landscape, the role of lysine-specific histone demethylase (KDM)-based pathways in atherosclerosis remains elusive. The aim of this study was to investigate the expression pattern of KDM subtypes in human and experimental atherosclerosis and to determine the potential role of KDM5B in mediating the up-regulation of key genes linked to oxidative stress and inflammation in vitro. Human non-atherosclerotic and advanced atherosclerotic arterial samples and $ApoE^{-/-}$ mice were used. Male $ApoE^{-/-}$ mice were fed a normal or a high-fat, cholesterol-rich diet for 14 weeks. In vitro studies were done on primary mouse monocyte-derived resting (M0), pro-inflammatory (M1) and anti-inflammatory (M2) macrophages (Mac). The occurrence of oxidative stress (NADPH oxidase/Nox) and inflammatory cells/markers (CD45, CD68, NOS2) in the plaques was confirmed by western blot and immunofluorescence microscopy. Microarray-based (SurePrint G3 Human/Mouse gene Expression v2 8x60K) genome-wide expression profiling and bioinformatics analysis predicted the implication of KDM-related signaling pathways in atherogenesis. Real-time PCR and western blot assays revealed the up-regulation of gene and protein expression levels of KDM1A, KDM1B, KDM2A, KDM2B, KDM3A, KDM3B, KDM4A, KDM4B, KDM4C, KDM5A, KDM5B, and KDM5C subtypes in human atherosclerotic tissue samples, atherosclerotic aorta of $ApoE^{-/-}$ mice, and in pro-inflammatory M1-Mac. Pharmacological inhibition of KDM5B by PBIT suppressed the up-regulation of TNFα, MCP-1, Nox1, Nox2, and Nox4 expression levels in M1-Mac. Isoform-specific KDM pharmacological interventions could represent an effective therapeutic strategy to correct the expression of dysregulated genes that are mechanistically linked to atherosclerosis. Work supported by PN-III-P2-2.1-PED-2019-2497, PN-III-P4-ID-PCE-2020-1898.

P-01.4-20
Structural basis for NTP specificity of non-canonical CutA nucleotidyltransferase

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During the course of its lifecycle, eukaryotic mRNA undergoes several types of modifications at different stages of its maturation. These modifications significantly affect the mRNA fate. One such modification is a post-transcriptional addition of untemplated nucleotides to mRNA 3'-end. Apart from well-known polyadenylation, the presence of homo- or heteropolymeric stretches containing nucleotides other than adenosine has been reported for protein-coding transcripts in multiple organisms, including fungal, plant and animal species. CutA is a non-canonical nucleotidyltransferase identified in filamentous fungi *Aspergillus nidulans*, which belongs to DNA polymerase β superfamily. *In vivo*, it adds short CUCU-rich extensions to 3'-ends of mRNA, which in turn leads to deadenylation-independent decapping and eventually mRNA degradation. Our previous biochemical studies carried out using recombinant CutA from *Thielavia terrestris* demonstrated that although the enzyme is able to processively polymerize only adenosines, it indeed displays an unusually high specificity towards cytidines as compared to other known terminal nucleotidyltransferases, and it synthesizes predominantly tails terminating with two cytosines. Our present work involves determination of crystal structures of *T. terrestris* CutA catalytic domain alone and in complex with substrate and product. The apo structure of CutA was solved by Se single-wavelength anomalous diffraction. Based on the structural analysis further biochemical characterization was performed on selected mutants to get deeper insight into catalytic properties of the enzyme: 1) high specificity towards CTP; 2) exclusive processivity towards ATP; and 3) a complete lack of activity for GTP as an incoming nucleotide and RNA substrate ending with guanines.

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P-01.4-21
Functional studies of *A. thaliana* MYST-family acetylases, HAG4 and HAG5, produced by cell-free translation

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Histone post-translational modifications (PTMs) play a crucial role in the regulation of gene expression in eukaryotes. Histone modifying enzymes, as master regulators, can add, identify and remove a wide array of modifications of core histones, namely histone H2A, H2B, H3 and H4. One of the most frequent PTM is acetylation, which is mainly associated with transcriptional activation. In *Arabidopsis thaliana* 12 histone acetyltransferases (HAT) can be found. We aimed to investigate two of the lesser-known MYST-family acetylases, HAG4/HAM1 (AT5G64610) and HAG5/HAM2 (AT5G09740). Previous *in vivo* studies confirmed their roles in plant fertility and significant phenotypic differences were observed in mutant plants. Despite the high similarity in their amino acid sequence, it was shown that HAG4 and HAG5 are a functionally redundant pair of genes. It is also known, that HAG4 and HAG5 primarily acetylate histone H4 (H4K5) and have a slight activity on H3. Previously, efficient *in vitro* expression and purification of HAG4 and HAG5 enzymes were not successful in *E. coli* expression systems. In the presented project, we show the successful expression and purification of *Arabidopsis thaliana* HAG4 and HAG5, histone H3, H4 by cell-free wheat germ translation system. We aim to perform acetylation assays with HAG4 and HAG5 on histone H3, H4 and other putative substrates, which can be confirmed by mass spectrometry. Additionally, we aim to investigate the binding of acetyl-CoA to the enzymes, and the mechanism of the predicted autoacetylation of HAG4 and HAG5. (This project is supported by 129083 grant of the National Research, Development and Innovation Office, Hungary.)

P-01.4-22
Dual effect of methylation of lncRNA genes on pathogenesis and metastasis of ovarian cancer

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Ovarian cancer (OvCa) is asymptomatic and still the most-deadly cancer of the female genital tract worldwide. The discovery of noncoding RNAs (ncRNAs) involved in epigenetic processes opens up new opportunities in the study of the pathogenesis of cancer. Aberrant DNA methylation is an important mechanism for regulating the expression of both protein-coding and ncRNA genes. However, the role of methylation in the regulation of genes of long noncoding RNAs (lncRNAs) is still very little studied. So, the aim of this work is to identify new hypermethylated lncRNA genes in ovarian tumors and their effect on OvCa metastasis. Using quantitative methylation-specific PCR and non-parametric Mann-Whitney test we observed a multiple and statistically significant ($P < 0.001$) increase in the methylation level of a group of lncRNA genes: MEG3, SEMA3B-AS1, ZNF667-AS1 and TINCR. All our results we found for the first time. Analysis of 19 samples of peritoneal metastases in comparison with paired primary tumors unexpectedly revealed a statistically significant decrease in the methylation level of the same 4 genes: MEG3 ($P = 0.004$), SEMA3B-AS1 ($P = 0.002$), TINCR ($P = 0.002$), and ZNF667- AS1 ($P < 0.001$). This phenomenon, which we discovered, is apparently associated with the participation of these lncRNAs in the regulation of plastic reversion of EMT– MET. So EMT– MET reprogramming of cells, a decrease in methylation of lncRNA genes activates the suppressive functions of lncRNAs, and may play a role in stabilizing the epithelial properties of secondary tumors. Thus, long ncRNAs have a dual effect on OvCa tumors and metastasis which is important for understanding the pathogenesis of OvCa and for finding new targets for therapy. The described effects of hypermethylated lncRNA genes on tumors and metastases (secondary tumors) of OvCa are under validation via expression studies. The work was supported by the grant from the Russian Science Foundation #20-15-00368. *The authors marked with an asterisk equally contributed to the work.

P-01.4-23
Analysis of inter-chromosomal contacts of rDNA genes with DUX4 genes in human cells in different physiological conditions using fluorescence *in situ* hybridization

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Direct rDNA inter-chromosomal contacts with different chromosomal regions are involved in the regulation of genes activity. rDNA contacts with the subtelomeric region of chr4 possessing DUX4 genes were previously detected by the 4C (Circular Chromosome Conformation Capture) approach. The contacts disappear after the heat shock treatment (Tchurikov et al., 2020, Dokl Biochem Biophys. 490(1):50–53. doi: 10.1134/S1607672920010032). DUX4 genes specify transcription factors that play a key role in zygotic genome activation and are silenced at the later stages. Their expression at later stages leads to muscular dystrophy. The aim of this study was to study the contacts of rDNA and DUX4 genes using the independent approach – fluorescence *in situ* hybridization (DNA-FISH). We used HEK293T cells before and after heat shock treatment. On average, we observed about 4 foci of rDNA probe and 10 foci of DUX4 probe per cell, 34% of them were located in the area of rDNA contacts. The number of DUX4 genes contacts with nucleoli decreased approximately twice (to 17%) after the heat shock treatment and the distances between hybridization foci increased. The proportion of hybridization sites of DUX4 genes not associated with the rDNA region increased by almost 10 times. Cells also were stained with antibodies to nucleolin, that is required for RNA polymerase I transcription, to determine the transcriptional activity at rDNA hybridization foci. We detected that the intensity of immunostaining of rDNA loci decreased after the heat shock treatment. The data indicate the changes of transcriptional activity in nucleoli induced by the treatment. In RT-PCR experiments we detected that the treatment causes nearly a 2-fold decrease the levels of pre-rRNA. FISH results support the results obtained by 4C approach and provide data

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on diversity of inter- chromosomal contacts in individual cells. The study was supported by the grant from Russian Science Foundation No. 21-14-00035.

P-01.4-24
RASSF1A and PRSS21 as future diagnostic biomarkers for testicular germ cell tumors

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Testicular germ cell tumors (TGCTs) are the most common malignancies in young men with an overall increasing incidence. Therefore, it is crucial to identify biomarkers for early detection and diagnosis. TGCTs are divided into seminomas (SE) and nonseminomas (NS) that both arise from germ cell neoplasia *in situ* (GCNIS). RASSF1A is a tumor suppressor gene that influences tumor initiation and development. PRSS21 is expressed in normal testes and it is hypothesized to be a tumor suppressor gene. In contrast to RASSF1A and PRSS21 gene expression status and DNA methylation pattern, its protein expression is poorly investigated. Therefore, we performed a comprehensive *in silico* bioinformatics analysis at the DNA methylation level and mRNA level and compared it with data of protein expression in TGCTs. RASSF1A and PRSS21 protein expression was analyzed in 108 TGCT samples by immunohistochemistry in healthy testicular seminiferous tubule tissue (HT), GCNIS, TGCTs, SE, and all NS components (embryonal carcinoma, yolk sac, choriocarcinoma, teratoma). The immunoreactivity score (IRS) was calculated with a cut-off value of four for clinical diagnostic positivity. UALCAN was used for the analysis of promoter methylation levels in SE and NS. The highest RASSF1A and PRSS21 protein expression were in GCNIS, while in HT and TGCTs was significantly lower. In HT and TGCTs, RASSF1A and PRSS21 showed inverse protein expression to their mRNA levels. In SE, RASSF1A expression on mRNA level was significantly higher than in NS, while differences of PRSS21 in mRNA expression were not found. RASSF1A and PRSS21 show general hypomethylation in SE and hypermethylation in NS, although no differences in protein expression were detected. Regarding NS components, PRSS21 has shown increased protein expression in choriocarcinoma. PRSS21 and RASSF1A discriminate GCNIS from HT and TGCT, as well as SE from NS, showing significant potential as future TGCTs biomarkers on more than one molecular level.

P-01.4-25
BPM1 protein is involved in plant development and stress responses by regulating *de novo* DNA methylation

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A member of a small family of *Arabidopsis thaliana* MATH-BTB proteins, BPM1 functions as a substrate-specific adaptor of a cullin3-based E3 ubiquitin ligase complex. Its N-terminal MATH domain binds target proteins and designates them for ubiquitination and subsequent proteasomal degradation. Recent studies reported that BPM1 mediates proteasomal degradation of transcription factors (ERF/AP2, R2R3 MYB and Homeobox) and phosphatases (PP2Cs). By using immunoprecipitation, TAP-tag purification, fluorescence-lifetime imaging microscopy, yeast-2-hybrid, pull down and microscale thermophoresis we confirmed BPM1 interaction with DMS3 and RDM1, key regulators of de novo DNA methylation in *A. thaliana*. Chromatin immunoprecipitation was used to determine regions of overlap between DMS3 and BPM1 chromatin binding sites. The DNA methylation status of identified regions was analysed by pyrosequencing after bisulphite conversion in *A. thaliana* plants overexpressing BPM1, in plants with downregulated BPMs as well as in DNA methylation mutants. Methylation patterns were assessed during plant development and after exposure to different abiotic stresses. Our results indicate that BPM1 does not participate in degradation of DMS3 and RDM1. Rather, it seems BPM1 recruits DNA methylation machinery to specific chromatin positions for de novo DNA methylation establishment.

P-01.4-26
The role of heterochromatin proteins in imprinted paternal X chromosomes elimination in the development of *Sciara coprophila*

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The phenomenon of genomic imprinting was first investigated in fungus gnats *Sciara coprophila*. It is known that programmed elimination of paternal X chromosomes takes place during the early stages of embryogenesis in somatic cells, so that two paternal X chromosomes are eliminated in future males, and one is eliminated in future females [1]. The number of eliminated chromosomes can be determined by X chromosomes to autosomes ratio (X/A). Recently it was found that the region of X chromosome which is not exposed to elimination is associated with H3K9me3 and H4K20me3 epigenetic modifications [2]. Here we estimated the role of H3K9- and H4K20-specific methyltransferases (MTs) in X chromosomes elimination in early embryos. As a result of RNA-Seq and de novo transcriptome assembly 7 transcripts of specific MTs were found. Double stranded (ds) RNAs were synthesized against identified transcripts for RNA interference (RNAi). Embryos were incubated during 10 hours with dsRNAs against MTs genes and GFP as a control. DNA sequencing by

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Illumina MiSeq was used for reads counting. The number of eliminated X chromosomes was estimated by relation of X/A values obtained from experimental groups to X/A values got from controls. In our pioneer experiment with female embryos, it was shown that Su(var)3–9 RNAi probably leads to blocking of X chromosomes elimination and SetDB1 RNAi apparently leads to increased number of eliminated X chromosomes compared with controls. However, G9a and Hmt4–20 RNAi did not lead to significant differences compared with controls. Probably it can be associated with unsuccessful passing through pores of dsRNAs into embryos. Apparently, it is advisable to repeat the experiment with shorter siRNAs in order to achieve sustained effect. This study was supported by a grant of the Russian Federation Government #14.Y26.31.0024. Previously published in: [1]. Metz CW (1938) The American Naturalist 72, 485–520. [2]. Singh PB et al. (2019) Chromosoma 128(2), 69–80.

P-01.4-27
Methylation role of lncRNAs in breast cancer pathogenesis

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Epigenetic mechanisms, as promoter CpG islands methylation play a pivotal role in genes expression regulation, including long noncoding RNA (lncRNA) genes that involved in the pathogenesis of breast cancer (BC). The aim of this study was to assess the methylation status of 7 lncRNA genes in BC and the relationship with the morphological features of the tumor. Methylation analysis was performed using quantitative methylation-specific PCR in 30 paired (tumor/normal) breast cancer samples. The significance of the results was assessed by the R statistics (Mann-Whitney U-test, Kolmogorov-Smirnov test, SPSS 20; $P < 0.05$). The analysis of 7 lncRNA genes methylation level (MEG3, SEMA3B-AS1, HAND2-AS1, ZNF667-AS1, TINCR, MALAT, SNHG1) revealed statistically significant ($P < 0.001$) hypermethylation for MEG3, SEMA3B-AS1, HAND2-AS1, ZNF667-AS1; moreover, results for SEMA3B-AS1 and ZNF667-AS1 in breast cancer was shown for the first time. We also found the statistically significant ($P < 0.001$) increase of MEG3, SEMA3B-AS1, HAND2-AS1, ZNF667-AS1 methylation level on late stages (III-IV). A significant ($P < 0.001$) correlations between changes in the methylation level of the HAND2-AS1 lncRNA gene with tumor cells high size (T3/T4), the presence of lymph node metastases (NxMx) and a decrease level of differentiation (G3) were determined. Thus, our results supplement the "molecular portrait" of breast cancer and contribute to understanding its pathogenesis. The revealed methylation features of the studied genes can be applicant for new approaches to the prognosis, prevention and treatment tactics for breast cancer. This work was supported by the Russian Science Foundation (Grant No. 20-75-00126).

P-01.4-28
DNA methylation status and expression of pluripotency related genes in testicular development of rat

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SALL4 and LIN28A/B are pluripotency related markers, being highly expressed in testis in undifferentiated spermatogonia respectively, where they play an essential role in maintaining their pluripotent and self-renewal properties. However, data about their DNA methylation dynamics during testicular development are limited. The aim of this study was to analyze and compare expression dynamics with DNA methylation status of SALL4 and LIN28A/B in different stages of early fetal and neonatal testicular development of rat. DNA and RNA were isolated from the fresh fetal and neonatal samples of rat testis taken successively from GD20 to PND5,5. qPCR method was applied to determine expression status of SALL4 as well as LIN28A/B while the pyrosequencing method was used to determine the level of CpG methylation in promotor regions of mentioned genes. The results had demonstrated that SALL4 and LIN28A/B were expressed in all examined testicular developmental stages with significantly higher mRNA expression in the fetal compared to the early neonatal stages of testicular development. Furthermore, promoter regions of both genes were highly hypomethylated, however, without a difference in the level of methylation between the individual developmental stages. We conclude that the expression and related function of spermatogonial-related genes could be controlled at the posttranscriptional level, which is a much quicker way to activate/deactivate genes than the DNA methylation mechanism. This study was supported by the Scientific Center of Excellence for Reproductive and Regenerative Medicine, Croatia, and through the European Regional Development Fund, under grant agreement KK.01.1.1.01.0008. "Reproductive and Regenerative Medicine-Exploring New Platforms and Potentials".

P-01.4-29
Epiclonality alterations may contribute to adaptation of sticklebacks to different osmotic conditions

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The expansion of the methodological capabilities of modern molecular biology is the foundation for the birth of new hypotheses regarding seemingly well-studied cellular processes. For example, during tissue differentiation, the epigenetic profiles of some cells may be more effective for the processes of adaptation to new conditions, which will constitute the epigenetic clonality of the population. Three-spined stickleback is a convenient model organism for studying adaptation processes at epigenetic level due to its possibility to inhabit environments with different osmotic conditions. In this work, we investigate the epigenetic clonality of the freshwater and marine stickleback populations. For this purpose, bisulfite sequencing of 48 fishes was performed. 24 fishes were taken from a freshwater environment and 24 from a marine environment. On average, 65 million pairs of 100-bp reads were sequenced per sample. Paired reads were mapped to the gasAcu1 genomic assembly using the Bismark program with

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standard parameters. For each sample, the methylation (Shannon's) entropy was calculated with a window of 4, 5 and 6 CpG. The obtained values of entropy were compared between freshwater and marine conditions using the F-test (with Benjamini-Hochberg adjustment procedure). Thus, we obtained 6526 intervals, which shifted their level of epigenetic heterogeneity in the freshwater environment as compared to the marine one. Using the intervals, we obtained a list of genes whose promoters changed epiclonality. GO Enrichment analysis of these genes revealed set of terms mostly associated with positive and negative epigenetic regulation of gene expression. Overall, these data allow to make a hypothesis that the genes that carry out epigenetic regulation may themselves be under the pressure of the epigenetic component of selection. And this alteration in epiclonality may contibute to adaptive changes. This work was supported by Russian Science Foundation (RSF) grant 19-14-00347.

P-01.4-30
Deepening the regulation of PDYN gene, as a function of alcohol consumption

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For several years an important role in alcoholism research has been attributed to the endogenous opioid system [Koob et al., 1998]. Different groups analyzed genes system polymorphisms and transcriptional in alcoholics and in mammals exposed to alcohol. In particular, our group observed the epigenetic regulation of prodynorphin (PDYN) gene via gene promoter DNA methylation (D'Addario et al., 2017; Wille-Bille et al., 2018). Pattern-based prediction of transcriptional factors (TFs) binding to PDYN sequence analyzed show that the CpG site resulted to be differentially modulated by alcohol both in humans and in rats, is recognized by c-Jun, a TF that in combination with c-Fos forms the AP-1 (Activator Protein 1) complex. Moreover, PDYN rat sequence under study is recognized also by cAMP response element-binding protein-1 (CREB1). We here developed an *in vitro* assay to monitor the binding affinity of both c-Jun and CREB1 at PDYN gene promoter region, analyzing how DNA methylation can influence this binding usAlphaScreen® assay. In parallel, we quantitatively measured it in the Ventral Tegmental Area (VTA) of rats prenatally exposed to ethanol or vehicle, using Chromatin ImmunoPrecipitation (ChIP). The AlphaScreen® assay show a different binding affinity of c-Jun and CREB1 with PDYN sequence. Moreover, CREB1 binding results significantly affected by differential DNA methylation at the CpG site contained in its recognition motif. ChIP assay confirmed the differential CREB1 binding in VTA of rats, observing an increased expression of PDYN in animals prenatally exposed to alcohol compared to those not exposed. We used a new analytical method to quantitative monitor the effect of CpG methylation on the interaction of PDYN gene with TF in combination with an already well-characterized ChIP method to further describe the mechanisms behind PDYN gene regulation by alcohol. This approach would be of help for the design of new drugs targeting specific DNA sequences.

P-01.4-31
Possible effects of miR29a downregulation on keloid scar formation

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Abnormal wound healing can cause keloid scar formation. Keloids are fibrous benign tumours that continue growing beyond the wound boundaries. It is characterized by increased collagen accumulation. Therefore, targeting collagen synthesis may be the right approach for treatment. Evidence is emerging regarding the efficacy of the miR29 family in fibrotic diseases and extracellular matrix proteins are dominant among target molecules. In this study, we examined the role of miR29 family in keloids, HSP47 and LOX for collagen maturation and collagen synthesis through the TGF-b/Smad pathway. We determined the skin keloid and control tissue miR29 family gene levels via qPCR. After determining miR29 expressions in keloid scar tissues, we inhibited miR29a in primary keloid fibroblasts. We checked the protein levels related to collagen synthesis by using western blotting. TGF-b/Smad pathway gene levels were determined by using qPCR. Extracellular LOX activity was measured with a fluorescent kit, and TIMP-1 protein levels were assessed with ELISA. As a result, it has been found a significant increase in fibronectin, COL1A and LOXL2 protein levels, and LOX activity. These parameters proved that miR29a affects the collagen synthesis process and increases collagen synthesis. On the other hand, the downregulation of miR29a in keloids increased TGF-b/Smad pathway activity. TIMP-1 gene levels upregulated with this activity, but this was not reflected in the extracellular TIMP-1 protein level. This may indicate that miR29a affects TGF-b, TGF-b stimulates TIMP-1, but this stimulation may not be reflected in the protein level of TIMP-1 and may be a cause of the irregularity in keloid histopathology. These findings indicate that miR29a is effective in keloid formation and this activity is related to the expression level of miR29a.

P-01.4-32
Lysines acetylome profiling of H3 and H4 histones in TSA-treated stem cells

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The DNA in eukaryotic cells is packed in chromatin with nucleosomes as basic unit. Nucleosomes are composed by octamer of four histones, and the global chromatin structure is altered by histone post-translational covalent modifications. Several types of histone modifications are well known such as acetylation, methylation, phosphorylation, and ubiquitination that play a role in the regulation of transcription activity. Histone modifications dysregulation as well as disruption of chromatin remodeling machinery play a fundamental role in many pathologies and cellular mechanisms. The analysis of histone modifications with

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standard mass mapping procedures is complicated by the highest occurrence of basic residues mainly in the regions interested by the modifications (methylation and acetylation). We developed a methodology based on limited proteolysis coupled to MALDI-MS to achieve a complete sequence coverage; then by LC-MS/MS and ion extract procedures, we got a relative quantification of the modification. Once optimized the procedure on standard chicken core histones, we investigated the effects of TSA on H3 and H4 lysine acetylome in mice embryonic stem cells (ES14), treated with trichostatin A (TSA) by using the new, untargeted approach, consisting of trypsin-limited proteolysis experiments coupled with MALDI-MS and LC-MS/MS analyses. The proposed strategy was found in its simplicity to be extremely effective in achieving the identification and relative quantification of some of the most significant epigenetic modifications, such as lysine acetylation and methylation.

P-01.4-33
Analysis of prominent molecular biomarkers in tissue biopsies for TGCT diagnostics

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Testicular germ cell tumours (TGCT) make up 95% of testicular tumours and are divided into seminoma (SE) and non-seminoma (NSE), considered to originate from germ cell neoplasia *in situ* (GCNIS). GCNIS is driven by an interplay of genetic, epigenetic and micro-environmental factors leading up to an arrest of gonocyte differentiation. Currently used biomarkers for testicular cancer lack specificity and sensitivity; and are used as accessories in diagnostics. Due to the difference in treatment and outcome depending on diagnosis better biomarkers are needed, which molecular methods from tissue biopsies promise. We have analysed prominent TGCT biomarkers (POU5F1, NANOG, KIT, SALL4, HOXA9 and MGMT) *in silico* mRNA gene expression, DNA methylation and patient's gene expression on the protein level to see the diagnostic potential or limitations of certain methods. TGCT patient data from TCGA dataset and healthy adult testis data from GTEx dataset was analysed using XENA, UALCAN and cBioPortal platforms. For immunohistochemical detection, 108 FFPE non-seminoma TGCT's from KBC SM and 48 tumour-free testes were used. Slides were analysed semi-quantitatively by pathologists and analysed in GraphPad Prism using Mann-Whitey and Kruskal-Wallis tests. The results have confirmed the efficiency of molecular methods, discriminating TGCT from healthy tissue (mRNA levels of all investigated genes) and discriminating SE from NSE (mRNA level of KIT and DNA methylation of MGMT and HOXA9), however discriminating individual NSE components and GCNIS proves to be a challenge and is only possible using gene expression on the protein level. Relying purely on molecular methods could mask the presence of individual NSE components found in TGCT, making the pathologist's expertise irreplaceable in diagnostics. More detailed investigation of the molecular profile of individual NSE components is the last hurdle for novel molecular methods to find widespread clinical use in TGCT diagnostics.

Protein biosynthesis and expansion of genetic code

P-02.1-01
Biochemical approaches to developing spent media from industrial bioprocesses for new protein production in *E. coli* fermentation systems

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What if we could take waste byproducts from industrial processes and generate new value in secondary processes with that waste? Of the thousands of tonnes of hazardous chemical waste generated from the biopharmaceutical manufacture of protein-based drugs each year, spent cell culture media from bioprocessing represents one potential area for exploitation using applied biochemical approaches. Studies have shown that levels of up to 60% spent media added to cell culture systems helped to increase protein production by mammalian and insect cells. In this poster presentation we describe (i) the proof of principle experiments to determine viability of *E. coli* cultures using harvested spent media from CHO cell culture (ii) the expression analysis of a novel fusion protein by the cultures and (iii) the empirical approaches taken to optimise the culture conditions in comparison to rich bacterial media and nutrient limiting minimal media controls respectively. Our data shows that expression of a fluorescent mCherry fusion to a novel affinity construct of commercial value is maintained by the bacterial cultures in 100% spent medium from mammalian cell culture. The careful empirical optimisation of biochemical parameters in the expression culture system are presented to show how a spent medium from bioprocess can generate proteins of value in newly designed processes, including the titration of low cost additives such as glucose and glycerol, as well as gradients of temperature and expression time. A key aim of the circular bioeconomy is to maximise the utility of resources by ensuring waste can be used to generate new value and this study confirms the potential for the reuse of spent media from bioprocess as a food source for microbial expression cultures. *The authors marked with an asterisk equally contributed to the work.

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P-02.1-02
Stress-induced modulation of human antigen R by the apoptosis mediator cytochrome c and tyrosine kinase JAK3

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The post-transcriptional control of gene expression is mediated by the so-called RNA-binding proteins (RBPs). One of the best studied RBPs is the Human antigen R (HuR), which usually enhances the stability and translation of its mRNA ligands. Moreover, HuR binds to the histone chaperone ANP32B to export cargo mRNAs into the cytoplasm. Our group is currently developing two lines of research on the regulation of HuR function. The first one originated from pull-down assays in which we had detected the association of the apoptosis mediator cytochrome c (Cc) with HuR upon DNA damage stimulus. Interestingly, we already had evidence suggesting a physiologically relevant interaction between ANP32B and Cc. Therefore, our ongoing investigation aims to elucidate whether Cc directly binds to HuR or, alternatively, ANP32B acts as a molecular bridge linking the other two proteins. Furthermore, we are also examining the biological significance of the above-mentioned interactions in the context of programmed cell death. On the other hand, our second research line focuses on the role of Janus kinase 3 (JAK3) in the modulation of HuR binding to cognate mRNAs. Indeed, stress-induced phosphorylation of HuR at Tyr200 by JAK3 has been related to a reduced interaction of this RBP with its target transcripts. To get a deeper insight into this observation, we mimicked Tyr200 phosphorylation by co-expressing a tRNA/aminoacyl-tRNA synthetase pair specific for the non-canonical amino acid p-carboxymethyl-L-phenylalanine (pCMF) together with an HuR construct. Through several biophysical assays with phosphomimetic Y200pCMF HuR and single-stranded DNA oligonucleotides, we want to assess the impact of JAK3 activity on HuR affinity for mRNA. Importantly, HuR is considered an oncoprotein and its dysregulation has been implicated in several diseases. Thus, a better understanding of the molecular mechanisms controlling HuR function could provide valuable data for the design of new therapies.

P-02.1-03
Isoleucyl-tRNA synthetase carrying antibiotic resistance cannot support sporulation and biofilm formation in *Bacillus megaterium*

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Isoleucyl-tRNA synthetase (IleRS) catalyzes covalent coupling of isoleucine and $tRNA^{Ile}$ for protein synthesis. Several Bacilli species have two distinct types of ileS gene. While ileS1 seems to be following species phylogeny tree, ileS2 distribution among species is best explained by horizontal gene transfer. To understand requirements for having both ileS genes, we used *B. megaterium* as a model organism and performed kinetic analyses on the isolated enzymes and in vivo analyses on the constructed knockout strains. We found that BmIleRS2 exhibits 25-fold higher K_M for isoleucine than BmIleRS1 and at the same time a 1000-fold higher K_i for antibiotic mupirocin. Interestingly, while mupirocin acts as a fast-on/fast-off competitive inhibitor of BmIleRS2, it displays a slow-tight binding type of inhibition with BmIleRS1. Our data further indicate that interaction of BmIleRS1 and BmIleRS2 with isoleucine is modulated by $tRNA^{Ile}$ but in a different way. Whether and how distinct mupirocin interaction with BmIleRS1 and BmIleRS2 is related to the observed different K_M values of these enzymes toward isoleucine is still not clear, however, it may suggest that aminoacylation mechanisms could have distinctly evolved in these enzymes to account for the trade-off between mupirocin resistance and amino acid affinity. Knockout strains lacking either bmileS1(ΔileS1) or bmileS2 (ΔileS2) were created to address cellular demands for both ileS genes. Although both knockout strains were viable, ΔileS1 exhibited slower growth, inefficient sporulation and deviation in biofilm formation compared to the wild-type strain. In a minimal medium, ΔileS1 strain is outcompeted by ΔileS2 strain, suggesting bmileS1 in under constant selective pressure to remain in the genome. These results show that bmileS2, carrying antibiotic resistance, can complement bmileS1 as a sole housekeeping gene, however, BmIleRS1 is essential for overall fitness of B. megaterium.

P-02.1-04
Isoleucyl-tRNA synthetase editing domain accepts broad range of amino acids that are efficiently discriminated at the synthetic active site

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Aminoacyl-tRNA synthetases (aaRSs) activate amino acids and transfer them to cognate tRNAs. Some aaRSs cannot establish a required specificity in amino acid recognition and thus may erroneously activate noncognate amino acids with a frequency higher than 10^{-3}. These enzymes evolved a separate editing domain to hydrolyze formed misaminoacylated tRNAs (post-transfer editing). An initial model of discrimination at the editing domain proposed that binding of the cognate amino acid is prevented by a steric clash. Yet, we showed that the cognate amino acid may bind at the editing site, but unproductively (previously published in Dulic et al. J Mol Biol (2018) 430, 1–16). To understand better what shaped selectivity of the editing domain we have used isoleucyl-tRNA synthetase (IleRS) as a model enzyme in our recent (previously published in Bilus et al. J Mol Biol (2019) 431, 1284–1297; Zivkovic et al. FEBS J (2020) 287, 800–813, doi: 10.1111/febs.15053) and novel work. We tested a broad range of substrates belonging to i) proteinogenic (Ile, Ala, Val, Leu, Thr, Ser and Met), ii) nonproteinogenic (α-aminobutyrate, norvaline (Nva) and norleucine), and iii) synthetic (di- and tri-γ-fluoro-α-aminobutyrate) amino acids. Among them, only Val and Nva mimic well the cognate Ile and were poorly discriminated (< 200-fold), while the others were well discriminated at the IleRS synthetic site (500- to 10^6-fold). Nevertheless, we prepared misacylated tRNAs with all tested amino acids and followed their hydrolysis in an independent assay. Surprisingly, all misacylated tRNAs were hydrolyzed by IleRS at similar rates (35–70 s^{-1}). Thus, how efficient amino acids were discriminated at the synthetic site and consequently whether these amino acids posed an evolutionary threat to translation fidelity does not determine the efficiency of their post-transfer editing. Only the cognate $Ile\text{-}tRNA^{Ile}$ was hydrolyzed slowly (0.058 s^{-1}), suggesting that this is

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the main requirement that shaped specificity of the editing domain.

P-02.1-05
Investigation of interaction between SecM stop peptide and *E. coli* ribosome by MD

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The nascent peptide exit tunnel (NPET) participates the translation regulation. So-called stop peptides are able to bind NPET walls, arresting translation. SecM is a such kind of peptides that precedes the SecA translocase code in the mRNA: with SecA deficiency the arrest of the ribosome by SecM leads to unwinding of the mRNA hairpin hiding the SecA start codon and SecA synthesis. On the contrary, if SecA is abundant, the arrest of the ribosome by SecM is terminated by binding its N-terminus to the Sec translocon and the subsequent completion of SecM synthesis without initiating SecA translation. SecM is to contain the F_{150}XXXXWIXXXXGIRAGP$_{166}$ sequence to be able to arrest translation. Mechanism of SecM translation arrest was studied by MD simulations and cryoEM. Zhang et al. presented the structure of SecM complex with *E. coli* ribosome, in which the conformation of the critical GIRAG sequence contains two cis-peptide bonds. It is unlikely that anything in this region of the NPET is able to create such a conformation of the nascent peptide, although the published electron density indicates the general path of the peptide chain. Based on it, we modeled the structure of the SecM complex with the *E. coli* ribosome, which is characterized by stable interactions between the amino acid residues critical to SecM activity and NPET, such as A164 and R163 hydrogen bonds with U2585 and C2063 residues, hydrophobic contact of I162 and A2062 base, stacking interaction and hydrogen bonds of W155 with A789 residue. Thus, the structure simulated by us is consistent with the known experimental data. It can be assumed that these interactions enable SecM to bind strongly to NPET impeding translocation. But the external force pulling the peptide from the NPET is capable of breaking them, thereby translation arrest is reversible. All simulations were performed with the Lomonosov II supercomputer of Moscow State University using GROMACS 5 and PLUMED 2 packages and AMBER14SB force field.

P-02.1-06
Proteome-wide identification of proteoforms induced by adenosine-to-inosine mRNA editing in fruit fly, mouse and human brains

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Effects of A-to-I RNA editing by ADAR enzymes which leads to non-synonymous mRNA substitutions were identified proteome-wide using publicly available and in-house shotgun mass spectrometry proteomic data for fruit fly, murine and human brains. This type of RNA editing plays its primary role in immunity regulation, and adaptive significance of protein recoding due to this type of editing is still being discussed. Using a modified database for containing edited protein sites predicted from RNA-seq data, we have shown that edited forms are generally depleted in proteomes in comparison to genomically encoded sequences. Thus, of thousands non-synonymous edited sites in fruit fly, mouse and human transcriptomes, we identified 1–2% sites at the proteome level in each brain proteome. In the fruit fly brain, edited sites were shown to be enriched in proteins of SNARE presynaptic complex and other vesicle trafficking components (Kuznetsova et al. (2018) J Proteome Res 17, 3889–3903). Selected findings were confirmed by targeted mass-spectrometry which also showed the dynamics in editing of some protein sites during insect ontogeny. Re-analysis of deep proteomes of murine and human brains could identify as few as 20 and 37 editing sites in mouse and human, respectively, of them eight sites in six proteins were conservative between species (Levitsky et al. (2019) Proteomics 19, e1900195). These findings were in accordance with a previous art, where GRIA2-3 glutamate receptor subunits, COPA coatomer protein and FLNA filamin alpha were recognized as RNA editing targets. Of these extensively edited sites, a functional significance was only known for the GRIA2 glutamate receptor subunit in brain and, preliminary, for the filamin alpha in vascular tissues. Thus, we have identified RNA editing sites at the level of shotgun proteomes. Adaptive significance of these sites, as well as their possible role in animal and human pathology, should be further elucidated by functional studies.

P-02.1-07
Effect of temperature on heterologous expression of lectin CnSLB in minimal growth medium

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Lectins are proteins that specifically and reversibly bind carbohydrates without modifying them. CnSLB is a new lectin discovered in *Clitocybe nebularis* with interesting biochemical properties and insecticidal activity but currently unknown structure. The purpose of research was to yield enough labelled protein for studies of its three-dimensional structure. We used *Escherichia coli* BL21 (DE3) and *E. coli* BL21 (DE3) pTTRX cells as heterologous expression systems. Using autoinduction defined minimal growth medium and incubation at different temperatures (15°C, 18°C, 20°C and 22°C) we analyzed the solubility of the expressed proteins with polyacrylamide gel electrophoresis. As observed at all examined temperatures CnSLB was expressed as insoluble protein in inclusion bodies. The yield of CnSLB expression was proportional to increasing temperature, with 22°C as the highest observed yield. In conclusion, lectin CnSLB is in the bacterial expression system expressed in inclusion bodies regardless of the temperature in the process. The yield was, however, higher with higher temperature used. Therefore, to obtain the labelled protein for structural studies heterologous expression at higher temperatures (25°C and 27°C) will be performed to increase the yield even further and finally the refolding process will be developed.

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P-02.1-08
Introducing BglBrick gene assembly in lactic acid bacterium *Lactococcus lactis*

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Lactic acid bacteria (LAB) as cell factories are important hosts for production and delivery of biomolecules of interest. As they have considerable biotechnological potential and increasing interest, novel tools for engineering of complex phenotypes are in demand. A lot of new DNA assembly methods have been developed in the last two decades to facilitate and advance the gene construction. One of the introduced assembly standards is BglBrick, which emerged from optimisation of BioBrick™ assembly. It is a method that consists of iterative DNA digestion and ligation using two different restriction enzymes that generate compatible cohesive ends. These can be ligated, thereby generating a scar sequence in DNA that cannot be digested with either of previously used enzymes. BglBrick assembly was so far limited to *Escherichia coli*; however, in this study it was introduced to a model LAB *Lactococcus lactis*. We constructed a new plasmid pNBBX, on the basis of pNZ8148, that employs BglII and BclI restriction enzymes, which produce GATC sticky ends. After ligation, a TGATCT scar sequence is formed between each of the two consecutive cassettes. Altogether, our plasmids encode NheI-BglII-gene-BclI-XhoI cassettes. We applied three model proteins to test their concomitant expression in *L. lactis*, namely near-infrared fluorescent protein (iRFP), NanoLuc™ luciferase and and affibody with the affinity for human epidermal growth factor receptor 2. We aim to clone all three model protein-encoding genes in pNBBX plasmid by using BglBrick assembly, resulting in 6 different theoretical assembly possibilities. We will determine the quantity of the three expressed model proteins obtained with plasmids thus assembled. The ultimate goal of the research is to develop an alternative gene assembly option for *L. lactis*, thereby facilitating multiple protein expression.

P-02.1-09
Heterologous expression of fungal lectins in bacterial expression system

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Lectins are proteins which bind reversibly and specifically to carbohydrates. CnSL is a new fungal lectin with an unknown structure and unique characteristics. We assessed heterologous expression of the lectin in a bacterial expression system using a rich growth medium (RGM) and a defined minimal growth medium (MGM) with the goal to prepare a labelled protein for studies of its three-dimensional structure. Two variants of CnSL lectins (CnSLA and CnSLB) were expressed in *Escherichia coli* using the rich autoinduction medium RGM (3,5 h at 37°C, 19 h at 22°C). Alternatively, the CnSLB variant was expressed in the minimal autoinduction medium MGM (2 h at 37°C, 19 h at 22°C). Finally, we analysed the solubility of heterologously expressed CnSL with SDS-PAGE. Successful expression of CnSL in the bacterial expression system was confirmed in all cases. Lectin CnSLB expressed at a higher yield compared to CnSLA. There was no significant difference in protein expression between RGM and MGM, which is encouraging as we are required to use MGM for labelling of the lectin. Both lectin variants were expressed as insoluble inclusion bodies that were solubilized in 8 M urea. In conclusion, no significant difference was observed in heterologous protein expression between RGM and MGM. However, lectins CnSL form inclusion bodies and their purification will require optimization of the refolding process to obtain the proteins with correct tertiary structure. Furthermore, we will explore the possibility of their expression in soluble form in the bacterial periplasm.

P-02.1-10
The role of poly(A) sequence length in eukaryotic termination of translation

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There is a lot of evidence of the ability of 3' area below the stop codon, in particular, poly(A) tract, to influence translation termination efficiency. It was also shown that an approximation of poly(A) binding protein PABP and peptide release factor eRF3a leads to termination efficiency increase. Moreover, an amount of mRNA-connected PABP molecules is in direct dependency on the length of poly(A) tail. It was also demonstrated recently that poly(A) tail length affected efficiency of translation, but the data about termination dependency on poly(A) length is still poor. At the same time, investigation of this dependency is important, as about 10% of genetic diseases are caused by nonsense mutations, and the effect of poly(A) tract can modulate the cure. An appearance of small closed-loop mRNA structure as a result of interconnection between PABP and eRF3a was proposed. However, experimental evidences of such a structure are controversial. To reveal the mechanism of its formation, we used reconstituted system of eukaryotic translation. We obtained purified pretermination complexes on mRNAs with CDSs and poly(A) tracts of different length, containing PABP. Then we estimated the efficiency of translation and peptide release, as well as the efficiency of stop codon recognition by the release factor. We revealed that an increase of poly(A) length leads to stimulation of translation termination efficiency. The work was supported by the RSF grant № 19-74-10078.

P-02.1-11
Crystallization of chimeric proteins based on human glycyl-tRNA synthetase

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The translation initiation of entheroviral mRNA is occurred by cap-independent pathway, with the help of special structural elements – internal ribosome entry site (IRES). All enteroviruses have first type of IRES. At the moment, there is no complete understanding of the mechanisms of the IRES functioning. Moreover, not all protein factors participating in translation initiation on type I IRES have been discovered. It was shown recently that human glycyl-tRNA synthetase (GARS) is one of these factors (IRES trans-acting factors – ITAFs). It interacts with an apical part of the domain V of poliovirus IRES and

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stimulates its activity. We've obtained the anticodon-binding domain (ABD) of GARS in isolated form and showed its ability to specifically interact with IRES type I. However, working with isolated ABD is difficult because it's highly prone to aggregation and has low affinity to RNA for getting crystals of such complex. In order to solve this problem, we decided to create a chimeric protein, containing ABD (specific recognition) and some small RNA-binding protein (strong binding abilities). In theory, affinity of the chimeric protein to IRES should be higher than that of the intact protein. At present we have isolated, purified in a preparative scale and crystallized first chimeric protein. High-resolution diffraction data were collected at the ERSF in Grenoble. Currently, the structure determination and refinement is in progress. The results of our work will help to understand the mechanisms of viral initiation translation. This work was supported by the RFBR grant No. 19-34-90135

P-02.1-12
Reconstruction of lantibiotic gene cluster in methylotrophic yeasts *P. pastoris*

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The number of new cases of multidrug-resistant infections increases annually. Thereby, the discovery of new antibiotics becomes a high-priority challenge for biomedicine. Lantibiotics are ribosomally synthesized and post-translationally modified peptides that carry noncanonical thioether amino acids lanthionin and methyllanthionin. Lantibiotics mediate their antibacterial activity by blocking the cell wall synthesis or forming pores in a bacterial membrane. However, their clinical application is limited due to low stability and solubility, insufficient production levels and narrow activity spectra. Nevertheless, the genetically encoded architecture of lantibiotics allows the creation of various peptides that could be modified with lantibiotic biosynthesis machinery resulting in a more advanced antimicrobial drug. In this work, we focused on the development of a heterologous expression system for lantibiotic. The biosynthetic cluster composed of pre-lantibiotic sequence, methyltransferase and lantibiotic synthetase (LanL) was transferred into methylotrophic yeast *Pichia pastoris*. We found that particular pre-lantibiotic sequences were essential for efficient lantibiotic production. We substituted the original lantibiotic core sequence with a panel of natural lantibiotics to understand whether LanL is substrate-tolerant toward lantibiotics of different classes and architecture. We analyzed structures and antimicrobial activity of the purified lantibiotics comparing recombinant lantibiotics with native once. The recombinant lantibiotics displayed pronounced antibacterial activity against Gram-positive bacteria including pathogenic *S. aureus* MRSA strain. We consider our yeast-based system for lantibiotic production combined with gene-editing techniques and high-throughput screening methods could serve as a useful tool in the discovery of the next-generation antimicrobial agents. This work is supported by RFBR grant 18-29-08054.

P-02.1-13
High-level homogeneous production of fluorescent proteins in *E. coli* provides a sensitive reporter for antibiotic activity detection

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The uncontrolled use of antibiotics both in the medicinal practice and agriculture demands the development of highly sensitive probes for their preventive detection. Otherwise, this misuse results in the global spread of multidrug-resistant strains. That is particularly critical in the case of gram-negative bacteria which are intrinsically resistant to the majority of novel antimicrobial agents. Recently we presented a concept of deep functional profiling of microbiomes providing a new powerful tool for the detection of biological activity of even minor components of bacterial communities. This concept is based on a combination of microfluidic encapsulation of single cells in droplets of double emulsion (MDE) and fluorescence-activated cell sorting (FACS) isolating phenotype of interest. The stumbling block of this technology is the requirement of a particular fluorescent reporter indicating the desired activity. Moreover, highly sensitive detection of antibiotics in various environments is an urgent task resulted from their uncontrolled use in medicinal practice and agriculture. The present study is dedicated to the development of an *Escherichia coli* strain as a Gram-negative reporter. We have tested a number of common laboratory *E. coli* strains for optimal growth conditions, cell morphology, and culture homogeneity. Different bright GFP derivative genes regulated by various strong promoters were examined to gain high levels of production, signal intensity, and narrow fluorescence distribution. Bulk culture fluorescence measurements supplemented with fluorescent microscopy and flow cytometry allowed us to identify a combination of strain, gene, and promoter providing optimal biosensor parameters. We believe our results will be applied for various applications including food safety-related studies, antibiotic pollutant tracking, and antibiotic discovery. This work was supported by RSF grant 19-14-00331.

P-02.1-14
Role of the isoforms of human eukaryotic release factor 1 in termination of translation

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Eukaryotic release factor 1 (eRF1) is a protein that carries out translation termination in eukaryotes. In humans, eRF1 is encoded by the ETF1 gene located on chromosome 5. There are six known transcriptional variants (TV) of the ETF1 gene resulting from alternative splicing. TV1 (NM_004730.4) encodes the major isoform eRF1 – iso1. More than 95% of all ETF1 gene transcripts expressed in healthy tissues are TV1. Our analysis of the Ribo-Seq data of human cells and tissues showed that all the other TVs of the ETF1 gene encode an isoform shortened from the N-terminus by 33 amino acid residues – eRF1 iso2. In addition, Ribo-Seq data shows that transcript TV1 contains an upORF, from which a small peptide (35 amino acid residues) is

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translated. Interestingly, this peptide is encoded in the same frame as eRF1iso1, and their sequences are separated by the stop codon UAG and the "linker" GGGGEK. It is known that UAG can be suppressed by some tRNAs. Thus, with a certain probability, eRF1iso1 can be extended from the N-terminus (eRF1 long). In this study, we obtained recombinant eRF1 iso1 and eRF1 iso2, as well as a peptide with upORF and a hypothetical longer isoform of eRF1 and tested their translational activity in in vitro systems. Our data show that eRF1 iso2 recognizes stop codons but is unable to hydrolyze peptidyl-tRNA. eRF1 iso2 binds to eRF3, but the resulting complex is functionally inactive. The activity of longer eRF1 in translation termination is comparable to that of eRF1 iso1. A peptide with upORF in high concentrations suppresses translation termination. We assume that eRF1 isoforms are involved in the regulation of translation termination. This work is supported by the Russian Science Foundation grant № 19-14-00349.

P-02.1-15
Structural organization of 3′UTR modulates the activity of PABP in translation termination

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It is assumed that in the process of translation, an mRNA structure with closely spaced 5' and 3' ends (so-called the closed-loop mRNA) can be formed. It forms via interaction of initiation translation factor eIF4G and poly(A) binding protein (PABP). Additionally, it was suggested that stop codon and poly(A) tail are also closely located in this structure as PABP is able to interact both with eIF4G and release factor eRF3. It is confirmed by the fact that PABP activates both initiation and termination of translation. We proposed that structure of 3' untranslated region (3'UTR) can be critical for such activation, since the formation of hairpins in the 3'UTR can facilitate the physical convergence of the poly(A) tail and the stop codon. To reveal that, we determined the translation efficiency of mRNA with different structure of 3'UTR in the presence of PABP using *in vitro* reconstituted translation termination system. For this purpose, we used two alternative approaches of obtaining linear 3'UTR. First approach implied using long antisense oligonucleotide complementary to 3'UTR of mRNA. This oligonucleotide interacting with 3'UTR, prevented formation of hairpins in the 3'UTR. Second approach implies using repeated CA motive, unable to form secondary structure. As a result, we observed the dependence of the termination efficiency on the mRNA structure. In particular, when the stop codon is spatially distant from the poly(A) tail, PABP associated with poly(A) tail does not activate translation termination. Thus, the spatial coupling of stop codon and poly (A) tail, which may occur during the formation of a closed-loop structure, modulates the efficiency of in translation termination. The work was supported by Russian Science Foundation (grant No. 19-74-10078).

P-02.1-16
Phosphostate of the ribosomal P-proteins

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Protein phosphorylation is the most common post-translational modification (PTM) which regulates protein activity. It can be responsible for on/off switching of their activity, can change the interaction pattern of proteins or can modulate their subcellular localization or dynamics. Phosphorylation has been described for many ribosomal proteins (RPs) found both within the 40S (12 proteins) and 60S (15 proteins) subunits. The phosphorylation is a dynamic process that can appear in different cell conditions like in mitosis as well as in response to external stress stimuli, e.g. hypoxia. Growing evidence has shown an enormous potential of phosphorylation of ribosomal proteins as a regulatory mechanism turning the activity of the translational apparatus. For example, the recent report has revealed that translation can be regulated during mitosis by uL11 phosphorylation. Despite the advent of high-throughput analyses devoted to mapping PTM's of ribosomal proteins, the phosphostate and phosphorylation impact of ribosomal proteins are not described exhaustively. Among RPs that undergo phosphorylation are the P-stalk proteins, namely uL10 protein and P1-P2 proteins. They are responsible for the stimulation of translational GTPases activity during all stages of ribosomal action. Although it is known that the P-stalk proteins are phosphorylated by CK2 kinase. Here, using Pro-Q staining, the method which selectively detects protein phosphorylation, we show that many ribosomal proteins are phosphorylated. Moreover, to describe the detailed phosphostate of the P-stalk proteins in different conditions as well as in cell lines, we applied the Phos-tag technique. Our results indicate that the ribosomal P-proteins phosphostate is changing upon application of environment stimulus, which is consistent with the idea that they may provide another level of regulation for translational machinery.

P-02.1-17
Mechanism-based dual reporter screening system assists in identification of translational inhibitors

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In the last decade, many bacteria with outstanding resistance have been discovered and the already known pathogenic bacteria gained resistance genes. It caused a gradual decrease in the efficiency of the known antibiotics. According to a population-level modelling analysis in the European Union in 2015 it was estimated that 33000 deaths were caused by antimicrobial resistance. In our laboratory method, convenient for high throughput screening was

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invented. A broad-specificity system for detecting translation inhibitors is built upon the tryptophan attenuator trpL and Katushka2S fluorescent protein. DNA-damaging antibiotics can be detected by the expression of Red Fluorescent Protein (RFP) cloned under sulA promoter. Using this approach 50000 individual chemical compounds and 4000 cultural broth were tested. Among tested substances we have found chemicals that induce translational reporter system. Molecule «Y020-7126» from ChemDiv compound library inhibits translation in the non-cellular system and *in vivo* incorporation of C^{14}-valine in cells proteins, stops translation on the stage of elongation according to the toe-printing assay. Also, two cultural broth which contains inhibitors of translation were found—the compound auroplanin from a cultural broth of Actinoplanes sp. VKM Ac-2862. It has the empirical formula $C_{23}H_{25}NO_4$ and, according to the structure solved by Nuclear Magnetic Resonance, is a novel molecule. *E. coli* strains resistant to this active compound have mutations in 560 helices of 16S rRNA. These mutations were not described in the literature before. Another interesting broth sample of bacterium *Amycoloplasis* sp. This culture produces aromatic polyketide antibiotic tetracenomycin X (TcmX), which is a potent inhibitor of protein synthesis and does not induce DNA damage as previously thought. TcmX binds to a novel site within the polypeptide exit tunnel on the large subunit. The reported study was funded by RFBR according to the research project №20-34-90048.

P-02.1-18
Programmed cell death protein 4 affects translation termination and undergoes proteolysis in cell lysate

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Programmed cell death 4 (PDCD4) was originally identified as a gene whose expression was increased during apoptosis. The PDCD4 expression decrease accompanied the development of a number of malignant tumors, including lungs, colon, liver, breast cancer, and glioblastoma. PDCD4 encodes a highly conserved phosphoprotein, which subcellular localization (in the nucleus or cytoplasm) is controlled by the protein kinase. PDCD4 also affects two stages of translation: cap-dependent initiation and elongation of oncogenic mRNAs: c-myb and a-myb. PDCD4 prevents initiation by binding to eukaryotic initiation factor 4A via MA3 domains located in the middle and C-terminal part of the protein. Elongation of c-myb and a-myb is thought to be affected by the interaction of PDCD4 with the N-terminal domain of poly(A)-binding protein (PABP). We previously showed that PABP is able to stimulate translation termination by recruiting the release factor 3a (eRF3a). After that, we found that PDCD4 binds to the release factors independently of PABP and observed stimulation of termination activity by PDCD4. Thus, PDCD4 increased the termination efficiency by stabilizing termination complex formation and stimulating the GTPase activity of eRF3a. In addition, we found out that PDCD4 is proteolyzed in a cell-free translation system based on rabbit reticulocytes lysate. This fact suggests the presence of a mechanism for the PDCD4 regulation modulated by proteases. This study was supported by Russian Foundation for Basic Research № 19-34-90048. *The authors marked with an asterisk equally contributed to the work.

P-02.1-19
The role of glycogen synthase kinase-3 beta in the regulation of ribosome biogenesis in rat soleus muscle under hindlimb unloading

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It is well-established that mechanical unloading results in a significant reduction in the rate of muscle protein synthesis and subsequent fiber atrophy and loss of muscle mass. To date, new therapies are needed to tackle the problem associated with disuse-induced skeletal muscle atrophy. However, molecular mechanisms involved in the regulation of translational capacity under disuse conditions are poorly explored. Glycogen synthase kinase-3 beta (GSK-3beta), which is known to negatively regulate protein synthesis (PS), is activated in rat soleus muscle under unloading conditions. We hypothesized that inhibition of GSK-3-beta activity during hindlimb unloading (HU) would reduce unloading-induced downregulation of ribosome biogenesis in rat soleus muscle. Wistar rats were randomly divided into 3 groups: 1) vivarium control (C), 2) 7-day HU, 3) 7-day HU + daily injections (4 mg/kg) of AR-A014418 (GSK-3beta inhibitor). GSK-3-beta and glycogen synthase 1 (GS1) phosphorylation was measured by western-blotting. The key markers of ribosome biogenesis were assessed via agarose gel-electrophoresis and RT-PCR. As expected, 7-day HU resulted in a significant decrease in the inhibitory Ser9 GSK-3beta phosphorylation and an increase in GS1 (Ser641) phosphorylation compared to the C group. Treatment of rats with GSK-3beta inhibitor prevented HU-induced increase in GS1 (Ser641) phosphorylation which was indicative of GSK-3beta inhibition. Administration of GSK-3-beta inhibitor also prevented unloading-induced downregulation of c-Myc expression as well as decreases in the levels of 45S pre-rRNA and 18S+28S rRNAs. These AR-A014418-induced alterations in the markers of ribosome biogenesis were paralleled with partial prevention of a decrease in the rate of PS. Thus, inhibition of GSK-3beta during 7-day HU is able to attenuate a decrease in translational capacity and the rate of PS in rat soleus muscle. The study was supported by the Russian Science Foundation grant No. 17-75-20152.

P-02.1-20
ERA GTPase of *Staphylococcus aureus*: cloning, expression, purification and preparation for structural research

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ERA, is a widely conserved and essential GTPase in bacteria playing a significant role in the regulation of protein synthesis. In bacteria, ERA is required for the maturation of the 30S ribosomal subunit and coordination of cell growth and division cycle. However, the contribution of ERA to protein biosynthesis is not yet fully understood. The study of the effect of ERA on the bacterial protein-synthesizing apparatus will help in finding new targets in the fight against a number of human bacterial pathogens such as *Staphylococcus aureus*. In this study, we have cloned the era gene from the genome of *S. aureus* and obtained the construct expressing ERA with six histidine residues on C-terminus. His_6-ERA was expressed in *E. coli*, purified, and concentrated to 10 mg/ml for

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further search of crystallization conditions. Crystals of ERA in a complex with GppCp were obtained using the hanging-drop techniques. We also showed that ERA forms a stable complex with a 30S ribosome subunit of *S. aureus*, confirmed using agarose gel electrophoresis and PAGE. Further studies of ERA molecule crystal structure by X-ray diffraction and cryo-EM studies of ERA-30S complexes will prompt active sites responsible for interaction with ribosomal subunit. This work was supported by the Russian Science Foundation (grant 21-74-20034).

P-02.1-21
Looking for the brightest one: fluorescent protein-based approach for identifying optimal coding sequence for recombinant protein expression in *E. coli*

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Due to the degeneracy of the genetic code, most amino acids are encoded by several codons. Different synonymous codons at the 5'-end coding sequence of mRNA (5CDS) has strong effect on protein expression. This often explained by different contribution of synonymous codons to the minimal free energy (MFE) of the 5CDS which correlates with probability of mRNA secondary structure formation. Strong secondary structure in this region interferes with ribosome binding and affects the process of translation initiation. *In silico* optimization of 5CDS can significantly increase the level of protein expression. However, this method is not always effective due to the uncertainty of the exact mechanism by which synonymous substitutions affect expression, thus it may produce numerous of non-optimal variants. An alternative approach is the generation of partially-randomized library comprising hundreds of selected synonymous variants fused to reporter gene with subsequent screening for most promising candidates according to reporter's signal intensity. For this work as a model protein, we used canine cystatin C (CCC) which is known for low expression level in *E. coli*. Thus, a library of 5CDS-partially randomized CCC fusions to superfolderGFP (CCC::sfGFP) was created. Colonies with the highest expression level of CCC::sfGFP were selected based on fluorescence intensity. Then sfGFP coding sequence was removed from the fusion and expression level of obtained non-fused CCC variants was measured. As a result, several optimized CCC sequences with an expression level exceeding the original version by ~20 times were obtained. Analysis of 5CDS from several optimal and non-optimal variants revealed that variants with highest values of MFE were the ones with the highest CCC expression levels. We suggest that this simple approach may provide efficient and inexpensive optimization method for poorly expressed proteins in prokaryotic system.

P-02.1-22
Studies on the structure and biosynthesis of posttranslationally modified isoforms of the bacteriocin BacSp222

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BacSp222 is a linear, 50-amino acid long peptide produced by a zoonotic strain *Staphylococcus pseudintermedius* 222. BacSp222 serves both as a bacteriocin as well as a virulence factor: it kills Gram-positive bacteria and is cytotoxic as well as immunomodulatory to selected eukaryotic cells. (Previously published in: Wladyka B. et al. (2015) Sci. Rep. 5, 14569, Nowakowski M. et al. (2018) Int. J. Biol. Macromolecules 107, 2715–2724.) We have recently discovered that BacSp222 is produced in three different posttranslationally modified isoforms. In the present research, we focused on the mechanism of modification and on the influence of modifications on the structure and activity of BacSp222. The identity of posttranslational modifications was determined by Edman degradation and mass spectrometry, and these studies showed that BacSp222 is produced as an unmodified isoform i1, an isoform i2 containing one butanedioic (succinyl) group, and an isoform i3 with two butanedioic groups. These modifications occupy epsilon-amino groups of lysine residues. We demonstrated that succinylation is a nonenzymatic reaction - in contrast to desuccinylation, which is a NAD- and cytoplasmic enzymes-dependent process. The only physiological donor of the butanedioic group able to modify BacSp222 is succinyl-coenzyme A. The nuclear magnetic resonance and circular dichroism studies did not show any significant differences between the conformation of i1 and i2. On the other hand, the antibacterial activity of i2 and i3 is significantly lower than i1. The level of modified isoforms depends on environmental factors such as different sources of carbon, culture temperature, and pH of the medium. In sum, BacSp222 is the first described bacteriocin which has posttranslationally succinylated lysine residues. Our results suggest that these modifications protect producer cells against the autotoxicity of the excreted peptide. Funding: National Science Centre, Poland (grant No 2018/31/B/NZ3/01226).

P-02.1-23
Influence of mistranslation stress on oxidative stress response in bacteria *Escherichia coli*

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Mistakes during translation can occur due to inaccurate amino acid selection by aminoacyl-tRNA synthetases (aaRSs). Some aaRSs possess editing domain and hydrolyze incorrect aa-tRNA product. Although mistranslation is usually toxic due to production of functionally aberrant proteins, there are few examples of its adaptive effect on cellular responses to subsequent stresses. Research on organisms with editing-deficient aaRS and their response to oxidative stress is scarce. In our experiments, we used *Escherichia coli* strain expressing isoleucyl-tRNA synthetase with

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inactivated editing domain which produces mistranslated proteins if amino acids structurally similar to isoleucine, such as proteinogenic amino acid valine (Val) or nonproteinogenic amino acid norvaline (Nva), are added to the media. To observe the effect of mistranslation and subsequent oxidative stress on cell morphology, proliferation and viability, cells were observed under microscope, growth curves were determined, and survival assays were performed. Mistranslation was induced by overnight incubation of bacteria with various concentrations of Val or Nva (0.25, 0.5, 0.75 and 1 mM), and cells were then exposed to oxidative stress by adding 1 mM H_2O_2. Prominent filamentation of the cells was observed with cell size increasing in Val or Nva concentration-dependent manner. Survival assays indicated increased survival of bacteria grown with 0.75 and 1 mM Val or Nva in the presence of H_2O_2. Growth curve measurements showed that after induction of oxidative stress, lag phase was shorter for cultures grown with 1 mM Val or 0.75 mM Nva. The results indicate that there is mistranslation-induced preadaptation to oxidative stress, albeit only in a narrow range of Val and Nva concentrations. Further work will be focused on identification of cellular mechanisms that allow better survival under oxidative stress due to misincorporation of valine or norvaline at isoleucine positions in proteins.

P-02.1-24
Cloning and heterologous expression of *Candida albicans* SC5314 deoxyhypusine hydrolase gene in *E. coli*

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Candida albicans (*C. albicans*) is a fungal pathogen that causes mucosal and systemic infections in immunocompromised people. Finding both selective and less toxic treatment against *C. albicans* is required and could be achieved by the regulation of transcription and translation [2]. Hypusination is a post-translational modification of eukaryotic translational factor 5a (eIF5A) in which unusual amino acid, hypusine is formed from a specific lysine residue [3]. This modification involves two enzymes, deoxyhypusine synthase (DHS) and deoxyhypusine hydroxylase (DOHH). At the first stage of hypusination, the aminobutyl fragment of spermidine is transferred to the ε-amino group of lysine by DHS and forms deoxyhypusine, which is then hydroxylated with DOHH to hypusine [1], [3]. This paper is about cloning and expression of DOHH gene in *E. coli* to obtain protein for biochemical study and crystallization. DOHH gene (Genbank KHC88093.1) was amplified from isolated total *Candida albicans* DNA by PCR using Tersus polymerase and specific primers (Evrogen, Russia). The resulting gene was ligated with the pETGB1a plasmid vector, and transformed into *E. coli* DH5a cells. Plasmid DNA (pETGB1a: dohh) was isolated and was further transformed into *E. coli* BL21 for expression, in LB medium at 37°C. Protein synthesis was induced by adding isopropyl-β-D-1-thiogalactopyranoside (IPTG) to a final 0.5 mM, at 18°C overnight. BL21 cells were pelleted, resuspended, and lysed. The lysate was centrifuged at 45000 rpm for 45 min. Protein 6xHis-GB1-CaDOHH, was purified by Ni-NTA agarose chromatography. The obtained protein was further purified to CaDOHH 43 kDa. DOHH catalyzes the last hypusination reaction that leads to the activation of eIF5A in all eukaryotes. The resulting construct and the developed protocol for CaDOHH isolation from E. coli make it possible to obtain a purified protein for structural studies. This work was supported by the Russian Science Foundation grant 20-65-47031

P-02.1-25
Aberrant protein N-terminal acetylation as a cause for congenital disease

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The major N-terminal (Nt) acetyltransferase NatA co-translationally acetylates nearly half of all human proteins at their N-termini. NatA consists of two core subunits; the catalytic subunit NAA10 and auxiliary subunit NAA15. In addition to NAA10's evolutionarily conserved role as part of the NatA complex, there also exists a cellular population of monomeric NAA10. Monomeric NAA10 has been reported to regulate proteins both through lysine acetylation and in an acetyltransferase-independent manner. A variety of hereditary and de novo missense variants in the X-linked NAA10 gene have been found to cause congenital disease in humans. Affected individuals display phenotypic heterogeneity, but phenotypes often include variable degrees of intellectual disability, developmental delay and cardiac anomalies. Importantly, the underlying disease mechanisms associated with pathogenic NAA10 variants are still poorly understood. In this project, we have investigated the biochemical profiles of novel and known NAA10 missense variants through immunoprecipitation and in vitro acetylation assays. While some missense variants appear to affect overall protein stability, some impair NAA10-NAA15 NatA complex formation and NatA mediated Nt-acetylation, and others appear to reduce monomeric NAA10 acetylation. Thus, different NAA10 missense variants are likely to affect different roles of the multifunctional NAA10 protein which may explain the heterogenous disease manifestations in affected individuals. Previously published in: McTiernan N et al. (2020) Int J Mol Sci 21, 8973. McTiernan N et al. (2020) Eur J Hum Genet 29, 280–288. Bader I et al. (2020) BMC Med Genet 21, 153.

P-02.1-26
Blocking IF3 N-terminal domain delays 30S initiation complex formation in bacterial protein synthesis

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Bacterial translation initiation factor IF3 has an essential role in 30S initiation complex (IC) formation. In the initiation phase,

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IF3 binds to the 30S ribosomal subunit rapidly and modulates the fidelity and speed of the codon-anticodon interaction between the mRNA and initiator tRNA. In canonical conditions, most of the IF3 functions are granted to the C-terminal domain, while the N terminal domain (IF3N) functions remain in debate. Here, we developed an aptamer for IF3N using the Systematic Evolution of Ligands by EXponential enrichment (SELEX) methodology and utilized biophysical methods to study aptamer-mediated blocking of IF3N during 30S IC assembly. Five potential aptamers were identified using purified IF3N from *Escherichia coli* as a target. Pull-down and Microscale Thermophoresis (MST) assays were used to identify Apt^{343} as an aptamer candidate that specifically binds to IF3N with a $K_d = 205 \pm 164$ nM. Rapid kinetics coupled to intramolecular Förster Resonance Energy Transfer (rkFRET) showed that Apt^{343} binds to a double-labeled IF3 drastically reducing by 13-fold the speed of IF3 binding to the 30S subunit. Additionally, the aptamer affected IF1- and IF2-depended conformational accommodations of IF3 on the 30S subunit. Furthermore, the canonical codon-anticodon duplex formation appeared to be delayed by Apt343. However, this delay does not affect the 50S joining reaction upon 70S IC formation. Altogether, our results suggest that IF3N positioning contributes to the accommodation of initiator tRNA and therefore, with canonical 30S IC formation. Additionally, the experimental scheme presented here provides a solid alternative for the development of new inhibitors of bacterial translation.

P-02.1-27
Amino acid substitutions in translation termination factor eRF3 of yeast *Saccharomyces cerevisiae* that lead to decreased GTPase activity

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The final step in protein synthesis, translation termination, occurs when one of three termination codons (UAA, UGA, or UAG) in mRNA reaches the ribosomal A-site. In eukaryotes, translation termination requires two factors: eRF1 (eukaryotic release factor) and eRF3. The eRF1 protein belongs to class 1 release factors, responsible for the recognition of the stop codon and peptidyl-tRNA hydrolysis, and eRF3 – to class 2 release factors, functioning to stimulate the work of class 1 factors due to its GTPase activity. In the yeast *S. cerevisiae*, release factors are encoded by the SUP45 and SUP35 genes. Factor eRF3 of *S. cerevisiae* (Sup35p) consists of three domains. C-domain is essential for translation termination and possesses GTP- and eRF1-binding sites. N-domain is responsible for Sup35p aggregation and $[PSI^+]$ prion formation. M-domain is involved in the maintenance of $[PSI^+]$. Both sup35 mutations and $[PSI^+]$ cause reduction of the translation termination fidelity and lead to the nonsense suppression. It was shown that sup35 mutations affecting the N-domain have an influence on $[PSI^+]$ appearance and maintenance. In this work, we have studied three sup35-m (missense) mutations located inside the C-domain of Sup35p. We reconstituted *in vitro* eukaryotic translation termination using purified ribosomal subunits, termination factors eRF1 and C-domain of eRF3 (wild type or mutant variants). It has been shown that amino acid substitutions in translation termination factor eRF3 of yeast *S. cerevisiae* lead to decreased GTPase activity. It is possible that these mutations disrupt the GTPase activity of eRF3, resulting in suppression and reduction of the translation termination fidelity. This work was supported by RSF grant 18-14-00050 "Genetic and epigenetic regulation of translation termination", RFBR grant 19-04-00173 and the State Research Program 0112-2016-0015. Part of experimental work was done in the resource centre of SPBU "Centre for Molecular and Cell Technologies".

P-02.1-28
Design of RNA polymerases recognizing synthetic nucleotides

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Nowadays, advances in gene modification and viral therapy have led to the development of various viral vectors, capable to replicate and express therapeutic proteins in specific tissues and even tumor cells. However, viral therapy still has some limitations due to the viral nature of genetic material delivery. First of all, the existing limitations are associated with the risk of uncontrolled viral replication and premature expression of therapeutic proteins. We have proposed a system that will include a reporter gene, carrying artificial nucleotides, and a mutant T7 DNA-dependent RNA polymerase capable of reading artificial nucleotides in genes and transcribing them into natural mRNA. Such a genetic construction will not be able to replicate, and the genes in it will not be transcribed by cellular DNA and RNA polymerases. Thus, its existence will be completely controlled. To choose positions for site-directed mutagenesis, we carried out molecular modeling, in particular, docking of DNA templates containing artificial nucleotides in the active site of T7 RNA polymerase. The ligand was a sequence consisting of natural and unnatural nucleotides. Having used molecular docking, we obtained the affinity of the oligonucleotide ligand with T7 RNA polymerases carrying various amino acid substitutions. Selected candidates were further tested in the proposed new method based on a double coupled (coupled2) cell-free transcription-translation system, in which we determine the activity of mutant T7 RNA polymerases by the luminescence of synthesized nanoluciferase (Nluc). As a result, we were able to evaluate the efficiency of transcription of unnatural templates with different mutants of T7 RNA polymerases. We have shown the high sensitivity and simplicity of the developed method, and also demonstrated the ability of the studied mutants of T7 RNA polymerase to recognize UBPs. Funding: This research was funded by RFBR, grant number 18-29-08044. *The authors marked with an asterisk equally contributed to the work.

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P-02.1-29
Modulation of eukaryotic release factors activity by eIF3j

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Translation termination has long been considered the shortest stage of protein biosynthesis, in which only two protein factors, eRF1 and eRF3, are involved. It turned out that in addition to the two main proteins, additional proteins can take part in translation termination. One such protein is human eIF3j. eIF3j is known as the labile subunit of eukaryotic translation initiation factor eIF3. The yeast homolog of this protein, HCR1, has been reported to participate in controlling the translation termination and stop codon readthrough. Also, it was shown that HCR1 functions in the recycling of the 60S ribosomal subunit *in vivo*. We revealed role of human eIF3j in translation termination using a reconstituted mammalian in vitro translation system. We showed that eIF3j stimulates peptidyl-tRNA hydrolysis induced by a complex of release factors eRF1–eRF3. Using toe-printing assay, we determined that eIF3j improves the efficiency of stop codon recognition by release factors. Experiments on the binding of eIF3j with eRF1, eRF3 and ribosomal complexes confirmed its direct involvement in translation termination. Moreover, we found that eIF3j could interact with eRF3 in solution. Thus, we have shown that the human translation initiation factor eIF3j, like its yeast homologue, is involved in the regulation of translation termination. This work is supported by the Russian Science Foundation grant no. 19-14-00349.

P-02.1-30
Possible role of mTORC1 in the regulation of ribosome biogenesis in rat soleus muscle at the initial stage of mechanical unloading

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It is well known that disuse-induced muscle atrophy caused by immobilization or real/simulated microgravity is associated with a downregulation of protein synthesis and a corresponding loss in muscle mass and size. The rate of protein synthesis in skeletal muscles is determined by both protein synthesis per unit RNA (translational efficiency) and the amount of ribosomes per unit tissue (translational capacity). mTORC1 is considered to be one of the key regulators of both abovementioned factors. Therefore, using a specific mTORC1 inhibitor (rapamycin) we aimed to determine to what extent mTORC1 would influence ribosome biogenesis in rat soleus muscle at the early stage of mechanical unloading (24 h). Wistar rats were divided into the 4 groups: vivarium control + saline injection (C); vivarium control + rapamycin injection (400 mg/kg) (CR); 1-day hindlimb suspension (HS) + saline injection (HS); 1-day HS + rapamycin injection (400 mg/kg) (HSR). The expression levels of both c-Myc and 45S pre-rRNA were assessed by RT-PCR. 18S rRNA and 28S rRNA contents were determined by 1.2%-agarose gel electrophoresis. RT-PCR analysis revealed that c-Myc mRNA expression did not change in the CR group compared with the C group, but declined by almost 50% ($P < 0.05$) in the HS and HSR groups relative to the C group. The expression levels of 45S pre-rRNA showed an 80% ($P < 0.05$) increase in the CR group vs. the C group, and almost a 30% decrease in the HS group and a 50% ($P < 0.05$) increase in the HSR group in comparison with the C group. The pattern of changes between the groups for the content of 18S and 28S rRNAs was similar to that observed for 45S pre-rRNA expression. Thus, the results of our study suggest that at the initial stage of mechanical unloading of the rat soleus muscle (24 h) the expression of 45S pre-rRNA, but not c-Myc expression, is apparently dependent on the activity of mTORC1. The study was supported by the Russian Science Foundation (project No. 17-75-20152). *The authors marked with an asterisk equally contributed to the work.

P-02.1-31
Tissue-specific landscape of translation machinery components

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Protein synthesis is vital for the majority of cell types. Diversely differentiated cells require specific translation regulation, which suggests specialization of translation machinery across tissues and organs. Using data from GTEx, FANTOM, and Gene Atlas we systematically explored the abundance of transcripts coding for translation factors and aminoacyl-tRNA synthetases (ARSases) in normal and cancer human tissues. We recovered a few known and identified several novel cases of a strict tissue-specific expression of particular transcripts. Among those there were eEF1A1, eEF1A2, PABPC1L, PABPC3, eIF1B, eIF4E1B, eIF4ENIF1, and eIF5AL1. Furthermore, our analysis revealed pervasive tissue-specificity of relative abundance of transcripts encoding components of the translation machinery (e.g. paralogs of PABP, eRF3, and eIF5MP, as well as eIF2B subunits and some ARSases), suggesting presumptive variance in composition of translation initiation, elongation, and termination complexes. These conclusions were largely confirmed by the analysis of proteomic data. Finally, we paid attention to a sex-specific difference in the repertoire of translation factors encoded in sex chromosomes (eIF1A, eIF2γ, and DDX3) and identified ovary, testis, and brain as organs with the most diverged expression patterns. N.M.K., A.A.E., and S.E.D. are part of the Interdisciplinary Scientific and Educational School of Moscow University «Molecular Technologies of the Living Systems and Synthetic Biology». The work was supported by the Russian Science Foundation (grant no. 18-14-00291 to S.E.D.). *The authors marked with an asterisk equally contributed to the work.

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Autophagy and protein recycling

P-02.2-01
Autophagy is involved in lipid droplet breakdown in serum-starved breast cancer cells

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Lipid droplets (LDs) are dynamic fat storage organelles present in most eukaryotic cells and involved in the regulation of lipid metabolism, cell signaling and protein trafficking. LDs accumulate in cells deprived of oxygen and nutrients, indicating their involvement in the cellular stress response. Recent studies have shown that LDs are engaged in a complex relationship with autophagy, the major cellular recycling and stress response pathway. Autophagy may participate in both LD biogenesis and breakdown, whereas LDs may provide essential lipids for the initiation of autophagy. It is not yet clear how this complex interplay is regulated and which molecular pathways are responsible for the execution of the multiple possible outcomes. These questions are particularly important in cancer cells, which are often exposed to nutrient and oxygen fluctuations and use both autophagy and LDs for protection against metabolic stress. In this work, we aim to determine if and how autophagy and LDs cooperate in the protection of breast cancer cells against starvation. We found that LD biogenesis is upregulated during severe amino acid deprivation in Hanks' balanced salt solution, whereas milder starvation in the absence of serum induces LD breakdown. The latter was dependent on cytosolic lipolysis mediated by adipose triglyceride lipase (ATGL). Intriguingly, autophagy was active during both severe and mild starvation. Using live-cell confocal imaging we show that autophagosomal and lysosomal structures colocalize with LDs during mild, but not during severe starvation, suggesting the involvement of "lipophagy", an LD-selective form of autophagy, in starvation-induced breakdown of LDs. In accordance, inhibition of autophagic flux with bafilomycin A1 or chloroquine further elevated the colocalization between LDs and autophagosomes. These results suggest that autophagy is active during mild starvation and is involved in the breakdown of LDs in aggressive breast cancer cells.

P-02.2-02
Degradation of autophagic bodies in sugar-starved lupin embryo axes: a transcriptomic and proteomic approach

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The degradation of autophagic bodies is one of the final stages of autophagy. Autophagic bodies are spherical structures formed in the vacuole of yeasts and plants. The newly formed autophagic bodies are rapidly degraded by vacuolar lytic enzymes. Based on our results, autophagy is significantly enhanced by sugar starvation in cells of lupin embryo axes but asparagine (a central amino acid in lupin seed metabolism) causes a clear inhibition of autophagic bodies degradation. Trying to describe the role of asparagine in a mechanism of autophagic body degradation, we performed transcriptomic and proteomic analyses of lupin embryo axes. The experiments were performed on embryo axes isolated from imbibed seeds of white and Andean lupin. Embryo axes were cultured in vitro for 96 h on a mineral medium supplemented with 60 mM sucrose, without the sugar, and on both the media mentioned above enriched in 35 mM asparagine. The quality of the libraries was verified by Sanger sequencing method, and the large-scale transcriptomic sequencing using Illumina HiSeq Next Generation Sequencing technology (NGS) was performed. The obtained sequence reads were aligned to reference transcriptome and counted in the aim to find differentially expressed genes. As transcriptome modulation could be manifested in proteomic changes, isobaric tags for relative and absolute quantitation (iTRAQ)-based proteomics was performed to screen the differentially expressed proteins. Our goal was to analyze the effect of asparagine on the expression of genes and accumulations of proteins involved in autophagy. First of all, we focused on changes in the level of transcripts of genes coding for ATG proteins and genes coding for vacuolar lytic enzymes (e.g., proteases) as well as on the changes in the accumulation of appropriate proteins. This work was financed by the National Science Centre, Poland (Grant no. 2016/23/B/NZ3/00735) and European Social Funds (Grant no. POWR.03.02.00-00-I006/17-00). *The authors marked with an asterisk equally contributed to the work.

P-02.2-03
Drosophila Sec20 regulates autophagy and endocytosis independently of the Golgi-to-ER retrograde transport

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Sec20/BNIP1 (BCL2/adenovirus E1B 19 kDa protein-interacting protein 1) is a SNARE protein first described in yeast as an endoplasmic reticular SNARE. It mediates the fusion between the ER and retrograde vesicles from the Golgi. Other studies have also suggested that it may have a role in many other cellular functions including autophagy, ER homeostasis and apoptosis. As the role of Sec20 in autophagy (and other lysosomal pathways) has yet to be elucidated we used *Drosophila melanogaster* as an *in vivo* model organism. Starved larval fat cells and garland nephrocytes were used to detect any defects in autophagy or endocytosis, respectively. We found that Sec20 and its partner Syx18 not only regulates autophagy, but it surprisingly has a role in endocytic transport as well. Ultrastructural analyses revealed abnormal lysosomal compartment and dilated ER in both fat cells and nephrocytes. Moreover, the latter contained enlarged late endosomes and was nearly devoid of endolysosomes. As we found that depletion of other SNAREs or tethering proteins (previously shown to regulate Golgi-to-ER retrograde traffic) did not cause any defect in either autophagy or endocytosis, we suggest that Sec20 has a novel role in lysosomal degradation that seems to be independent of Golgi-to-ER transport.

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P-02.2-04
A dual role of autophagy in the anticancer therapy

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In the development of cancer, autophagy demonstrates a dual role. It can both inhibit disease progression as well as protect cells and stimulate tumour growth. Many studies indicate that the main mechanism of cytotoxic action of cytostatic is induction of apoptosis process. However, the initiation of this process is often inhibited by triggering the process of autophagy – in this way the damaged organelles can be removed and the cell death induction inhibited. Based on these opposing properties of autophagy, attempts to inhibit or activate the process can be effective strategies in the fight against cancer. In the present study, it has been analysed whether the process of apoptosis will be induced by inhibiting autophagy in breast cancer cells MCF-7 and MCF-7/Dox using doxorubicin and its two nanoforms (N-DOX/ N-DOX-gh625). In order to determine the intensity of the autophagy process, a staining technique with monodansylcadaverine was used, whereas in order to analyse pro-apoptotic properties of the analysed compounds, the activity of caspase 3 was measured. The research showed that the drugs in question simultaneously trigger apoptosis and autophagy in cells. NH4Cl was used in the study and it was shown that this compound can inhibit autophagy. Ammonium chloride, which is a weak alkali can accumulate inside lysosomes, increase pH and inactivate hydrolyses. Ammonium chloride inhibited autophagy in MCF-7 and MCF-7/Dox cells. Interestingly, the process of autophagy was most effective after 48 hours of post-incubation culture, both in cells treated with doxorubicin and its two nanoforms, despite the use of antioxidants (NAC, Tiron, Trolox) in the cell were generated damage. This shows that the damage caused by doxorubicin and its two nanoforms was removed from the cell. By removing the damaged cellular organelles, which can be a source of free radicals, autophagy prevented the signals necessary to start the apoptosis process. *The authors marked with an asterisk equally contributed to the work.

P-02.2-05
Super-resolution imaging of macroautophagy in astrocytes

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Autophagy is a regulated pathway ending in lysosomal degradation of intracellular material, including organelles. It maintains cell homeostasis and enables cell survival under stress. Astrocytes are a type of glial cells, providing homeostatic support in the brain. They are essential for neuron function and also participate in synaptic signalling. However, the exact role of autophagy in astrocytes in different conditions, including reactive gliosis and viral infections are not known. Studying macroautophagy is challenging because it is a multistep process, involving structures with dimensions below the optical resolution. Plasmid constructs that encode fluorescent-tagged LC3 (microtubule-associated protein 1A/1B-light chain 3), a marker of autophagosomal membranes, have been successfully used to distinguish autophagosomes and autolysosomes in single cells. In our experiments we tested the efficacy of plasmid construct encoding LC3 in evaluating autophagic activity of murine astrocytes. To discern the autophagic intermediates with dimensions below limits imposed by diffraction, we have used super-resolution structured illumination microscopy (SIM). Therefore, murine astrocytes in culture were transfected with LC3-coding plasmid constructs and treated with known autophagy inhibitors (putatively affecting different stages of autophagy) and/or activators. Astrocytes expressing the constructs were examined with SIM. To estimate autophagic activity, we determined the total number of autophagic compartments and the fractions of autophagosomes and autolysosomes in single astrocytes. The results show that astrocytes expressing fluorescent-tagged LC3 have responded in expected ways to known autophagy activators and inhibitors. We conclude that murine astrocytes expressing fluorescent-tagged LC3 in combination with SIM, represent a suitable approach for the future studies of autophagic activity in reactive astrocytes and in the presence of different flaviviruses.

P-02.2-06
Autophagy contributes to the ethanol response in the *Saccharomyces cerevisiae* cells

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Baker's yeast *Saccharomyces cerevisiae* is the valuable ethanol producer important for biofuel manufacturing. During fermentation, yeast cells cope with several stresses induced by high ethanol concentration. Among these are a disturbance in membrane integrity, oxidative stress, and proteotoxic stress caused by intracellular protein aggregation. The ubiquitin-proteasome system (UPS) and autophagy are the main proteolytic systems that degrade intracellular protein waste. How crosstalk between these systems helps the cells to tolerate ethanol-induced stresses is not fully understood. We have shown that impaired proteasome function sensitizes yeast to high ethanol, isopropanol, and butanol concentrations. Surprisingly, the proteome analysis showed that yeast proteasomal subunits are not upregulated upon short-term ethanol treatment. However, the UPS and autophagy protein substrates' level is decreased in the mutant strain with the impaired Rpn4-dependent regulation of the 20S proteasomal subunit Pre1 (YPL), while less significant changes are observed for the RPN4 deletion mutant (rpn4-Δ) compared with the wild-type strain. Next, ethanol stress induces autophagy hyperactivation in the YPL strain, while no autophagy hyperactivation is seen in the rpn4-Δ strain compared with wild-type. Accordingly, the expression of important autophagy genes ATG7 and PRB1 is induced in YPL upon ethanol stress. PRB1 as an Rpn4 target is overexpressed due to Rpn4 stabilization in the YPL. Deletion or CRISPR/dCas9-mediated repression of Rpn4-dependent regulation of the PRB1 renders yeast cells more sensitive to ethanol than wild-type. We conclude that both ubiquitin-proteasome and autophagy systems are required for ethanol resistance in yeast cells. The compensatory

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autophagy induction upon proteasome dysfunction partially depends on the Rpn4. This work is supported by the Russian Science Foundation grant no. 17-74-30030.

P-02.2-07
EVOO polyphenols can relieve autophagy dysregulation in Alzheimer's disease

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Autophagy is a key process involved in the control of cell proteostasis, in the regulation of lipid metabolism and organelle turnover and in the clearance of materials of endogenous or exogenous origin. Autophagy efficiency declines with age, leading to accumulation of harmful protein aggregates and damaged mitochondria, with increased ROS production. These modifications contribute to several pathophysiological conditions, including Alzheimer's disease (AD). Indeed, several studies have reported that the maturation of autophagolysosomes and the inhibition of their retrograde transport creates favourable conditions for the accumulation of the Aß peptide, whose aggregation into extracellular plaques is considered a key responsible of neuronal damage in AD. Recent data have shown that oleuropein aglycone (OleA), a key component of olive oil, interferes with Aß aggregation, stimulates cell defences against plaque-induced neurodegeneration and triggers autophagy. After ingestion, OleA is metabolized to hydroxytyrosol (HT), the most powerful antioxidant compound in the olive tree. Based on these premises, we aimed to investigate the molecular mechanisms involved in autophagy activation by a mixture of OleA and HT. Therefore, we performed a set of in vitro experiments to extend and to deepen the knowledge on the molecular determinants of the beneficial properties of olive polyphenols. Our results show that a mix of OleA/HT activates the autophagic pathway more than the same amounts (in molar terms) of OleA or HT taken alone. Moreover, a reduction of ROS production with a significant recovery of cell viability was observed in cells exposed to toxic Aß oligomers following treatment with the mixture. These studies extend previous data and provide the rationale to consider these molecules as promising candidates for prevention and long-term nutraceutical treatment of neurodegeneration or as molecular scaffolds for further pharmacological development.

P-02.2-08
Cellular studies of the two main isoforms of human D-aspartate oxidase

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Human D-aspartate oxidase (hDASPO, EC 1.4.3.1) is a peroxisomal flavoenzyme that selectively degrades the D-enantiomers of acidic amino acids and is the only enzyme known to degrade D-aspartate (D-Asp). In mammals, D-Asp is present in the central nervous system, where it acts as a signaling molecule and is involved in neural development, brain morphology and behavior [1]. Studies performed in animal models (DASPO$^{-/-}$ knock out mice) demonstrated that, by regulating D-Asp concentration in the brain, DASPO impacts on glutamatergic neurotransmission, thus preventing precocious age-related deterioration processes [2]. The UniProtKB database reports three hDASPO isoforms, constituted by 369, 341 and 282 amino acids. To date the different isoforms have only been partially characterized and notably the properties of the longest putative isoform have never been studied. Here, we identified the additional N-terminal peptide of the hDASPO_369 isoform only in the hippocampus of female Alzheimer's disease (AD) patients, while peptides common to hDASPO_369 and hDASPO_341 isoforms were present in samples from both male and female healthy controls and AD patients. Unfortunately, the hDASPO_369 isoform was largely produced in E. coli as inclusion bodies, thus hampering its biochemical characterization. However, the functional properties, the degradation kinetics and the mechanisms involved in cellular turnover of hDASPO_341 and hDASPO_369 were investigated by ectopically expressing these isoforms in the U87 human glioblastoma cell line. This study demonstrated that both protein isoforms are active (showing similar kinetic properties), localize to the peroxisomes, are very stable (with an estimated half-life of approximately 100 hours) and are primarily degraded through the ubiquitin-proteasome system [3]. References: [1] Usiello A et al. (2020) Int J Mol Sci 21, 8718. [2] Cristino L et al. (2015) Neurobiol Aging 36, 1890-1902. [3] Rabattoni V et al. (2021) FEBS J, doi: 10.1111/febs.15797

P-02.2-09
Correlation between the modified expression of autophagy and endoplasmic reticulum stress markers and the continuous inflammation of the colon in ulcerative colitis

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Ulcerative colitis (UC) is characterized by an unknown cause of chronic inflammation of the colon. Among the reasons that could trigger inflammation, an impaired autophagy process could further affect the endoplasmic reticulum stress (ERS) response, hence leading to unfolded protein response activation. The aim of this study was to evaluate gene and protein expression of specific autophagy and ERS markers, in order to correlate their level of expression with their possible implication in the inflammatory state of UC. In this study we used colon tissue samples isolated from 4 mice conditions: BALB/C, 2,4,6-trinitrobenzenesulfonic acid (TNBS)-induced UC BALB/C, STAT6 knockout and TNBS-induced UC STAT6 knockout. For gene expression evaluation, quantitative Real Time PCR was performed using primers for key autophagy and ERS genes, whereas protein expression evaluation was possible by immunohistochemistry, with specific autophagy and ERS antibodies. When analyzing gene expression of autophagy markers, we identified an increased SQSTM1 and ATG16L1 expression, but an inhibited LC3-II expression in TNBS-induced UC BALB/C mice when compared to BALB/C. Similar results were obtained for TNBS-induced colitis STAT6 mice, but with an even more exacerbated expression of SQSTM1 and ATG16L1 than in the case of TNBS-induced UC BALC/C. XBP-1s, eIF2α and ATF6α ERS markers investigation displayed an increased level of expression in colitis BALB/C mice in comparison to control group. Colitis STAT6 mice displayed higher values for ERS gene expression when compared to colitis BALB/C mice. Gene expression results were confirmed by

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protein expression antibodies staining. Our results indicated a modified expression of key intestinal homeostasis markers, which leads to epithelial barrier dysfunction, hence supporting the idea of an aggravated UC in STAT6 knockout mice. This work was supported by UEFISCDI PN-III-P1-1.2-PCCDI-2017-0407/ INTELMAT.

P-02.2-10
Targeting autophagy via AMPK/ULK1/2 pathway inhibition affects survival and therapeutic susceptibility of chronic lymphocytic leukemia cells

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Chronic lymphocytic leukemia (CLL) is the most common hematologic malignancy in the Western world and is characterized by a progressive accumulation of mature-appearing CD5+, CD19+ B lymphocytes in the blood, lymph nodes, and bone marrow. We set out to investigate the role of autophagy in the survival and therapeutic susceptibility of CLL cells. The study was approved by Slovenian National Medical Ethics Committee; 0120-136/ 2019/46. Six different pharmacologic modulators of distinct targets in the autophagic process were evaluated on patient-derived CLL cells (n=17). Of the tested, AMPK inhibitor (dorsomorphin), ULK1/2 inhibitor (MRT68921), and autophagosome-lysosome fusion inhibitor (chloroquine) demonstrated the most prominent concentration and time-dependent cytotoxic effects against CLL cells. The mean EC50 values of MRT68921, dorsomorphin, and chloroquine after 24 hours were 3 μM, 5 μM, and 26 μM, respectively. Comparing the effects on CLL cells and PBMCs revealed that MRT68921, dorsomorphin, and chloroquine act selectively cytotoxic against malignant B-lymphocytes. However, only MRT68921 and dorsomorphin acted against CLL cells in low micromolar concentrations and through a caspase-dependent mechanism. Next, we inspected the potential of combining AMPK/ULK1/2 inhibitors with contemporary targeted therapy. MRT68921 and dorsomorphin augmented the action of targeted therapeutics ibrutinib, idelalisib, and venetoclax in patient-derived CLL cells. Moreover, MRT68921 alone induced cell cycle arrest at the G2/M phase, while in combination with ibrutinib and venetoclax it acted synergistically cytotoxic through a caspase-dependent mechanism. To conclude, we have demonstrated the antileukemic potential of targeting autophagy via AMPK/ULK1/2 pathway inhibition both alone and in conjunction with targeted therapy and thus provided the rationale for further investigation of autophagy as a target in CLL.

Proteolytic processing

P-02.3-01
The study of the main porcine muscle proteins and its modifications

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In recent years, the volume and scale of meat production is in trend to be arisen. This tendency causes serious investigations, which can be successfully attributed to livestock farming. Porcine muscle tissues were studied, as well as functional structural proteins were identified, including their modifications. 2D PAGE was carried out by O'Farrell method; scanned with Bio-5000 plus (Serva, Germany) images were analyzed with ImageMaster™ 2D Platinum software (GE Healthcare, Switzerland); protein fractions were identification by MALDI-TOF MS. A comparative analysis of 2-DE muscle samples using the software showed that on about 300 spots were detected on each gel. 56 standard protein fractions were observed in samples with various geographical origin, breed, age, as well as in samples with different technological processing and storage conditions of muscle tissue. Major proteins belonged to tropomyosin family, myosin structural light chains, phosphorylated myosin light chains, isoforms of muscle enolase and creatine phosphokinase, aldolase A, glyceraldehyde-3-phosphate dehydrogenase and myoglobins, stress-induced phosphoprotein, pyridoxine isoform, diethanolamine phosphate binding protein, troponin I and phosphoglyceratmutase. The most dynamic changes caused by various factors (storage conditions, degree of autolysis, breed, age) were concerned the following protein fractions: myosin light chains (21.5 kDa), tropomyosin chains (33.5 kDa), pyruvate dehydrogenase components (33.5 kDa), creatine kinase (41.0 kDa) and enolase beta (46.0 kDa). This work was supported by RFBR, project number 19-316-90056.

P-02.3-02
The impact of proteasome impairment on microglia function and neuroinflammation

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Cellular protein homeostasis is maintained by the ubiquitin proteasome system (UPS) via protein ubiquitylation and proteasomal degradation. In response to inflammation, catalytic β-subunits of the standard proteasome (SP) are replaced by the inducible subunits and form an alternative isoform, the immunoproteasome (IP). Proteasome (IP and SP) dysfunction results in accumulation of ubiquitylated proteins, the induction of type I interferons (IFNs), and systemic inflammation including neuroinflammation (Previously published in: Brehm A et al. (2015) J Clin Invest 125, 4196-211). Microglia are immune cells of myeloid origin in the brain which constitutively express IP. Since our understanding of the impact of proteasome impairment on microglia function is very limited, we sought to determine the molecular link between ubiquitin-conjugate accumulation following proteasome dysfunction and neuroinflammation. In order to mimic inflammation, we subjected primary microglia isolated from wild type and LMP7 knockout mice, which harbor a deletion of the PSMB8 gene

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encoding the IP catalytic subunit LMP7, to treatment with the proteasome inhibitor bortezomib (BTZ) and the toll-like receptor 4 ligand lipopolysaccharide (LPS). BTZ treatment induced type I IFNs dependent on the IRE1-arm of the unfolded protein response in wild type microglia (Previously published in: Studencka-Turski M, Çetin G et al. (2019) Frontiers Immunol doi: 10.3389/fimmu.2019.02900). LPS treatment caused an accumulation of ubiquitylated proteins in primary microglia of both genotypes, however cells with impaired IP function exhibited more of the ubiquitin-conjugates. Moreover, molecular analysis revealed significantly stronger induction of inflammation, as indicated by higher levels of type I IFNs and interferon-stimulated genes in primary microglia with IP impairment. In an attempt to identify the drivers of the inflammation, the ubiquitylated proteins will be characterized further by proteomic analysis.

P-02.3-03
Extracellular substrates of apoptotic and inflammatory caspases in cancer

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Programmed cell death describes several mechanisms that maintain homeostasis and consequently, impairments in these mechanisms are detrimental to the organism. Our research focused on caspases activated during apoptosis and pyroptosis. In the case of apoptosis, insufficient clearance of apoptotic bodies leads to the progression to secondary necrosis, which is morphologically similar to pyroptosis. In both cases, membrane integrity is disrupted and intracellular proteins can get released to the extracellular space. Caspase-3-like DEVDase activity has already been detected in the extracellular space of apoptotic cells and similarly, some evidence exists of caspase-1 activity in supernatants of pyroptotic cells. We checked whether released active caspases could, similarly to cathepsins, perform selective proteolysis of membrane proteins from cancer cells. To investigate the extracellular role of caspases-3, -7 and -1 we treated breast cancer cells with recombinant human caspases and used the mass-spectrometry proteomic platform to identify the proteins released from the cell surface. Furthermore, several cleavages were additionally confirmed using immunoblotting. Target analysis revealed that caspases can mostly cleave membrane proteins that act as cell adhesion molecules (e.g. CD44) or cell transmembrane receptors (e.g. NRP-1), but the exact consequences of caspase cleavages remain unknown. Additionally, we confirmed the presence of DEVDase activity in extracellular space during progression from apoptosis to secondary necrosis. Using immunoblotting we showed the presence of caspase-3 in the supernatants from apoptotic cells. As for caspase-1 activity, additional tests are needed to determine whether it can be detected in the supernatants from pyroptotic cells. In the future, we will try to validate the effect of extracellular caspase cleavages on the properties of cancer cells and try to determine the physiological relevance of caspases as potential sheddases.

P-02.3-04
Cystatin C deficiency is associated with increased NLRP3 inflammasome activation and LPS-induced sepsis

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Cystatin C (encoded by CST3 on human chromosome 20p11.21) is a potent cysteine protease inhibitor that plays an important role in various biological processes including cancer, cardiovascular diseases and neurodegenerative diseases. It is ubiquitously expressed and secreted from various cell types and is abundant in body fluids. Inflammasomes are multimeric protein platforms that mediate activation of pro-inflammatory caspase-1 and maturation of pro-inflammatory cytokines such as interleukin-1β (IL-1β) and IL-18. Autophagy, an intracellular process important for recycling of damaged organelles and destruction of intracellular pathogens, was reported to protect the host from excessive inflammation. In the present study we demonstrated that cystatin C-deficient mice were significantly more sensitive to the lethal LPS-induced sepsis. We demonstrated that pro-caspase-11 and pro-IL-1β are up-regulated in cystatin C-deficient bone marrow-derived macrophages (BMDMs) upon LPS stimulation. We examined the role of cystatin C-deficiency in Nlrp3 inflammasome activation and release of pro-inflammatory cytokines in BMDMs upon LPS and ATP stimulation. Our results show that cystatin C-deficient BMDMs secrete higher amounts of pro-inflammatory cytokine IL-1β due to increased caspase-1 and -11 activation upon Nlrp3 inflammasome activation, but is not mediated by elevated activity of cysteine cathepsins. Cystatin C-deficient BMDMs show decreased levels of autophagy, which might lead to increased inflammatory response.

P-02.3-05
Deciphering the functional properties of non-metazoan caspase homologues

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Caspases are enzymes, indispensable for execution of apoptosis in metazoa. However, no caspases were yet identified in other eukaryotes. Instead, structurally highly homologous proteins were identified and termed metacaspases and paracaspases. Additionally, caspase homologues were also found in prokaryotes and were termed orthocaspases. All members of this caspase superfamily contain the p20 domain, with the His-Cys dyad needed for hydrolysis of the peptide bond. Despite high structural similarities between the p20-containing proteins, only caspases cleave their substrates after negatively charged amino acid residues, while paracaspases, as well as type I/II metacaspases and orthocaspases favour the Arg or Lys at the position P1. No information was until recently available for orthocaspases and type III metacaspases, which are found in prokaryotes and algae, respectively. We have recently shown that genes for both types code for proteolytically active enzymes, which have the preference for

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cleavage after basic amino acid residues. The activity of type III metacaspases, just like for type I and type II metacaspases, strongly depends on the presence of calcium. Additionally, we show high functional relatedness of type III metacaspases to type I metacaspases and propose a new function of the p10 domain. This domain contains a well-conserved N-terminal region, which can only be found in type I/II/III metacaspases, but is absent in caspases and calcium-independent caspase homologues, which explains their calcium-independent activity. Despite their similar structural properties, the three metacaspase types exhibit remarkably contrasting activation, autoprocessing and proteolytic properties and vary in their substrate preferences, as we have determined by performing Proteolytic Identification of Cleavage Sites (PICS).

P-02.3-06
Interaction of murine cathepsin B and DARPin and its prospects

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Cysteine cathepsins are lysosomal proteases that influence many cellular processes. They are activated by other active cathepsins in the lysosomal acidic milieu by cleavage of the propeptide. They are essential for homeostasis in healthy tissue and are involved in processes such as bone remodeling, antigen processing and presentation, hormone processing, and overall protein turnover. Their aberrant expression and activity play major roles in many pathologies including in the progression, invasion, and metastasis of solid tumors. Among them, cathepsin B (CtsB) has been the most studied in the context of cancer. During cancer progression, it can be found at the surface of and in membrane invaginations of tumor cells where it was linked with extracellular matrix degradation and metastasis spread. Designed ankyrin repeat proteins (DARPins) are genetically engineered antibody mimetic proteins based on natural ankyrin proteins. DARPins can be used as diagnostic or therapeutic agents but also as agents for co-crystallization due to their high selectivity and affinity for the selected target. We designed a new inhibitory DARPin (4m3) that shows a high affinity for murine, but not human, cathepsin B. Currently, the structure of murine cathepsin B remains unresolved due to lack of success in crystallization efforts but DARPin 4m3 could be used for chaperone-assisted crystallization since its binding is expected to sufficiently stabilize murine CtsB. Due to similar biochemical and physiological properties between human and murine CtsB it is sensible to resolve the structure of murine variant which could help in structure-based drug design. Additionally, the resolved structure could help understand the potential difference between binding of antibodies and related proteins to murine and human CtsB, which could be helpful in the design of human CtsB-targeting molecules.

P-02.3-07
Phosphoproteomic analysis of legumain deficient mice

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Legumain, an asparaginyl specific protease, is a member of the cysteine proteinase family. Normally it is confined to the endolysosomal system, though, when specific physiological conditions arise, it can also be found in the cytoplasm, cell nucleus or extracellular space. The enzyme's primary structure is highly conserved among a wide variety of species, implying its significance in evolutionary and physiological aspects. Interestingly, legumain knock out mice exhibit a very mild phenotype. They are viable, fertile and don't display any behavioural aberrations. However, their phenotype includes lowered body mass, irregular kidney function and enhanced inflammatory response. The molecular basis for the observed phenotype is largely unknown, due to the fact, that no system-wide studies on legumain-deficient mice have been published to date. It has been shown that levels of EGF receptor are significantly increased in legumain null mice. An effect which may cause global changes in cellular signalling. Apart from that, legumain could also be able to influence the function of other receptors and kinases. To reveal these possible connections, we carried out a phosphoproteomic analysis of legumain-deficient mice tissue samples to elucidate changes in protein phosphorylation caused by legumain. We also immunologically validated selected targets. Obtained evidence will enable us to gain an understanding of role legumain has in the physiology of the organism and its possible involvement in pathological states.

P-02.3-08
Intracellular cathepsin B inhibition with DARPin

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Cysteine cathepsins are endolysosomal proteases which over the recent years were established as key players in many intra- and extracellular physiological processes. Their intracellular role is often linked with cell death regulation following release into the cytoplasm, where they act as activators of regulatory proteins. However, their broad substrate specificity which is often overlapping between different members of the cathepsin family makes it hard to design specific inhibitors and substrates, as demonstrated by many failed attempts in clinical studies. Therefore, when elucidating cellular mechanisms it is important to critically interpret the results obtained with inhibitors which are generally considered specific but in reality are not, especially in high concentrations which are usually used when studying processes in cell culture. The use of intracellularly expressed specific binders has a potential advantage over traditional knock-out experiments and inhibitors, because they can be targeted to a specific location and

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therefore, do not disturb the physiological function of their target elsewhere. We, therefore, set out to compare an inhibitor considered specific for cathepsin B (CA074-OMe) with a cathepsin B specific DARPin (designed ankyrin repeat protein) expressed intracellularly.

P-02.3-09
Optimized expression and purification of the ectodomain of the disintegrin metalloprotease ADAM17 and the design of a cleavage assay for EpCAM

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The disintegrin metalloprotease ADAM17, also known as the tumor necrosis factor converting enzyme (TACE), is an essential regulator of several signaling pathways that include development, immunity, inflammation, and cancer progression. This is possible because it cleaves a number of different substrates including cytokines, growth factors, and adhesion molecules. Even though TACE has been extensively studied, molecular mechanisms that govern cleavage of some substrates have yet to be elucidated. One of these substrates is the transmembrane glycoprotein EpCAM, which has been identified as a tumor-associated antigen due to its high expression level in rapidly growing epithelial tumors. *In vivo* studies have shown that TACE cleaves EpCAM at two alpha sites, which are both located in a groove formed by two subunits in a dimer – the native oligomeric state of EpCAM. The cleavage sites are thereby inaccessible to TACE. To better understand the mechanism by which TACE is able to cleave EpCAM we designed various full-length and extracellular constructs of TACE and expressed them in an Sf9 insect cell line. We optimized the expression and purification methods for the construct containing the entire wild-type ectodomain (TACE-EX) and performed the first *in vitro* cleavage of EpCAM. We also designed and tested the experimental procedure to analyze the cleavage of the soluble EpCAM ectodomain. Our optimized expression and purification protocols will enable further studies of in vitro cleavage of different TACE substrates and allow us to better understand the underlying mechanisms of EpCAM cleavage.

P-02.3-10
Insights in peptidyl substrate binding to cysteine cathepsins

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Cysteine cathepsins are lysosomal peptidases involved in numerous physiological and pathological processes, such as protein degradation, protein processing, antigen presentation, cancer and CNS (central nervous system) disorders. We are trying to understand how these proteases select their endogenous substrates. Analysis of proteomic study of the cell lysate, which was enriched with selected cathepsins, suggested representative peptides as a model of protein substrates based on cathepsin's specific cleavages. Using a structural approach, we attempted to validate the peptide model with the crystal structures of active-site mutant human cathepsin V in complex with those peptides. The results showed that binding to cathepsin was affected by primary sequence of the peptides, their terminal modification, crystallization conditions and cathepsin active site mutation. Further on, those same peptides were treated with native cathepsin V, K and L and cleavage sites were identified by HPLC-MS. We showed that peptide binding or cleavage don't always match with the binding and/or cleavage of protein substrate. Hence, peptides are not (always) the model to rely on when studying substrate specificity at least of cathepsins V, K and L, but likely also other endopeptidases.

P-02.3-11
Enhanced cytotoxic effect of NK-92 cells toward K562 target cells in the presence of propeptide dipeptides of granzymes A and B

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Cathepsin C (dipeptidyl-peptidase I) activates granzymes A and B by proteolytic removal of their N-terminal dipeptides. The possible physiological role of the cleaved dipeptides Glu-Lys and Gly-Glu is not yet understood. Our research showed that adding either of the two dipeptides to NK-92 cells resulted in enhanced cytotoxicity toward the targeted K562 cells and increased death rate of the target cells. Cathepsin C is known to generate cytotoxic polymers from various dipeptides, as seen for Leu-Leu-OMe. However, in the case of the dipeptides Glu-Lys and Gly-Glu, cathepsin C was unable to polymerize them. We showed that the enhanced cytotoxicity in the presence of the dipeptides is perforin depended. Additionally, the dipeptides were found to be inhibitors of the transferase activity of cathepsin C (IC50 < 20 mM), and weak competitive inhibitors of the peptidase activity with Ki values in the millimolar range.

P-02.3-12
N-terminal domain of EpCAM is involved in proteolysis-associated signaling

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EpCAM is a homodimeric transmembrane glycoprotein associated with enchanced cell proliferation and cancer through regulated proteolysis-associated signaling. This involves a series of cleavages carried out by three membrane-bound proteases, TACE (TNFα converting enzyme), BACE1 (β-secretase 1) and γ-secretase, and results in the release of the EpCAMs intracellular part, a crucial component for the formation of the transcription complex responsible for increased mitogen synthesis. The signaling is initiated by the cleavage of the extracellular part by either

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TACE or BACE1, however the details of molecular events leading to cleavege at sites buried in the dimerization surface of EpCAM remain unknown. One possible factor regulating the accessibility of the cleavage sites is interaction of EpCAM with other proteins which can lead to the exposure of the cleavage sites, i.e. by causing the dimer dissociation. In order to test this hypothesis, we performed interaction interface mapping by mutating surface-exposed residues of EpCAM's extracellular part (EpEX). A set of 28 of such mutants was studied *in vivo* and the extent of the cleavage was assessed with the quantitive western blot. The analysis revealed three distinct surface patches with the same cleavage pattern compared to the wild type, however the extent of the cleavage at certain sites was substantially different. These surface regions represent possible interaction sites with proteins affecting the cleavage and are located mostly on the membrane-distal N-terminal domain. Those interaction partners will be identified using a proximity ligation assay involving promiscuous biotin ligases BioID2 and/or TurboID fused to the extracellular part of EpCAM and will enable us to determine which interaction partners bind to which surface patch and hopefully reveal their underlying effect on the signaling initiation.

P-02.3-13
Ectodomain shedding of epidermal growth factor receptor by cysteine cathepsins

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Cysteine cathepsins are lysosomal proteases, also known to be secreted to the extracellular space. Secreted cysteine cathepsins can cleave ectodomains of membrane proteins including receptors, growth factors, cytokines, and adhesion proteins. Among the receptors, the epidermal growth factor receptor (EGFR) was identified as a cysteine cathepsin substrate candidate with high physiological relevance. EGFR belongs to the ErbB family of receptor tyrosine kinases and plays an important role in cell differentiation, migration, proliferation, and metabolism. This receptor is found to be overexpressed in many cancers, showed to be consequently more aggressive and resistant to chemotherapeutics. Signaling through EGFR is commonly triggered by ligand binding, however, deletions in the extracellular region of EGFR can also cause ligand-independent constitutive activation. Using mass spectrometry-based proteomics, we determined that extracellular cysteine cathepsin L cleaves domain II of EGFR. Moreover, our results revealed that this ectodomain truncation activated the receptor and changed the phosphorylation profile of cellular kinases. By phosphoproteomic analysis, we were able to identify and quantify significant differences in cellular phosphorylation in cells expressing truncated EGFR. Significant pathways suggest that expression of truncated EGFR causes changes in mitogen-activated protein kinases (MAPK) pathway, connected with cell cycle and proliferation, and changes in RNA metabolism. Since EGFR is one of the important anticancer drug targets, our finding could lead to a better understanding of EGFR and possibly to more effective strategies in anticancer therapy. Additionally, our results confirm the increasingly recognized important role of extracellular cysteine cathepsins in cancer.

P-02.3-14
Modulation of the 20S proteasome activity by the interplay between ATP and Mg^{2+} ions

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The proteasomes represent elements of the ubiquitin-proteasome system (UPS) directly responsible for the degradation of cellular proteins. There are several mechanisms that regulate activity of proteasomes including association with regulators, cofactors and via posttranslational modifications of proteasome subunits. Interestingly, the activity of proteasomes was shown to be influenced by divalent ions Ca^{2+} and Mg^{2+}. Magnesium forms a key complex with intracellular ATP and most intracellular ATP is bound to Mg^{2+}, however in certain conditions including stress, when Mg^{2+} concentrations are decreased the amount of free ATP may rise. Herein, using purified preparations of 20S constitutive and immunoproteasomes and a set of short fluorogenic proteasome substrates we demonstrated that chymotrypsin- and caspase-like proteasome activities were gradually decreased from 90 to 50% with the rise of free ATP concentration from 0.25 to 10 mM. In contrast, Mg^{2+} concentrations in range from 0.5 mM to 20 mM dose dependently increased the chymotrypsin-like activity of purified 20S proteasomes from 17% to 67% correspondingly. Concordantly, when proteasomes were incubated with combinations of ATP and Mg^{2+} the chymotrypsin-like activity was directly proportional to the molecule ratio, being equal to control values when identical concentrations of Mg^{2+} and ATP were used. At the same time, when casein labeled with Cy5, or Rhodamine was used as a proteasome substrate instead of short peptides minimal effect of free ATP on the substrate turnover was demonstrated. Taken together, obtained results indicate that free ATP and Mg^{2+} differently influence the degradation of different substrates likely relevant to their size and charge. Furthermore, proteasome regulatory mechanism based on the balance between free ATP and Mg^{2+} may exist in cells. The work was supported by Russian Science Foundation grant #17-74-30030.

P-02.3-15
Structural mechanisms underlying maturation of papain-like cysteine protease

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Papain-like cysteine proteases (PLCPs) are synthesized by various living organisms and have been widely implicated in biotechnology and medicine. Normally, these enzymes consist of a signal (leader) peptide, a pro-peptide domain and a catalytic domain harboring catalytic triad Cys-His-Asn. Being expressed as inactive zymogen, PLCPs become maturated via autocatalytic cleavages, which separate their pro-peptide and catalytic domains. In

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this study we address structural mechanisms regulating timely and efficient maturation of PLCPs by the example of human protease cathepsin L and wheat protease triticain-α. We demonstrate that activation of these proteases is a multistep pH-dependent process, which is governed by formation of a proper molecular complex enabling productive autocatalytic processing. Structural and biochemical analysis along with molecular modeling suggested that formation of such complex is mediated by multiple electrostatic interactions in the interface between the enzyme subunits. These findings expand general understanding of PLCPs functionality, and may be used upon developing PLCPs-based products for pharmaceutics and biotechnology. This work was supported by the Russian Science Foundation (grant No. 21-75-30020).

P-02.3-16
Selective and reversible cathepsin X inhibitors: development and structure-activity relationships study

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Cathepsin X is a carboxymonopeptidase found mainly in immune cells, where it regulates migration, adhesion, proliferation, maturation, phagocytosis and signal transduction through cleaving different substrates. Cathepsin X is highly elevated in various types of cancer, neurodegenerative disorders, inflammatory and other diseases and became an object of interest as a possible therapeutic target. Until recently, an epoxysuccinyl-based inhibitor AMS36 was the only one that showed certain selectivity toward cathepsin X. It is an irreversible inhibitor that also partially inhibits cathepsin B. However, for treatment of pathologic conditions associated with excessive proteolytic cleavage, reversible small molecular inhibitors could be a preferred option. In our study, 579 compounds from the in-house library were tested for the relative inhibition of cathepsin X, following further characterization. A reversible and specific inhibitor Z9 (1-(2,3-dihydrobenzo[b][1,4]dioxin-6-yl)-2-((4-isopropyl-4H-1,2,4-triazol-3-yl)thio)ethan-1-one) with K_i 2.45 ± 0.05 μM was further validated on PC-3 prostate cancer cells and PC-12 pheochromocytoma cells revealing substantial inhibition of PC-3 migration and PC-12 neurite outgrowth (previously published in: Pečar Fonović U et al. (2017) Sci Rep 7(1): 11459). Both processes are under the control of cathepsin X carboxypeptidase activity. Next, a concise series of inhibitor Z9 modifications on its benzodioxine or triazol moieties and ketomethylenethio linker were synthesized to study the structure-activity relationships. Several of the most promising candidates were further characterized and tested in cell-based assays.

P-02.3-17
In-gel profiling of protease cleavage specificity

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Determination of protease specificity is of crucial importance for understanding protease function and also for the design of selective synthetic peptide substrates. Despite several available proteomic methodologies, protease cleavage specificity profiling still remains a labor-intensive and experimentally challenging task. To tackle these drawbacks, we developed a gel-based label-free proteomic approach for reliable determination of protease cleavage specificity (Vidmar et al., 2017, EMBO J). The presented methodology is based on in-gel digestion of the gel-separated proteome with the studied protease, enrichment of cleaved peptides by gel extraction and subsequent mass spectrometry analysis combined with a length-limited unspecific database search. We developed an optimized and easy-to-implement workflow that enables the identification of both N- and C-terminal cleavage sequences, which are aligned to establish a global protease cleavage specificity profile. The methodology was validated by profiling ten proteases from highly specific caspase-7, legumain, endoproteinase GluC and trypsin to broad specific matrix-metalloproteinase-3, thermolysin and cathepsins K, L, S and V. Specificity profiling of thermolysin was performed at its optimal temperature of 75°C, which confirmed the applicability of DIPPS to extreme experimental conditions. Furthermore, DIPPS enabled us to perform a first global cleavage specificity profile of legumain at a pH as low as 4.0, which confirmed a pH-dependent cleavage specificity switch. The obtained data of >50 000 cleavages showed excellent correlation with known protease specificities, demonstrating the applicability of this approach to all major mechanistic classes of proteases under a broad range of experimental conditions. This approach can be easily implemented by any proteomic laboratory and thus provides an excellent starting point for protease characterization and development of specific protease substrates and inhibitors.

P-02.3-18
Role for the S1 pocket in substrate specificity of papain-like cysteine proteases

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Papain-like cysteine proteases (PLCPs) are well-recognized as enzymes responsible for low-specificity digest of different proteins in lysosomes and other acidic cellular compartments. Growing evidence indicates that PLCPs have additional specific functions under ambient pH conditions characterized for the cytoplasm. In this study, we examined structural aspects ensuring pH-dependent substrate specificity of PLCPs. Mass-spectrometry analysis of the natural products of plant PLCPs-catalyzed digestion revealed that the enzymes cleave polypeptides with a hydrophobic residue in the P2 position. Meanwhile, experiments employing

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fluorogenic peptide substrates demonstrated that plant PLCPs and human cysteine cathepsins additionally possess a pH-dependent specificity for the residue in the P1 position. X-ray crystallographic studies and molecular simulations allowed overall structure determination of the enzymes and predict residues in the S1 binding pocket which can form electrostatic contacts with the substrates. Sequence analysis established variability of these residues among PLCPs. Based on these data, we conclude that the S1 binding pocket may govern specific pH-dependent recognition of substrates by PLCPs, thereby ensuring multiple physiological functions of these enzymes. This work was supported by the Russian Science Foundation (grant No. 21-75-30020) and by the UK-Russia Young Medics Association.

P-02.3-19
Characterization of ubiquitin metabolism in mammalian cells by fluorescence tracking

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Despite almost 40 years having passed from the initial discovery of ubiquitin (Ub), fundamental questions related to its intracellular metabolism are still enigmatic. Here we utilized fluorescent tracking for monitoring ubiquitin turnover in mammalian cells, resulting in obtaining qualitatively new data. We report (1) short Ub half-life estimated as 4 h; (2) for a median of six Ub molecules per substrate as a dynamic equilibrium between Ub ligases and deubiquitinated enzymes (DUBs); (3) loss on average of one Ub molecule per four acts of engagement of polyubiquitinated substrate by the proteasome; (4) direct correlation between incorporation of Ub into the distinct type of chains and Ub half-life; and (5) critical influence of the single lysine residue K27 on the stability of the whole Ub molecule. Concluding, our data provide a comprehensive understanding of ubiquitin-proteasome system dynamics on the previously unreachable state of the art. The reported study was supported by Russian Scientific Foundation project # 19-14-00262.

P-02.3-20
Expression of (pro)cathepsin X and cystatin C in PC-12 cell line affected by amyloid beta fragment (25-35)

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Cathepsin X is a unique cysteine protease expressed mainly in immune and neuronal cells. In addition to the non-specific degradation of proteins in lysosomes, it also specifically regulates inflammatory and immune responses by cleaving protein targets outside lysosomes. Increased expression and activity of cathepsin X in neurodegenerative diseases such as Alzheimer's disease (AD) and multiple sclerosis suggest its role in disease onset and development. Cystatin C is an endogenous cysteine protease inhibitor found among others in the senile plaques of patients with AD. It shows anti-amyloidogenic activity by binding to amyloid beta and inhibiting formation of its neurotoxic forms. Undifferentiated PC-12 cells that represent a good model for neurodegeneration, were incubated with Aβ(25-35) at 3 hour and 12 hour time intervals with and without continuous shaking. The quantities of intracellular (pro)cathepsin X and cystatin C were determined by ELISA in cell lysates, while a kinetic method was used to measure the amount of active cathepsin X. The amount of total cathepsin X (pro and active form combined) increased significantly within 3 h of incubation and remained elevated for the next 9 h. The level of cystatin C initially decreased during the first 8 h, subsequently increased to a peak at 12 h and then slowly decreased back to normal in the next 48 h. The amount of active cathepsin X intracellularly was not affected during the observed time points. Cathepsin L, which is responsible for the activation of procathepsin X into its active form, might be inhibited by cystatin C intralysosomally, thus hindering the boosting of cathepsin X in spite of increased procathepsin X expression.

P-02.3-21
Characterization of the protease with keratinolytic activity produced by *Aspergillus giganteus*

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Proteases are in demand in various fields of science and bioeconomy. In recent years, a substantial part of studies has been devoted to investigation of keratinases. Expanding the accumulated knowledge about biochemical and physicochemical properties of keratinolytic enzymes, will increase the efficiency of processes that are significant for humanity, such as biodegradation, treatment of certain skin diseases, and production of bioplastics from organic waste. In this regard, this work was focused on study of the keratinolytic protease synthesized by *Aspergillus giganteus*. A complex preparation of proteins secreted by A. giganteus was fractionated by isoelectric focusing. Enzymatic activity was examined with keratin suspension. Also, reactions were carried out with various chromogenic peptide substrates para-nitroanilides (CPS) to determine the substrate specificity of the enzyme. For inhibitory analysis, the enzyme was treated (1 h, 25°C, pH 8.2) with the following inhibitors: PMSF (1.5 mM), EDTA (1 mM), PCMB (1 mM), TPCK (0.5 mM), TLCK (0.5 mM), soybean trypsin inhibitor (0.5 mg/ml). Reaction conditions are 37°C, pH 8.2, 600 rpm. The amount of formed products in all types of reactions was measured spectrophotometrically. The purified enzyme was analyzed by SDS-PAGE. Qualitative reaction was performed for determination of glycoproteins using periodic acid and Schiff's reagent by dot blotting on nitrocellulose membranes. The keratinolytic enzyme of *A. giganteus* is a protein with molecular weight about 27 kDa and pI 9.2–9.3. The highest activity in reactions with CPS was shown with Z-Ala-Ala-Leu-pNA, and protease inhibition occurred upon the addition of PMSF and EDTA. These results may indicate the subtilisin-like nature of the enzyme. No glycosylation of the enzyme was detected, which will simplify the cloning work. Thus, some characteristics of new keratinolytic protease that can be promising for practical applying and produced by *A. giganteus* were investigated.

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P-02.3-22
Sumoylation of neurofibromin and its SecPH domain plays a role in their functions and implies unexpected structural requirements

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Neurofibromin (Nf1) is a large, multi-domain protein encoded by the tumor-suppressor gene NF1. NF1 is mutated in a frequent genetic disease, Neurofibromatosis type I, which is mainly characterized by the development of tumors of the nervous system and it is also mutated in various types of cancers. The best described function of Nf1 is its Ras-GTPase activity, carried out by its central GRD (GAP related domain), which negatively regulates the Ras-MAPK pathway. But Nf1 exerts other functions in the regulation of cAMP production and actin cytoskeleton dynamics. Nf1 has been identified as highly regulated by post-translational modifications (PTM), particularly by phosphorylation and ubiquitination. Data of our team previously demonstrated a partial colocalisation of Nf1 with PML (ProMyelocytic Leukemia) nuclear bodies thereby suggesting a possible Nf1 sumoylation. In this work we demonstrated that endogenous Nf1 is preferentially sumoylated by SUMO-2. We then focused on a specific Nf1 domain, SecPH, a bipartite phospholipid binding module immediately adjacent to GRD which connects Nf1 to diverse signaling pathways by interacting with different partners. We identified a typical SecPH sumoylation pattern and a specific lysine as a major SUMO acceptor site. We demonstrated that this sumoylation is important for Nf1 function because it affects the Ras-GAP activity of the adjacent GRD and the interaction of SecPH with one of its partners, furthermore SecPH sumoylation pattern is disrupted in different pathogenic NF1 missense mutations. We further characterized SecPH major sumoylation and demonstrated that it is independent of a consensus site but requires unexpected structural elements and could be used as a readout of SecPH folding or conformation to affect its stability and functions.

P-02.3-23
The role of ACRC/GCNA in the repair of DNA–protein crosslinks

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DNA–protein crosslinks (DPCs) are DNA lesions which occur when a protein becomes irreversibly covalently linked to DNA. DPC formation is very common in cells, as it can arise from both endogenous (aldehydes and reactive oxygen species) and exogenous sources (ionizing radiation, UV, chemotherapeutics). Due to their bulky nature, DPCs impair all DNA transactions (replication, transcription, and repair). They have adverse effects on the organismal level including cancer, premature aging, and neurodegenerative diseases. Several groups have identified novel proteases, Wss1 in yeast and SPRTN in higher eukaryotes, which initiate the removal of DPCs through the proteolytic digestion of crosslinked proteins. Considering that they are a common type of DNA damage, a second potential DPC protease in higher eukaryotes might exist. Phylogenetic analysis of the SPRT family in metazoans identified a SPRT-like protein family, ACRC (acidic repeat containing). In line with the phylogenetic proximity, the 3D structure of the protease core within the Sprt domain of ACRC is very similar to that of SPRTN. The goal of our study is to determine if ACRC is proteolytically active and what is its relation to SPRTN, using zebrafish model and CRISPR/Cas9 gene manipulation. To address the role of ACRC *in vivo* we introduced mutation in the ACRC putative protease active site (E451 deletion) with the aim of creating an enzymatic dead version of ACRC. We also compared both proteases using phylogenetic and syntenic analysis and in regard to mRNA and protein expression across different tissues in zebrafish. Our study will reveal actual contribution of ACRC to the DPC removal on the organismal level.

P-02.3-24
Identification of a novel putative interaction partner of dipeptidyl peptidase 3, SH2 domain-containing protein 3C

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Dipeptidyl peptidase 3 (DPP3) is a zinc metallopeptidase that sequentially cleaves off dipeptides from the unsubstituted amino-termini of 3–10 residue long peptides *in vitro*, showing broad specificity for 4–8 residue substrates. It is involved in the regulation of Nrf2/KEAP1 signalling pathway through its interaction with KEAP1 protein, which is so far the only confirmed protein interaction partner of DPP3. We have conducted SILAC-MS study on the whole proteome of HEK293T cells and identified novel putative interaction partner of DPP3, SH2 domain-containing protein 3C (SH2D3C). SH2D3C is one of the three members of the family of proteins which contain both SH2 domain and a domain similar to guanine nucleotide exchange factor domains for Ras family GTPases (Ras GEF-like domain). Several different length isoforms of SH2D3C protein are expressed in different cell types, where they have different functions, including acting as an adapter protein involved in the regulation of cell adhesion and migration, tissue organization, and the regulation of the immune response. The interaction of DPP3 with the isoforms 2 and 3 of SH2D3C was confirmed by Co-immunoprecipitation of overexpressed proteins in HEK293T cells and by GST-pulldown with both wild type DPP3 and catalytically inactive DPP3 variant, E451A. The colocalization of EGFP-DPP3 and SH2D3C-mCherry was analysed by confocal microscopy in NIH 3T3 cells and detected in cytosol and on the membrane, with weak staining signal in the nucleus. Preliminary bimolecular fluorescence complementation (BiFC) investigation displays the interaction in the cytosol and in the membrane ruffles. Present knowledge about the DPP3 and SH2D3C proteins indicate that their interaction might represent a link between Nrf2/KEAP1 mediated oxidative stress response and the regulation of cell migration, and further investigations that will elucidate the potential physiological implications of this interaction are in progress.

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P-02.3-25
Specificity of cysteine cathepsins through the eyes of large-scale proteomic data analysis

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Cysteine cathepsins are a group of endolysosomal proteases involved predominantly in protein turnover with a number of specific roles. Their redundancy and broad specificity make it difficult to understand their functional differences. To get an insight in the redundancy and specific protein degradation patterns we undertook a proteomic analysis of SH-SY5Y cell lysates with human cathepsins K, V, B, L, S, and F. A survey of approximately 30000 cleavage sites determined by the COFRADIC method showed that the majority of cleavages can be performed by several cathepsins, with a small fraction of specific cleavages, performed by a single cathepsin in the intact protein only once. Redundant cleavages often appear in groups of short sequence spans. Statistical analysis of the cleaved peptides revealed differences in the specificity-determining substrate binding sites and their span. We call these sites heterogeneous, in contrast to homogeneous, which contain normal distribution of amino acids. The cleavage sites of heterogeneous substrate positions were clustered using the BLOSUM weighted substitution matrix and served us to extract refined training sets for generating the support vector machine models for cathepsins K, V, B, L, S, and F. We applied this methodology to successfully predict cathepsin cleavages in a number of viral proteins known to be associated with cysteine cathepsin activation, including the S-protein of SARS-CoV-2. The computational methodology developed here can be applied to provide an insight and predict interactions in a broad range of protein substrates and their processing enzymes. *The authors marked with an asterisk equally contributed to the work.

P-02.3-26
The role of P97 segregase in the repair of DNA–protein crosslinks *in vivo* using CRISPR/Cas9 gene editing in zebrafish

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DNA-Protein-Crosslinks (DPCs) are DNA lesions which occur when proteins become irreversibly covalently bound to DNA, thereby physically blocking all DNA transactions including chromatin remodeling, replication, transcription and repair. DPC repair (DPCR) is a specialized DNA damage repair pathway in which crosslinked proteins are proteolytically or nucleolytically cleaved. Recently, the specific roles of several enzymes in DPCR have been uncovered, and their dysfunction has been linked to ageing, cancer and neurodegenerative diseases. To quantify DPCs, we are developing a new method using stably transfected HeLa cells expressing a GFP-tagged Histone which can form model DPCs upon e.g. formaldehyde treatment. The presence of GFP in particular cellular fractions, which depends on the presence and activity of DPCR enzymes, can then be analyzed, either by fluorescence or by western blot. Once the method is established in cell culture, we aim to develop an in vivo assay in zebrafish. Currently, we are creating zebrafish mutants for DPCR factors using CRISPR/Cas9 gene editing with the aim of studying the function of target proteins in DPCR *in vivo* during embryonic development and in adults. In particular, we are generating a zebrafish mutant line by introducing the K524A mutation in the p97 gene using the CRISPR/Cas9 knock-in method to elucidate the role of the segregase P97 specifically in DPCR. We will investigate the epistasis of P97 with Sprtn, which is a central component of DPCR and for which we are generating equivalent mutant zebrafish lines. Taken together, our results will unravel the role of P97 during the orchestration of DPC repair *in vivo*.

Protein folding and misfolding

P-02.4-01
Biochemical characterization of short collagen-like otolin-1 involved in otoliths and otoconia formation

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Many distinctive proteins regulate the process of tissue mineralization. These macromolecules control the deposition of biominerals and have significant influence on the nucleation, growth, localization and morphology of growing crystals. Many of biomineralization related proteins are intrinsically disordered (IDP) considered as the major regulators of the process. However, significant role of matrix proteins involved in biomineralization cannot be neglected. These proteins provide an organic scaffold and surface for mineral deposition. In the formation of fish otoliths and higher vertebrate otoconia a short collagen-like protein, otolin-1, is involved as the scaffold providing and tethering element of these calcium carbonate inner ear structures. In this work we present the preliminary biochemical characterization of two homologs of otolin-1. *Danio rerio* and *Homo sapiens* otolin-1 was obtained as a recombinant protein from bacterial culture. The estimation of secondary structure content, the influence of calcium ions on the stability of these proteins and its oligomeric state show many differences in molecular properties between homologous proteins originated from these two species. Further investigation will be mostly focused on the resolution of protein structure. Acknowledgments: This work was supported by the National Science Center (Poland) [UMO-2015/19/B/ST10/02148] and in a part by statutory activity subsidy from the Polish Ministry of Science and High Education for the Faculty of Chemistry of Wroclaw University of Science and Technology.

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P-02.4-02
Lactoferrin structural stability in association with enzymatic hydrolysis

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Lactoferrin is an 80-kDa glycoprotein that has an ability to reversibly but strongly bind two iron ions. It is most abundantly present in mammalian milks but also in other biological secretions and white blood cells. Since its discovery in 1960, it has been widely studied primarily because of its variety of biological functions from antitumor, anti-inflammatory, immuno-modulatory, antioxidant to antimicrobial. Lactoferrin can exist in iron depleted (apo) form and iron loaded (holo) form. Apo form has open conformation and is more prone to denaturation. When incubating lactoferrin in the presents of iron ions, it adopts holo form, which has more closed conformation and exhibit greater resistance to denaturation. In our study, the pH and temperature stability of lactoferrin was measured. Lactoferrin was stable in pH range from 4.0 to 11.0. At pH below pH 4.0 and above pH 11.0 lactoferrin undergoes protein denaturation process. Temperature stability of lactoferrin was measured over a wild pH range. At pH 4.0 lactoferrin exhibited lower temperature stability. The higher temperature stability was observed at neutral pH. However, saturation of lactoferrin with iron thermally stabilize the structure. As we have observed, lactoferrin unfolds at pH lower than pH 4.0 what makes it more prone to enzymatic proteolysis by pepsin. Using pepsin for lactoferrin hydrolysis, lead to discovery of peptide with higher antimicrobial effect on bacteria. Enzymatic hydrolysis of lactoferrin with pepsin at different pH (1.0–4.0) was studied. SDS PAGE analysis showed peptides smaller than 10 kDa at pH range from pH 1.0 to pH 3.0, while at pH 3.5 and 4.0 peptides larger than 10 kDa were observed. This indicates poor enzymatic activity of pepsin at higher pH. By exposing lactoferrin and proteolytic enzyme to different incubation conditions can affect structure and enzymatic activity of an enzyme or protein which can reflect in generating new bioactive peptides.

P-02.4-03
The complex phosphorylation patterns that regulate the activity of Hsp70 and its cochaperones

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Proteins must fold into their native structure and maintain it during their lifespan to display their functions. Cells synthesize a wide variety of molecular chaperones that assist folding of other proteins and avoid their aggregation to ensure proper folding and stability, and avoid generation of misfolded conformations that can be potentially cytotoxic. A protein machinery in metazoa composed of Hsp70, Hsp40 and Hsp110 chaperone families can reactivate protein aggregates. Although Hsp70s are the heart of this system, Hsp40s drive much of the versatility, since depending on which Hsp40 interacts with Hsp70, the machinery will be driven to one function or another. Although the interaction of a specific Hsp40 with the main chaperone must be well regulated, the lack of structural information about this complex severely limits the understanding of its functional mechanism. Post-translational modifications (PTMs) constitute a main route used by cells for simple and reversible regulation of protein functions. Phosphorylation is the most studied PTM and triggers different biologically important effects, such as induction of structural changes, protein labelling for cellular translocation and regulation of protein-protein interactions. In this work, we focused on the phosphorylation sites found in a representative of Hsp40 (DnaJA2) and the functional consequences associated with some of them. Experimental results of different phospho-mimetic forms of DnaJA2 might indicate that phosphorylation of this protein could regulate not only its affinity for Hsc70, but also its Hsc70-independent capacity to bind misfolded substrates to avoid their aggregation. These results suggest that phosphorylation of DnaJA2 co-chaperone, and in general phosphorylation of any component of this versatile and multifunctional system, could be a specific, reversible and fast way to modulate their functions, and thus, to rapidly adapt to different conditions ensuring cell proteostasis and survival.

P-02.4-04
The effect of chemical chaperones on test systems with different kinetic regime of aggregation

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The kinetic regime of the protein aggregation process, which includes the stages of the protein molecule unfolding, nucleation and growth of aggregates, is determined by the rate-limiting stage of this process. In the presence of chemical chaperones, the kinetics of target protein aggregation can change significantly. In this work, we propose a method for analyzing aggregation curves obtained by dynamic light scattering, which allows us to quantitatively characterize the effect of chaperones on individual stages of the model protein aggregation process. The test systems characterized by the order of aggregation with respect to the protein (n) equal to 0.5, 1 or 2 and based on thermal aggregation of muscle glycogen phosphorylase b (Phb) at 48°C (n = 0.5), UV-irradiated Phb (UV-Phb) at 37°C (n = 1) and apo-form of Phb (apo-Phb) at 37°C (n = 2) were used. It has been shown that the effects of the chemical chaperones betaine and lysine are not the same for test systems with different aggregation kinetics, while arginine stimulates the formation of aggregates for all proteins under study. Betaine protects Phb and apo-Phb from aggregation, but accelerates aggregation of UV-Phb. Lysine increases the rate of Phb aggregates formation, but slows down the process of UV-Phb and apo-Phb aggregation. The protective effect of chaperones is accompanied by a slowdown of the stages of nucleation and aggregates growth, while accelerating the target protein aggregation results in a decrease in the duration of the nucleation stage and an increase in the rate of protein aggregates growth. The mechanisms of chaperones action on the aggregation kinetics of various test systems have been discussed. This work was supported by the Russian Science Foundation (grant 16-14-10055) and the Ministry of Science and Higher Education of the Russian Federation.

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P-02.4-05
The function of the molecular co-chaperone prefoldin during mitochondrial stress

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Proteins must obtain their correct fold to fulfill their cellular functions. If proteins cannot be folded properly, they tend to aggregate causing a burden for protein homeostasis. Failure to maintain a correctly folded proteome is linked with numerous diseases. Molecular chaperones are dedicated machinery that folds newly synthesized proteins, but also protect unfolded proteins from aggregation, help to rescue the function of proteins by facilitating disaggregation and guide proteins for degradation. The co-chaperone prefoldin is a hexameric protein complex also called the Gim complex in the yeast *Saccharomyces cerevisiae*. It is mainly known to assist the folding of the newly synthesized cytoskeleton proteins actin and tubulin. However, recent research indicates that prefoldin might play a role during protein homeostasis maintenance. We analysed the consequences of the loss of prefoldin subunits for yeast growth under various stress conditions. We found that prefoldin is necessary to withstand a variety of exogenously added stressors. Moreover, we are investigating the role of prefoldin during the physiological processes of mitochondrial biogenesis. The function of mitochondria is linked with cellular protein homeostasis depending on the import of proteins, which are encoded by genes in the nucleus and synthesized on cytosolic ribosomes. While much is known about the import of mitochondrial proteins, little knowledge exists on the fate of mitochondrial destined proteins in the cytosol under basic and stressed cellular conditions. Our data show that the loss of some prefoldin subunits compromises mitochondrial morphology. Thus, we aim to understand mechanisms of how prefoldin is involved in the maintenance of cellular and/or mitochondrial protein homeostasis upon mitochondrial stress conditions. The prefoldin substrate landscape might also include mitochondrial precursor proteins. This work is supported by the National Science Centre grant 2018/31/B/NZ1/02401.

P-02.4-06
The existence of the dissociation stage in the heat-induced aggregation of bovine liver glutamate dehydrogenase

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The detailed mechanism of heat-induced aggregation of bovine liver glutamate dehydrogenase (GDH) has been studied. The initial stages of the aggregation process at 50°C were investigated using analytical ultracentrifugation. The sedimentation velocity analysis showed that the native protein at the concentration of 0.2 mg/mL contained hexameric GDH and larger native associates. The samples preheated at 50°C during 3 min contained the oligomeric forms of larger size than in the native sample, along with hexameric GDH. Further heating (5 min) produced the dissociated forms smaller than hexamers, although the previous oligomers and the native protein remained. The thermal treatment up to 10 min resulted in the loss of 83% of protein due to precipitation of large aggregates. These data allowed us to refine the mechanism of the heat-induced GDH aggregation studied in our laboratory. GDH denatures upon heating with the formation of molten globule-like intermediate [1]. These minor structural changes are apparently enough to initiate protein aggregation. The irreversible dissociation step occurs after unfolding. The further growth of aggregates proceeds by attachment of dissociated forms to the existing associates and by the aggregate-aggregate sticking. These data clarify the mechanism of GDH aggregation and directly confirm the existence of the protein dissociated forms after its denaturation at the elevated temperatures. The specified information about GDH aggregation and stability can be useful in developing of GDH-based biosensors and the studies of GDH function in vivo under thermal stress conditions. This work was supported by the Russian Science Foundation (grant 16-14-10055) and the Ministry of Science and Higher Education of the Russian Federation. 1 Singh N, Liu Z & Fisher HF (1996) The existence of a hexameric intermediate with molten-globule-like properties in the thermal denaturation of bovine-liver glutamate dehydrogenase. Biophys Chem 63, 27–36. *The authors marked with an asterisk equally contributed to the work.

P-02.4-07
SAXS analysis of intrinsically disordered protein with a tendency to aggregate

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Small angel X-ray scattering (SAXS) is commonly used for the low-resolution structure analysis of the macromolecules in solution. SAXS is especially useful for intrinsically disordered proteins (IDPs) elongated and flexible chains studies, where other methods fail. We performed SAXS analysis to determine the character of *D. melanogaster* Germ-cell expressed protein C-terminal fragment (GCEC). The GCE, as the juvenile hormone (JH) receptor, mediates JH function in preventing the precocious development of *D. melanogaster* during metamorphosis. What important, GCEC is probably responsible for specific modulation of protein action. During irradiation GCEC experienced severe radiation damage what resulted in aggregation. Since the SAXS scattering signal is a function of molecular weight, this technique is sensitive to the presence of a very small amounts of aggregates, higher oligomers or larger impurities. These significantly affect the measurements results and make it not interpretable. Finally, a qualitative diffraction patterns were obtained for several samples measured directly after protein purification. Initial scans, collected for each of the samples, were combined and used for further analysis. The obtained Kratky plot for GCEC does not present a maximum and reaches plateau at higher values of scattering vector. Such a shape is characteristic for IDPs. The calculated radius of gyration (R_g=56 Å) is significantly higher as 26.5 Å calculated for GCEC with the assumption of globular structure. The maximal intramolecular distance within the molecule (D_{max}) is 174.5 Å. All parameters indicate highly asymmetric and expanded GCEC conformation. Finally, we performed EOM analysis to generate exemplary conformers adopted by GCEC. All determined structures present characteristic bend in the middle of the sequence and significant tangling at both N- and

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P-02.4-08
Structural characterization and stability analysis in the folding of the second bromodomain of the BRD2 protein

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Bromodomains (BRDs) are conserved structural motifs involved in a variety of cellular processes such as chromatin remodeling, post-translational modifications or transcriptional control, acting as "reader" of histones acetylation sites. Mutations or chromosomal rearrangements affecting BRDs have been linked to cancer onset. Previous results have shown that BRD domains follow a three-state folding mechanism [1]. In this work, we aim to investigate in detail the structure of the folding transition state of the second bromodomain of BRD2 protein (BRD2d2), by exploiting site-directed mutagenesis and rapid folding kinetics experiments. For this purpose, we generated, expressed and purified, a set of 25 single mutants of BRD2d2, selecting the mutations in the perspective to maintain the native structure of the protein. The structural integrity of the mutants was tested by circular dichroism technique and by evaluating the thermal stability of every mutant. We obtained the thermodynamic stability for every mutant, performing urea-induced denaturation experiments at equilibrium condition, and subsequently we analyzed the (un)-folding kinetics of BRD2d2 mutants by stopped flow experiments. We then calculated for each variant protein a parameter (phi value) derived from the equilibrium and kinetic data. Since the phi-value is a parameter expressing the destabilization of the folding transition state upon mutation, determining a complete set of phi values allows depicting the structure of the transition state for folding. Overall, our analysis sheds light on the kinetic role of different residues that, in some cases, could be important not only during the protein folding pathway of BRD2d2, but also in the interaction with its binding partners. [1] Petrosino M, Bonetti D, Pasquo A, Lori L, Chiaraluce R, Travaglini-Allocatelli C. (2017) "Unveiling the folding mechanism of the Bromodomains" Biochemistry and Biophysics Reports 11; 99–104.

P-02.4-09
Analysis of helix-to-coil transitions in human serum albumin

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Human serum albumin has quite intriguing distribution of amino acid residues and combinations of hydrophobic and hydrophilic residues (in pentapeptides) among structurally stable (remaining in all 104 analyzed 3D-structures) and structurally instable fragments of its alpha helices (that may turn to random coil at least in one of them). According to the alpha-helical pattern of PentaFOLD algorithm (chemres.bsmu.by), both stable and instable alpha helices are predicted with approximately the same success: 57.5% of helices, 16.4% of beta sheet and 26.1% of random coil vs. 52.28%, 4.6%, and 43.15%, respectively. Unexpectedly, beta-structural pattern of PentaFOLD algorithm predicted 54.3% of residues in structurally stable fragments of alpha helices as beta sheet, while just 29.0% of residues from structurally instable alpha helices, where 44.4% were predicted as random coil. Stable alpha helices are significantly enriched by both amino acid residues and their combinations prone to form beta sheet (34.0% vs. 22.8%, $P < 0.05$; 38.6% vs. 22.8%, $P < 0.05$, respectively) relatively to structurally instable alpha helices. Residues from structurally stable fragments of alpha helices show significantly lower average value of surface accessibility calculated by DSSP (https://www3.cmbi.umcn.nl/xssp/) compared to fragments of alpha helices prone to change their conformation to random coil ($33.5 \pm 5.0\%$ vs. $50.46 \pm 4.8\%$, $P < 0.05$). Indeed, the usages of strong beta sheet formers are much higher in stable than in structurally instable fragments of alpha helices (Val: $10.6 \pm 0.8\%$ vs. $5.9 \pm 0.4\%$; Phe: $7.3 \pm 0.6\%$ vs. $4.6 \pm 0.3\%$; Thr: $6.0 \pm 0.5\%$ vs. $3.8 \pm 0.2\%$). Alpha-helical hydrophobic buried core of HSA incorporates significant number of residues known to be strong beta-formers. In contrast, fragments of alpha helices on a surface of that protein are protected from alpha to beta transitions by the decreased usage beta-formers, but they are prone to helix-to-coil transitions.

P-02.4-10
Alpha-B-crystallin modulates physical properties and cytotoxicity of lysozyme amyloids

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The main functions of chaperones are maintaining the native tertiary or quaternary structure of monomeric proteins, as well as the formation and dissociation of protein complexes. The role of chaperones in fibrillogenesis is currently being actively investigated. At the same time, the effect of chaperones on mature amyloid fibrils is paid much less attention. Given that progressive amyloidosis is currently being detected at the stages when mature amyloid fibrils have already formed, the study of the chaperones effect on these protein aggregates are of high relevance for searching the therapeutic agents. It is known that small heat shock protein alpha-B-crystallin, which is found in large quantities in the body of patients as part of amyloid plaques, is able to inhibit the proliferation of amyloid fibrils. We investigated the effect of this chaperon on mature lysozyme amyloid fibrils prepared under various conditions. Transmission electron microscopy of the tested objects indicated the chaperon-induced degradation of lysozyme amyloid fibrils into the large disordered aggregates at the physiological conditions. Simultaneously with the decompaction of amyloid fibrils, a partial degradation of denatured disordered aggregates into smaller aggregates and monomers occurs. We showed that degraded aggregates obtained by treatment with alpha-B-crystallin have a more pronounced cytotoxic effect on the HeLa cell line in comparison to mature fibrils. According the results of MTT test, the presence of the degraded aggregates in the culture medium for 24 h reduced the

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number of living cells by about 30% ($P < 0.05$) in comparison with mature fibrils, while chaperone itself had no toxic effect on the cells. Thereby our data support the notion that physical modifications of amyloid fibrils by alpha-B-crystallin are sufficient to enhance their cytotoxicity. This work was supported by grant from Russian Science Foundation (No. 18-74-10100). *The authors marked with an asterisk equally contributed to the work.

P-02.4-11
Structural analysis of the PDZ domain of MEG in a complex with a viral protein E6

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High-risk human papillomaviruses (HPVs) cause a variety of cellular hyperproliferation-involved diseases, which include cervical cancer. E6, one of the two HPV-encoded oncoproteins, is known to recruit p53, the master regulator of cell cycle, and induce its degradation. E6 also provokes tumorigenesis in a p53-independent pathway through its C-terminal tail region that recognizes a PDZ domain of diverse host proteins. Herein, we determined the crystal structure of the PDZ domain of MEG in a complex with a peptide derived from the C-terminal region of HPV16 E6, which interact with each other with a dissociation constant of 18.8 μM. Structural analysis exhibited that the complex formation between the two proteins depend on hydrophobic interaction mainly mediated by Leu158 of HPV16 E6 and also on a number of intermolecular hydrogen bonds. Introduction of structure-based mutation and modification into the C-terminal region of HPV16 E6, including L158A, phosphorylation of Thr156, and amidation of the C-terminal end region, disrupted the complex formation, supporting the relevance of our crystal structure. Our study therefore provides a structural and biochemical basis for elucidating an additional target of HPV E6 in causing cell transformation and tumorigenesis.

P-02.4-12
Investigation of antimicrobial propensities of amyloidogenic peptides derived from sequences of bacterial ribosomal proteins S1

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The prospects for the use of natural and artificially synthesized antimicrobial peptides (AMPs) are currently being discussed. However, the amyloidogenic properties of such antimicrobial peptides are rarely considered. Recent work has examined development strategies based on the amyloidogenic properties of AMPs [Previously published in: Kurpe SR et al. (2020) Int J Mol Sci 21(24), 9552.] Prediction and theoretical analysis of amyloidogenic regions based on the sequences of bacterial ribosomal proteins S1 was performed using the FoldAmyloid, Aggrescan, Waltz and PASTA 2.0 programs. The predicted peptides were synthesized using the Fmoc methodology. The amyloidogenic properties of the synthesized peptides were experimentally studied using electron microscopy and fluorescence spectroscopy. [Previously published in: Grishin SY et al. (2020) Int J Mol Sci 21(15), 5199]. The antimicrobial effect of peptides on bacterial cells was assessed by spectrophotometry. The proteome of peptide-treated and intact cells was compared by mass spectrometry. Determination of toxicity for eukaryotic cells was assessed using a cell viability assay with resazurin. [Previously published in: Kurpe SR et al. (2020) Int J Mol Sci 21(17), 6382]. The described specific amyloidogenic regions can be potential targets for modulating the aggregation properties of bacterial ribosomal S1 proteins. The obtained results are important for understanding the process of fibrillogenesis of amyloidogenic peptides with antimicrobial activity and can be used to develop new AMPs against pathogenic microorganisms. This study was supported by the Russian Science Foundation (project no. 18-14-00321).

P-02.4-13
Structural characterization and stability of proteins in solid forms

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Protein therapeutics are becoming increasingly important as an alternative treatment for a variety of diseases. For better stability, proteins are often formulated as solid dosage forms, the most common of which are lyophilizates. Their stability depends on the preservation of the native structure during lyophilization, as well as in the lyophilizates. During lyophilization cycle, proteins are exposed to various stress factors that, in combination with the excipients, can affect the protein structure in the final solid form. If the native structure of the protein is not maintained during lyophilization, this may be reflected in an unstable final pharmaceutical product and consequently in its quality, safety and efficiency. Characterization of proteins in solid form is less established, as most methods evaluate critical properties in solution, which is not necessarily indicative of adequate stabilization in the solid phase and thus long-term stability of the pharmaceutical form. Characterization of proteins in solid form can be used to evaluate both secondary and tertiary structure during formulation development. In addition to structural characterization, monitoring protein aggregation is very important. Together with denaturation and surface adsorption, aggregation can affect the activity and stability of lyophilized proteins. Only formulations that retain their structure in the solid state are included in stability studies, which are of particular importance for the development of protein drugs [1]. In this work, the study of protein structure and stability in solid dosage forms using analytical methods such as FTIR, NIR, Raman, solid-state fluorescence, UV-Vis and NMR spectroscopy, as well as circular dichrosim, DSC and X-ray powder diffraction is presented. Aggregation phenomena were also studied by size exclusion chromatography and dynamic light scattering [2]. [1] Bolje, A.; Gobec, S. (2021) (under peer-review). [2] Bolje, A. et al. (2021) (unpublished results).

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P-02.4-14
Study on the aggregation of PHC3 protein in microbial and human cells

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Amyloids are insoluble highly ordered protein aggregates of a fibrillar nature, which are held together by intermolecular cross-beta sheets. The importance of amyloids is related to their role in a variety of human diseases, such as Alzheimer's, Huntington's and Parkinson's diseases, prion diseases and type II diabetes. Recent data also uncover the existence of functional amyloids in various organisms. By using the yeast-based test system described previously (Chandramowlishwaran et al. 2018 J. Biol. Chem. 293: 3436), we have identified new amyloidogenic proteins in the human proteome. Among them, short isoforms of the human PHC3 protein were found, that demonstrate amyloid properties both in yeast cells and when produced in *E. coli*. The full-length PHC3 protein is one of the key components of the polycomb complex (PcG), that is necessary for maintaining the repressed state of a variety of genes, including Hox-genes, during the development of an organism. The function of short isoforms of PHC3 remains unknown thus far. We have shown that short isoforms of the PHC3 protein, fused with a fluorophore, form detergent-resistant aggregates during overproduction in HEK293T human cell culture. Using antibodies to the full-length PHC3 protein, we have demonstrated that full-length PHC3 is colocalized with the aggregated short isoforms. We propose that aggregation of the short isoforms of the PHC3 protein can result in sequestration of its full-length isoform, altering the assembly and activity of the polycomb complex and leading to a change in the expression a variety of genes. Ongoing experiments are aimed at testing this model. This work was performed with the support from RFBR grants 19-34-51054 and 19-34-90153, and from the SPbSU project 51140332, and with the technical help of the SPbSU Resource Centers "Chromas", "Molecular and Cell Technologies" and "Biobank".

P-02.4-15
Structural-dynamic studies of D3-peptide interaction with membrane bound Aβ-peptide precursor

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Alzheimer's disease (AD) is a devastating neurodegenerative disease resulting in severe dementia. Despite huge efforts to combat the problem currently, there is no appropriate treatment of the disease. Amyloid Aβ-peptides forming plaques in brain during AD are the products of sequential cleavage of amyloid precursor protein (APP). Recently we developed D3-like peptides, which destroy the Aβ-aggregates, inhibiting AD, and currently one of them is about to undergo a phase II clinical trial. Here, we report that D3-peptide directly interacts with Aβ precursor, transmembrane APP fragment 672–726 (APPmc), solubilized in membrane-mimicking DPC micelles and studied by means of protein engineering, microscale thermophoresis and high-resolution NMR. Molecular dynamics relaxation of the D3/APPmc-complex in explicit lipid bilayer revealed that D3-peptide can fold into a nascent helix and stabilize juxtamembrane helical region of APP in a manner of so-called IDP-IDP (intrinsic disorder-based protein) interactions. Also, we find that AD familial mutations (D693N and E694G) placed in the C-terminus of APP juxtamembrane helix are considerably decrease the strength of D3/APPmc-interactions, specifying the hotspot of APPmc/D3-interaction and assuming the importance of local Coulomb contacts in the complex formation. The structural data in agreement with ELISA and Western-blot analyzes, which did not reveal any influence of D3 on the APP cleavage by the secretases, which is consistent with the flexible IDP/IDP nature of the D3/APP interactions. The data suggest that D3 can recognize the amyloidogenic region of APP before its processing, restricting conformational diversity generally favoring its α-helicity via prevention of intermolecular hydrogen bond formation, inhibiting early steps of Aβ conversion into β-conformation that precedes toxic oligomerization, thus targeting early stages of AD development. This work was supported by the Russian Science Foundation (project 20-64-46027).

P-02.4-16
Nonsense mutations in the prionogenic domain of the yeast SUP35 gene induce prion conversion of the Sup35 protein

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Sporadic forms of prion diseases can be considered as a result of violation of folding of corresponding prionogenic proteins. These disorders, which are traditionally considered accidental, can also result from rare and difficult to track events - in particular, certain mutations in the genes of these proteins that could occur in individual somatic cells. In this paper, we simulated the early stages of the process described above using the prionogenic protein Sup35 of the yeast Saccharomyces cerevisiae. It is known that the emergence of the prion state of proteins requires the overproduction of these proteins. We searched for mutant variants of Sup35 protein inducing prionogenesis at the normal expression level. Using error-prone PCR, we mutagenized the prionogenic N-domain of the SUP35 gene and selected two mutant SUP35 alleles, which, being located on a low-copy plasmid, caused a significant increase in de novo formation of the [PSI+] prion. Nonsense mutations were present in both mutant alleles leading to the synthesis of shortened Sup35 fragments of 98 and 108 amino acid residues (a.a.), respectively. Then we obtained a set of 12 alleles that encode Sup35 N-terminal fragments 19 to 240 a.a. long. It turned out that the fragments of 75

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to 112 a.a. led to a great increase of prionogenesis, while fragments shorter than 73 a.a. or longer than 123 a.a., as well as wild type Sup35 protein, did not promote prion formation. This process depended on the presence of the [PIN+] prion. Importantly, the [PSI+] variants selected in the study were able to be maintained in the cells after loss of the mutant SUP35 allele. We also discovered that SUP35 mRNA could undergo splicing into transcript encoding the shortened Sup35 isoform, which was also highly prionogenic. Our data evidence that truncated forms of amyloidogenic proteins might initiate the spontaneous formation of amyloids, which can then spread via prion-like mechanism, resulting in sporadic amyloid disease.

P-02.4-17
Obtaining and characterization of SPA–β-lactamase fusion protein

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Staphylococcus aureus protein A (SPA) is well known for its capacity to interact with Fc-fragments of IgG. Thus, it is widely used for capture and purification of antibodies and Fc-fusion proteins, for separation of the blood of patients with autoimmune diseases from autoantibodies and circulating immune complexes. Our research was aimed to obtain fusion protein SPA–β-Lac for its application in immunoassays as a secondary immunoreagent. Recombinant DNA technology assures the elaboration of genetic constructions without disadvantages of chemical conjugation, such as high heterogeneity of the final product or necessity of separating full-size conjugates from non-conjugated components. β-Lactamase was selected due to its advantages, such as stability and the possibility of obtaining high concentrations in bacterial systems of expression in soluble active form. Besides, genetically fused with the IgG-binding protein (SPA), it allows quantitative and qualitative antibodies detection. The DNA sequence of *E. coli* β-lactamase was subcloned into plasmid vector pET24-SPA. E. coli BL21(DE3) cells were transformed by obtained plasmid vectors. SPA–β-Lac expression was induced by adding IPTG and also by the autoinduction protocol. The protein of interest was accumulated in the cytoplasmic fraction of *E. coli*. The functional activity of SPA–β-Lac was confirmed with ELISA. The possibility of long-term storage of the protein at –20°C without loss of its functional activity was shown. Application of SPA–β-Lac as a universal secondary immunoreagent allows to extend the range of primary antibodies for antigen detection in ELISA or blot analysis (SPA detects Fc-fragments of IgG of different animal species and human IgG).

P-02.4-18
Epitope composition of recombinant fragment of orthopoxvirus p35 protein

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Oncolytic virotherapy – a revolutionary tool for cancer treatment. One of these viruses is the vaccinia virus. But the use of oncolytic viruses has a number of disadvantages associated with the appearance of neutralizing antibodies even after a single use. Therefore, approaches are needed to reduce the immune response in the body of a patient with oncolytic virotherapy without reducing the oncolytic properties of viruses. A possible solution to this problem will be to reduce immunogenicity by disrupting the structure of epitopes recognized virus neutralizing antibodies. Earlier we obtained full-sized human antibodies capable of neutralizing various orthopoxviruses, and one of the virus-neutralizing epitopes of p35 protein was localized using synthetic biology and phage display. It shown that the localized virus-neutralizing epitope of the p35 protein is discontinuous, and amino acid residues in the 15–19 aa and 232–237 aa regions located on the ^{13}VIDRLPSETFPNVHEHINDQKF^{34} and ^{231}DNAAKYVEH^{239} loops, respectively, are involved in binding. To understand which critical amino acid residues are involved in the interaction with virus neutralizing antibodies, a fusion protein containing both regions of localized epitope (1–34 aa and 228–239 aa), united by a flexible peptide linker, was constructed. Based on this combined protein, a panel of 5 mutant proteins containing substitutions in both regions was obtained. Analysis of the specificity of the interaction of the obtained mutant variants of the main immunogenic protein of vaccinia virus p35 with existing neutralizing full-length human antibodies fh1A and fh8E, recognized the defined neutralizing epitope, showed that the changes introduced lead to the absence of antibody binding. The sera of donors immunized with vaccinia virus also did not detect deletion mutant variants of the p35 protein of orthopoxvirus in Western blot analysis. This study was supported by the Russian Scientific Foundation (Project #20-74-00135).

P-02.4-19
Targeting protein processing in acute myeloid leukemia

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Acute myeloid leukemia (AML) is an aggressive hematologic malignancy of which 40% of the cases have mutations in the Fms-like membrane receptor tyrosine kinase 3 (FLT3). This receptor in synthesized and processed in the endoplasmic reticulum (ER), and controls cell survival. Mutations in FLT3 prevent its correct processing and it is aberrantly retained in this organelle. This has led us to hypothesize that there are differences in the normal functioning of the ER in FLT3 wild-type or mutated cell lines. Since the ER is responsible for the folding and protein processing in the cell, we blocked these processes using reducing agents (dithiothreitol and 2-mercaptoethanol) and an N-glycosylation inhibitor (tunicamycin), on two wild-type and FLT3 mutant AML cell lines. After 48 hours of treatment, wild-type cells were severely

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affected by protein folding inhibitors. On the contrary, they were less sensitive to glycosylation inhibition compared to mutant lines. 24 hours exposure to a low dose of tunicamycin affected cellular levels of glycosylated proteins and FLT3 signaling, in addition to potentiation of the antitumor effect of kinase inhibitors (midostaurin and SGI) only in mutated cell lines. We could verify by western blot that doses of tunicamycin that are nontoxic to wild type cells trigger endoplasmic reticulum stress-induced death in AML mutant cell lines, supported by an increased expression of BiP and CHOP. Further studies on basal endoplasmic reticulum protein levels revealed differences in those involved in the process of protein folding (PDI, ERO, calnexin), and found increased in mutant lines. This could explain their higher tolerance to reducing agents. Current AML treatments are mainly based on the administration of FLT3 inhibitors but, in many cases, patients suffer a relapse. Our data suggest that wild-type and mutant cell lines differ in ER biology, and that the process of glycosylation and protein folding could be therapeutic targets for AML treatment.

P-02.4-20
Protein folding *in vivo* is a non-equilibrium process

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Protein re-folding experiments have led to the thermodynamics hypothesis according to which the native structures of proteins are uniquely defined by their primary sequences. However, a growing body of experiments has revealed the important role ribosomes have in co-translational folding, and there is even direct evidence that folding in vivo is a non-equilibrium, kinetic, process (published in: Alexander L et al. (2019) Nat Commun 10, 2709). In a dynamical process it is possible to ensure that the structural outcome is always the same, first, if the starting structure is always the same and, second, if the pathway followed from the initial structure is always the same as well. A non-equilibrium, dynamical pathway for protein folding in vivo that follows those two rules has been previously proposed in: Cruzeiro L (2020) Pure Appl. Chem. 92, 179–191. The idea is that the nascent chain of proteins is helical and that the first step in the folding pathway is the bending of that helix at specific amino acid sites. In this presentation, statistical evidence (previously published in: Cruzeiro L (2021) Biomolecules 11, 357), and dynamical simulation evidence will be presented in support of the proposed pathway.

P-02.4-21
An investigation into the role of turn-supporting motif in polyglutamine binding peptide (QBP1) in Huntingtin aggregation inhibition

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Huntington's disease (HD) is a progressive neurodegenerative disorder characterized by cognitive decline, motor dysfunction, and neuropsychiatric disturbances. The most prominent HD symptoms are related to chorea's, which is an involuntary and sudden muscle movement. It is caused by the expansion of the CAG tract from a threshold limit (>36) in the exon 1 region (HDx1) of the huntingtin gene IT-15, which results in the transformation of ubiquitously expressed huntingtin protein into the pathogenic huntingtin protein. It is imperative to devise strategies that could inhibit huntingtin aggregation. Polyglutamine binding peptide 1 (QBP1) Ac-SNWKWWPGIFD-am is a potent therapeutic peptide known to bind with pathogenic polyQ regions and prevent their transition monomeric protein to amyloid-like structures. QBP1 peptides harbor a Pro-Gly dipeptide motif, a characteristic feature of potential β-turn regions. This study shows that this turn-supporting dipeptide motif is essential for QBP1-mediated inhibition of huntingtin aggregation by using single amino-acid substitutions to generate analogs that could support, introduce, or eliminate the β-turn. Besides, this study identified, somewhat serendipitously, a minimal QBP1 analog that induces an α-helical conformation in the Trx-HDx1 protein. Previously published in Belwal et al. (2020) FEBS letters 594 (17), 2894–2903

P-02.4-22
Assembly of N-acetylated α-synuclein at the air–aqueous interface

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Alpha-synuclein (αS) is a Parkinson's disease biomarker and remains as intrinsically disordered protein in aqueous solution. Here, we demonstrate the self-assembly of N-terminally acetylated α-synuclein (Ac-αS) at the air–water interface. The protein is highly surface active and attains a surface pressure of ~22 mN/m when introduced in the sub-phase. In a compression-expansion cycle of Langmuir monolayer the protein attains a highest surface pressure of ~30 mN/m. A hysteresis is seen in the pressure-area (π-A) isotherm which signifies the self-assembly of the protein at the air-aqueous interface. The Langmuir-Blodgett thin film of the protein shows an α-helical signature in circular dichroism spectroscopic analysis. The anisotropy of the thin film is illustrated using linear dichroism spectroscopy. Previously published in: Mohapatra et al. (2021) Int J Biol Macromol 174 (2021), 69–76

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P-02.4-23
β2-microglobulin – a trigger for NLRP3 inflammasome activation in tumor-associated macrophages promoting multiple myeloma cell progression

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Pro-inflammatory macrophages, as significant constituents of the tumor microenvironment in multiple myeloma (MM), are key promoters of disease progression, bone destruction, and immune-impairment. Consequently, the identification of endogenous mediators of these inflammatory processes open novel therapeutic avenues against major pathological features of MM. We identify beta-2-microglobulin (β2m) as an important driver in the initiation of inflammation in myeloma-associated macrophages (MAMs). Lysosomal accumulation of phagocytosed β2m in patient derived MAMs promoted β2m amyloid aggregation, resulting in lysosomal rupture and ultimately in the production of active interleukin (IL)-1b and IL-18. Interestingly, this process strictly depended on the activation of the NALP3 inflammasome after β2m accumulation. Moreover, depletion or silencing of β2m in MM cells abrogated inflammasome activation in a murine MM model. Finally, the specific disruption of NLRP3 or IL-18 diminished tumor growth and osteolytic bone destruction normally promoted by β2m-induced inflammasome signaling. Taken together our results provide novel mechanistic evidence for β2m's role as an NALP3 inflammasome activator during MM pathogenesis. Moreover, inhibition of NALP3 highlights one potential novel therapeutic approach to combat this severe malignancy. *The authors marked with an asterisk equally contributed to the work.

P-02.4-24
The expression of small heat shock protein AllbpA from mycoplasma *Acholeplasma laidlawii* in *E. coli* cells promotes the formation of amyloid structures

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The main function of small heat shock proteins (sHSPs) is the prevention of protein aggregation in the cell. Besides, sHSPs have been shown to be involved in many other processes including the biofilm formation. Thus, in *E. coli*, two sHSPs IbpA and IbpB indirectly influence biofilm formation. When absent, cells are subjected to endogenous oxidative stress and consequent overproduction of indole, that in turn inhibits formation of the biofilm. Here we show that the overproduction of sHSP AllbpA from phytopathogenic mycoplasma *Acholeplasma laidlawii* restores at high temperatures the biofilm formation and increases the amyloid structures content in *E. coli* cells lacking their own IbpB. The full-length AllbpA and proteins with deletions of putative functional terminal motifs (AllbpAΔN12, AllbpAΔC14, AllbpAΔN12C14) were overexpressed in *Escherichia coli* wild-type and strains with deletions of own sHSPs (ΔEcIbpA or ΔEcIbpB). The crystal violet staining of the biofilms revealed that the biofilm formation was restored in ΔEcIbpB cells producing the full-length AllbpA. Furthermore, the Thioflavin S and Congo red staining of the biofilms revealed that the removal of one of sHSP in *E. coli*, with the simultaneous expression of AllbpA, leads to increased formation of amyloid structures. Increased amyloids were also observed during overexpression of AllbpAN12 in the *E. coli* ΔEcIbpA and AllbpAC14 by cells of the *E. coli* ΔEcIbpB, respectively. Amyloids in the matrix were also detected during overexpression of AllbpA with double deletion (AllbpAΔN12C14) in both knockout *E. coli* strains. Thus, the sHSPs can be involved in biofilm formation also via amyloids synthesis, however, the molecular mechanism of regulation requires further study. The work was supported by RFBR grant 20-34-90066.

P-02.4-25
Intrinsically disordered regions of alanine: glyoxylate aminotransferase shape its fitness and function

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Intrinsically disordered regions (IDR) play a key role in shaping the plasticity of proteins and often define their function. However, how protein evolution co-opts IDRs to impact on the population of native protein conformers and their individual fitness has remained unexplored. Alanine:glyoxylate aminotransferase (AGT) is a liver pyridoxal 5'-phosphate-dependent enzyme involved in the detoxification of glyoxylate and the cause of primary hyperoxaluria type I (PH1) when dysfunctional. In Caucasian populations, AGT is present in two allelic forms, the major (AGT-Ma) and the minor (AGT-Mi) alleles, the latter increasing the susceptibility of AGT to PH1-causing mutations. By solving the crystal structure of AGT-Mi we identified three distinct regions exposed to the solvent that have a defined structure in AGT-Ma but are disordered in AGT-Mi. Molecular dynamics showed that AGT-Mi samples more flexible conformations than AGT-Ma supporting the hypothesis that IDRs originate from an enhanced plasticity of the entire structure. Characterisation of variants from a library of these three regions shed light on their effect on enzymatic activity and intrinsic stability of AGT. In addition, the analysis of the behaviour of selected hits from the library in human cells, paired with determination of the interactome of AGT-Ma and AGT-Mi, revealed the impact of IDRs on protein fitness and function at a cellular level. This work establishes that naturally occurring conformers generating by taking advantage of subtle instability of a protein can modulate its function and intracellular fitness. *The authors marked with an asterisk equally contributed to the work.

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P-02.4-26
Conservative amyloidogenic regions in nucleoporins with FG-repeats

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Amyloids are a group of protein aggregates possessing a set of unusual features. Many functional amyloids required for different biological processes have been discovered. In these cases, protein aggregation seems to be beneficial for an organism. Therefore, this property might be conservative for orthologous proteins. At least one known example supports this hypothesis. The amyloid aggregates of the CPEB protein were suggested to play an important role in the long-term memory formation in *Aplysia californica*, *Drosophila melanogaster*, and *Mus musculus*. We suppose that the list of functional amyloid candidates can be extended with orthologous proteins which possess conservative regions with amyloid properties. A perspective example is nucleoporins with FG-repeats. For several of them from different species were demonstrated amyloid properties. We speculate that this feature may be common for the whole protein family. Nup49, Nup57, Nup100, Nup145, and Nsp1 are the most abundant proteins with FG-repeats in the yeast nuclear pore complex. For each of these nucleoporins, we retrieved at least 170 orthologous sequences of different *Opisthokonta* species from the EggNOG database. Further, we analyzed them with the ArchCandy program in combination with the IUPred tool to find unstructured aggregate-prone regions. According to the results, almost all orthologs of investigated nucleoporins can potentially form amyloids. We aligned protein sequences of the nucleoporin orthologs and assigned amyloidogenic scores to the alignment positions to find conservative aggregation-prone regions. Such fragments were found in Nup49, Nup57, and Nup145 proteins, and their orthologs, but not in Nup100 and Nsp1. Thus, we suggest that only three nucleoporins with FG-repeats possess conservative amyloid regions, which might reflect the potential functional role of their aggregation. The research was supported by the RFBR grant 20-34-70073.

Protein localization and dynamics

P-02.5-01
Mapping the network regulating transcription factor activity and dynamics by TF-FRAP

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Cells respond to their environment via an intricate cellular signaling network. Insight into this network regulating cell fate is important for controlling stem cell differentiation, understanding disease pathology and defining better regenerative medicine strategies. Changes in cell fate are characterized by changes in gene transcription. These changes are dictated by changes in (master) transcription factor (TF) activity. SOX9 is the master TF of cartilage development. However, its dysfunction is associated with diseases, such as cancer, osteoarthritis (OA), fibrosis, sclerosis, etc. Here, we present a new method to directly monitor changes in TF activity. We developed Transcription Factor – Fluorescence Recovery After Photobleaching (TF-FRAP) to measure SOX9 dynamics and activity in primary human chondrocytes (hPCs) to understand its role in OA pathology. We found that changes in SOX9 dynamics as measured by TF-FRAP correlated to its transcriptional activity. Higher DNA binding and longer residence time of SOX9 on DNA increased its target gene expression levels and vice versa. SOX9 dynamics studies on hPCs showed that its residence time and DNA binding is significantly lower in OA as compared to healthy hPCs. We cross-validated TF-FRAP data with ChIP-qPCR and quantified gene expression changes with RT-qPCR. Moreover, TF-FRAP also identified subpopulations of cells within a donor, based on distinct dynamic rates of SOX9. Distinct and diffused SOX9 nuclear localization patterns were observed in the healthy and OA hPCs respectively. Distinct nuclear localization patterns correlated to higher DNA binding rate and longer residence times in healthy hPCs as compared to OA hPCs. Our data indicate a differential response of SOX9, depending on the disease state of the hPCs. This may have implications for treatment strategies that aim to restore SOX9 function. We show for the first time that our TF-FRAP method enables monitoring TF activity in real-time in primary cells.

P-02.5-02
Molecular basis for the DNA damage-induced interaction between cytochrome c and the histone chaperone SET/TAF-Iβ

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During the DNA damage response, nucleosome eviction by histone chaperones provides access of repair machinery to DNA injuries. The histone chaperone and oncoprotein SET/template-activating factor-Iβ (SET) engages DNA repair response. Upon DNA insults, mitochondrial cytochrome c (Cc) reaches the cell nucleus, where it binds SET so as to inhibit its histone chaperone activity. SET functions as a homodimer in which each monomer consists of an N-end α-helix dimerization domain (residues 1–80), a globular α/β domain (a.k.a. earmuff; 81–225) and a low-complexity acidic region (LCAR; 226–277). Our previous data demonstrate that SET dimerization and earmuff domains are sufficient to bind both histones and Cc. To further characterize the SET-Cc complex, we deployed a methodological approach combining Electron Paramagnetic Resonance (EPR) and Nuclear Magnetic Resonance (NMR) with Small-Angle X-ray Scattering (SAXS). For such purpose, we have designed five single cysteine mutants across a SET construct lacking its disordered region, named SET-ΔC (residues 1 to 225). Cysteine residues were bound

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to either nitroxide spin or ^{19}F probes. Continuous-wave EPR spectra of the spin probe and chemical-shift perturbations of ^{19}F resonances were assessed to determine those regions from SET-ΔC interacting with Cc. Paramagnetic Relaxation Enhancement NMR (PRE-NMR) measurements induced by the SET spin probe onto the Cc surface deciphered those residues of the heme-protein involved in the complex formation. SAXS experiments enabled to obtain a low-resolution model of the SET-Cc complex. Altogether, our findings indicate that Cc recognizes the globular domains of SET, where histones bind to, so providing a molecular basis for histone chaperone inhibition activity.

P-02.5-03
Antimicrobial compounds of farm animal mucous membranes and glands

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Cattle (tongue and larynx, nasal and oral cavities, rectum, as well as submandibular salivary and lymph glands) and pork (larynx, tongue, labial and nasal cavities, rectum and sublingual salivary glands) mucous membranes were used as sources of antimicrobial substances. Histones of different types were found in studied bovine and pork samples, which themselves possess an antimicrobial activity and can make a great contribution to the formation of peptides with the same action as well as a lot of other antimicrobial proteins. Among bovine tissues, the highest content of tissue-specific peptides was observed in the oral mucosa (77), among pork tissues – in the mucous membranes of the nasal cavity (178), rectum (150) and larynx (166). Special attention was paid to the adaptation of the flow cytometry method to the determination of antimicrobial activity during the whole period of the project. A number of algorithms were worked out using such dyes as EvaGreen, PI, Sybr Green, Syto 9, cFDA, which allowed to form the main and alternative approaches to determining the number of living and dead bacterial cells. The efficiency of enzymatic treatment (collagenase, elastase) was observed for unpacking AMPs. In accordance with the selection criteria (the value of antimicrobial activity against *Staphylococcus aureus* and *Pseudomonas aeruginosa* in the short-term and long-term periods), 16 samples of animal origin were selected amount of living cells in relation to the control was not exceed 50%, often was not higher 25%). The obtained data confirmed that efficiency of use some slaughter waste as a source of natural AMPs, which are safe for usage. In addition, this raw material is easily available and cost-effective, and facilitates an improvement of ecological situation due to reduction of waste volumes by their processing.

P-02.5-04
Structure and dynamics of the intracellular domain of toll-like receptor 1: the basis for Zn^{2+} modulating the receptor signaling

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The family of Toll-Like Receptor (TLR) refers to the first type of membrane proteins. TLRs play a critical role in innate immunity as the first line of host defense. The medical and biological significance of the TLR signaling is obvious, since the dysregulation of the TLR system causes various autoimmune diseases and septic shock, and some therapeutic strategies targeting TLRs have already emerged. Despite the fact that the general scheme of the operation of TLR receptors is known and even there are structures of individual fragments for some proteins of this family, the detailed mechanism of receptor functioning remains unclear. The research presents the results of the structure and dynamics of the TLR1 toll-interleukin-like (TIR) cytoplasmic domain in crystal and in solution. The work demonstrates data on specific binding of the TLR1 TIR with the zinc ions with nanomolar affinity. Cysteine residues 667 and 686 are mediated interactions between TLR1 TIR and Zn, and C667 is required for Zn binding. Using functional assays for the heterodimeric TLR1 /2 receptor, the effect of Zn addition and Zn depletion on TLR1 activity was shown, and also was shown the key role of 667 cysteine: the C667A mutation disrupts the activity of the receptor. Analysis of the data presented in the work suggests that the ability of the TIR domain of TLR1 to bind zinc is critical for receptor activation. The work was supported by the grants of Russian Foundation for Basic research (#20-34-70024, NMR analysis), National Natural Science Foundation of China (21877106, 21807098), Pioneer Hundred Talents Program (CAS) and by the Ministry of Science and Higher Education of the Russian Federation (agreement #075-00337-20-03, project FSMG - 2020-0003, X-ray crystallography).

P-02.5-05
Novel approach to the expression of the mitoBKCa channel

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Ischemia/reperfusion (I/R) injury of brain and heart tissue is one of the most common causes of death in most Western countries. Activation of mitochondrial potassium channels is a promising direction for development of therapeutic strategy reducing tissue damage after I/R induced insults. The mitochondrial large conductance calcium activated potassium ($mitoBK_{Ca}$) channel is present in both heart and brain. Experimental data show that

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activation of the channel with potassium channel openers results in cardioprotection. Expression of the mitoBK$_{Ca}$ channel in HEK293T cells as a model system. The pore forming subunit of the channel is encoded by KCNMA1 gene and BK-DEC splice variant has been demonstrated to localize to mitochondria. However, it was not known whether this isoform forms an active channel in mitochondria. After transient transfection of HEK293T cells with plasmid encoding this splice variant we were able to record electrophysiological activity of this channel in isolated mitoplasts. The recorded channel had all properties typical for mitoBK$_{Ca}$. Interestingly, the activity of this channel was not detected in wild-type cells. Additionally, in untransfected cells we detected expression of the regulatory ß4 subunit of the channel. The presence of this regulatory subunit was verified using both western blot and quantitative PCR reaction. Our data also suggests that ß4 might localize to mitochondria. Results of our experiments show that BK-DEC splice variant forms a fully functional channel in the inner mitochondrial membrane. Moreover, the wild-type cells might express ß4 subunit of the channel. The presence of the ß4 in wild type cells raises the question about the role of this protein in mitochondria. Additionally, our data shows that the HEK293T cells can be used as a model for mitochondrial potassium channel research. This project was supported by Polish National Science Centre grant No.2015/18/E/NZ1/00737 and the Nencki Institute of Experimental Biology.

P-02.5-06
Argonaute's distribution in protrusional structures reveals its implementation in intercellular trafficking and in cell segregation

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Argonaute (AGO) proteins are at the hub of all the fundamental complexes in RNA bioprocesses. They play a key role in gene regulation through silencing, by guiding translational repression, in a post-transcriptional level. In the canonical RNAi pathway, AGO2 functions as the catalytic enzyme that cleaves mRNA-targets through miRNA-loading whilst AGO3 has a recently uncovered, complicated, and selective slicer activity regarding its substrates. AGO2 is compartmentalized into structures such as GW- and P-bodies, stress granules and adherens junctions as well as the midbody. Here we show using immunofluorescence, image- and bioinformatic analysis and cytogenetics that AGO2 also resides in membrane protrusions such as open- and close-ended tubes. The latter are cytokinetic bridges where AGO2 colocalizes at the midbody-arms with cytoskeletal components such as α-Tubulin and Aurora B, and various kinases. AGO2, phosphorylated on serine 387 is located together with Dicer at the midbody ring in a manner dependent on p38 MAPK activity. We further show that AGO2 is stress sensitive and important to ensure the proper chromosome segregation and cytokinetic fidelity. We suggest that AGO2 is part of a regulatory mechanism triggered by cytokinetic stress to generate the appropriate micro-environment for local transcript homeostasis.

P-02.5-07
Increased sensitivity of MS- based proteomics methods obtained by sample fractionation technique

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The Chromosome-centric Human Proteome Project (C-HPP) aims to find high-stringency evidence for all proteins encoded by the human genome, the major splice forms of each protein. The Russian part of this project consists of detecting proteins encoded by the 18th human chromosome. In this work HT-29 human cell line was used as a biological object of interest. Whole-cell protein extract was prepared and digested by trypsin protease. Fifteen and sixty-three proteins encoded by the 18th human chromosome could be detected in complex peptide sample by shotgun mass-spectrometry (MS) and Multiple Reaction Monitoring with stable isotope-labelled standards (MRM SIS) respectively. MRM SIS is 6-fold more sensitive compared to shotgun MS, but due to the high complexity of peptide sample (4359 unique peptides identified by shotgun MS, related to 1200 proteins), some of the identifications detected by MRM SIS were doubtful (low signal, signal interference). To decrease peptide sample complexity and increase the sensitivity of shotgun mass-spectrometry and MRM SIS methods peptide sample fractionation technique was used. It was shown that reversed-phase liquid chromatography (RP-LC) in alkaline conditions performed prior to MS analysis allowed to confirm all the doubtful identifications detected by MRM SIS and to detect 11 additional proteins encoded by the 18th human chromosome with shotgun MS (11750 unique peptides identified, related to 2837 proteins). Thus, using RP-LC in alkaline conditions allowed to detect 63 proteins by MRM SIS and 34 proteins by shotgun MS (18 protein identifications were common for both methods), 45 proteins were detected by MRM SIS only, 16 proteins were detected by shotgun MS only. In summary 79 proteins encoded by the 18th human chromosome were detected. Applying RP-LC in alkaline conditions in combination with shotgun MS and MRM SIS allows deeper proteome coverage in chromosome-centric way.

P-02.5-08
A structural mechanism of DNA polymerase lambda action that promotes error-free bypass of bulky modifications in DNA

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Different environmental factors such as ultraviolet, ionizing radiation and air pollutions are influence on the genomic DNA damage that negatively impact on the human cells. The products of incomplete combustion of hydrocarbon fuels and waste of various industries are main sources of polycyclic aromatic hydrocarbons. DNA can be damaged by several metabolites of these compounds through forming the bulky DNA adducts that can lead to cancer and mutagenesis. Benzo(a)pyrene is the most widely known polycyclic aromatic hydrocarbon. BaP after metabolic activation, to toxic and reactive intermediates, reacts with DNA to form bulky adducts with mutagenic, and carcinogenic

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properties. Such damages can be repaired. DNA pol λ is a eukaryotic enzyme belonging to the pol X family. Pol λ consists of two domains: 31 kDa polymerization domain (bearing the three conserved subdomains: fingers, palm, thumb) and 8 kDa domain. Pol λ has a dRP lyase activity, and play an important role in base excision repair (BER). Also, DNA pol λ has been suggested to play a role in meiotic recombination and DNA repair. In order to find out what effect on the localization of the protein in the active center of the enzyme affects the presence of the total number of BPDE-N2-dG residues that are in different regions, we carried out molecular modeling using the molecular dynamics method of the complexes of the enzyme with DNA duplex containing BPDE-N2-dG in the central part of the duplex. The complex also contains dCTP, forming a complementary pair with BPDE-N2-dG; triphosphate coordinated by the Mg^{2+} ion and is in a position of readiness for the reactions of incorporation of the nucleotide into the DNA chain. In summary, we have shown here how a family X polymerase utilizes subtle active site adaptations to carry out a critical repair reaction. The work was supported by RFBR grants (19-34-90052, 18-04-00596).

P-02.5-09
Atypical antipsychotic clozapine binds fibrinogen and affects fibrin formation

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Clozapine is an atypical antipsychotic used for the treatment of schizophrenia. Prescribed daily doses of clozapine may reach over 900 mg/day. Some studies reported a connection between clozapine usage and the development of thrombosis. Our *in vitro* study aimed to provide insight into molecular bases of this observation, investigating clozapine binding to isolated fibrinogen, the main protein involved in hemostasis. Fibrinogen/clozapine interaction was confirmed by protein fluorescence quenching, with affinity constant calculated to be 1.7×10^5 M^{-1} and the number of binding sites more than one. Direct interactions do not affect the structure of fibrinogen, as determined by UV-VIS spectrometry and Fourier-transform infrared spectroscopy, nor fibrinogen melting temperature, examined by fluorescence spectroscopy. However, clozapine binding affected fibrin formation, by reducing coagulation speed and thickness of fibrin fibers. This behavior suggests that in the presence of clozapine, fibrinogen may acquire thrombogenic characteristics. Although no difference in fibrin gel porosity was detected, other factors present in the blood may act synergistically with altered fibrin formation to modify fibrin clot, thus increasing the risk for development of thrombosis in individuals on clozapine treatment. By ORAC and HORAC antioxidant assays, we found that clozapine efficiently protects fibrinogen from free-radicals oxidation. Since the effect of clozapine on fibrin formation is dose-dependent, it seems that the dosage of the medication could be the main factor that determines if clozapine will have a more positive or negative effect on fibrinogen and coagulation process *in vivo*.

P-02.5-10
Glucose stimuli prompts insulin secretion by human spermatozoa

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Spermatogenesis is sensitive to metabolic alterations, where insulin is considered one of the most important regulators. Even 100 years upon its discovery, not much is known concerning the role of insulin in the testis. It is hypothesized that insulin plays a major role on human spermatozoa capacitation, a phenomenon where spermatozoa suffer morpho-physiological alterations in order to achieve fertilization capacity. However, the molecular mechanisms remain to be elucidated. Herein, we aimed to evaluate the insulin synthesis and secretion capacity of human spermatozoa, by assessing the expression of enzymes responsible for proinsulin cleavage, PC1/3 and PC2. In addition, our goal was to assess whether insulin secretion was responsive to glucose stimuli. For this purpose, human sperm samples from normozoospermic men were used (n=15). A density gradient protocol was performed and two fractions of spermatozoa were then collected according to its motility condition (high vs low motility). Gene expression of insulin, PC1/3 and PC2 mRNA was evaluated by RT-qPCR in both spermatozoa fractions. Protein expression of insulin, PC1/3 and PC2 in spermatozoa was evaluated by immunofluorescence. The fraction of highly motile spermatozoa was incubated in culture medium under capacitating conditions and supplemented with increasing glucose concentrations (in mM: 0, 5.5, 11 and 22). Insulin concentrations in the medium 6 h later were quantified by ELISA. Our results showed that insulin, PC1/3 and PC2 mRNA, as well as the respective proteins, are expressed in human spermatozoa. The mRNA expression was found to be higher in highly motile spermatozoa. Additionally, human spermatozoa released insulin to the medium in a glucose concentration-dependent manner. This study shows that insulin plays a role in human spermatozoa capacitation though the mechanisms mediated by insulin remain unknown, opening an exciting new line of investigation.

P-02.5-11
New potential role of Vps34 kinase in the control of the cell size

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Platelets, the smallest blood cells, are produced in the bone marrow by their precursors, megakaryocytes (MKs). One of the most characteristic features of the MK maturation is a substantial increase in size, together with the polyploidization of the nucleus. At the end of the maturation process, MKs generate prolonged cytoplasmic protrusions, termed proplatelets, which extend through the vascular sinusoids of the bone marrow and release

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platelets into the bloodstream. Phosphoinositides are small membrane phospholipids implicated in cellular signalling, organelle trafficking and cytoskeletal dynamics. Phosphatidylinositol 3-monophosphate (PI3P), which is mainly produced by the Vps34 kinase, is a key component in vesicular trafficking processes, as well as autophagy and mTOR signaling. Nucleolus is nuclear subcompartment rich in RNA and RNA–protein complexes. It is the site of different steps of ribosome biogenesis, including transcription of ribosomal genes (rDNAs) and processing of ribosomal RNAs (rRNAs). In this study we show that in immature small MKs majority of Vps34 kinase localizes in organized structures within nucleolus, in fibrillar center (FC), where transcription of rDNA occurs. Treatment of MKs with RNA Pol I inhibitors abolishes Vps34 localization in nucleolus. In addition, when we specifically inhibit Vps34 in immature MKs, they fail to increase in size, and express lower levels of GPIb, indicating failure in maturation. All together, these data indicate that Vps34 might play an important, still undescribed, role in the nucleolar structure organization and/or in the control of MKs size and maturation via ribosome biogenesis. Additional studies are underway for better understanding of these events. Acknowledgement: this work is supported by American Society of Hematology Global Research Award and University of Rijeka support grant no. 18-188-1343. I.B. is supported by Croatian Science Foundation (HRZZ-09-2016).

P-02.5-12
IQGAP-related protein IqgC involved in large-scale endocytosis regulates cell-substratum adhesion and migration in Dictyostelium

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The same building blocks and regulatory modules of the actin cytoskeleton are often utilized by the cell to perform multiple functions, but how their use is balanced between often competing processes is not well understood. Involvement of small GTPases from the Ras superfamily in the regulation of a variety of cellular processes, e.g. cell migration, large-scale endocytosis, cell division, and membrane trafficking, is a case in point. In Dictyostelium amoeba and other cells, Ras GTPases regulate the formation of transient actin-based assemblies such as pseudopodia, filopodia, ruffles and endocytotic cups by alternating between inactive (GDP-bound) and active (GTP-bound) forms. Thereby, they are primarily deactivated by GTPase-activating proteins RasGAPs. We recently characterized an untypical IQGAP-related protein from Dictyostelium, IqgC, as a RasG-specific GAP. Furthermore, we showed that IqgC localizes to macropinosomes and phagosomes and supresses RasG signaling during macropinocytosis and phagocytosis. Since it has been suggested that macropinocytosis and cell migration are negatively correlated, we proceeded to check whether IqgC has an effect on cell migration. Indeed, we determined that the speed of migration is positively correlated with overexpression of IqgC, which is exactly opposite to what we have previously shown for macropinocytosis. Interestingly, IqgC-null cells displayed a strongly diminished strength of the cell-substratum adhesion, as shown in a detachment assay using a rotary shaker. Consistent with this finding, TIRF microscopy showed that fluorescently labelled IqgC is localized to the stationary puncta at the ventral plasma membrane, which are known to harbour other proteins involved in cell-substratum adhesion such as talin and paxillin. Since RasG has previously been shown to positively regulate cell adhesion, our results indicate that IqgC regulates cell adhesion via a mechanism independent of its RasG-GAP activity.

P-02.5-13
Resolving DPC: analysis of repair pathway

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Common type of DNA lesions are DNA-protein crosslinks (DPC), a result of a covalent interaction of proteins involved in DNA maintenance and DNA molecule, due to endogenous and exogenous environmental conditions (previously published in: Stingele J, Jentsch, S (2015) Nat Rev Mol Cell Biol 16, 455–460). Spartan is one of the rare human proteins whose function is involved in resolving DPC (previously published in: Lopez-Mosqueda J et al. (2016) eLife 5:e21491). Mutations in SPRTN gene in humans cause hepatocellular carcinoma, as well as chromosomal breakage and Ruijs-Aalfs type of progeria (previously published in: Lessel D et al. (2014) Nat Genet 11,1239–1244). We hypothesized that Spartan protein regulates the activation of DPC repair pathway and that cells without Spartan function will have altered expression levels of genes related to DNA maintenance and repair. To test our hypothesis, we have compared cells with endogenous Spartan protein function with cells silenced for Sprtn gene and with or without reconstitution of Spartan with exogenous wild-type or mutated Spartan. Cells were exposed to different types and intensities of genotoxic stress and the level of gene expression was measured on mRNA level using RT^2 profiler PCR array, as well as RNA sequencing. The results obtained were further confirmed with protein mass spectrometry, pull-down, western blot and flow cytometry analysis. The aim of our study was to determine how Spartan influences the activity of other DNA repair genes expression and to elucidate the mechanism of DPC resolving pathway. *The authors marked with an asterisk equally contributed to the work.

P-02.5-14
Dihydro-alpha-lipoic acid binds and protects fibrinogen from oxidation and affects fibrin formation

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A reduced form of the alpha-lipoic acid, dihydro-alpha-lipoic acid (DHLA) is a potent, naturally occurring antioxidant that is found in higher amounts in plants like spinach, broccoli, potatoes, tomatoes, carrots, beets and rice. DHLA can be consumed as a food supplement as well, at doses up to 600 mg/day. DHLA has an inhibitory effect on coagulation as it can reduce concentrations of some coagulation factors. This study investigated a direct interaction between DHLA and fibrinogen, the main protein in coagulation and hemostasis. DHLA binds fibrinogen with a moderate straight. Calculated constant from spectrofluorimetric titration for DHLA/fibrinogen complex was 10^4 M^{-1}. Fibrinogen

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stability remains the same with only marginal structural changes in its secondary structure favouring more ordered molecular organisation upon DHLA binding, as determined by Fourier-transform infrared spectroscopy. Coagulation assay showed that fibrinogen with bound DHLA forms fibrin with thicker fibres, as measured by coagulation assay and is protected from oxidation to a certain extent. Docking analysis showed that DHLA may bind fibrinogen in its D regions, which are directly involved in the fibrin formation. Obtained results support beneficial effects of DHLA on fibrinogen and consequently on coagulation process, suggesting that DHLA supplementation may be indicated for persons with an increased risk of developing thrombotic complications, particularly those whose fibrin is characterised by increased oxidative modification and formation of thinner and less porous fibres. Although further investigation is needed, our results suggest that DHLA in complex with fibrinogen can be located at the site of injury where it may exert antioxidant effects. This work was supported by the Ministry of Education, Science and Technological Development of the Republic of Serbia

P-02.5-15
The impact of glycosylation and phosphorylation on cellular localization of cystatin F active form

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Cystatin F is a cysteine peptidase inhibitor. Among other differences that distinguish it from other members of type II family, cystatin F possesses three glycosylation sites and is localized in endo/lysosomes. Cleavage of 15 N-terminal amino acids enhances monomerization and turns cystatin F into an active form in which it becomes a potent inhibitor of cysteine cathepsines C, H and L, localized in the endo/lysosomes. It has been shown that degree of glycosylation influences secretion and uptake of cystatin F inactive form and that transition is facilitated by binding of phosphorylated glycans to M6P-receptor. The aim of this thesis was to determine whether the degree of glycosylation has an effect on secretion, reuptake and cellular localization of active form of cystatin F. For this purpose, we prepared single, double and triple non-glycosylated mutants of active form and examined their expression, secretion and reuptake on HeLa cells, that normally do not express cystatin F. We determined whether mutants are secreted and taken up by the cells by western blot analysis. Using fluorescent confocal microscopy, we analyzed the intracellular localization of our mutants. As a control, glycosylated active form was used, which is secreted from cells as well as localized in endo/lysosomes. Our results show that only active cystatin F, non-glycosylated on asparagine 115 can be taken up to the cell and is localized in endo/lysosomes. Other non-glycosylated mutants are not localized in the endo/lysosomes and cannot be taken up by the cells. Secretion of mutants that are not glycosylated on asparagines 61 and 62 shows us that glycosylation on asparagine 115 affects secretion of the protein. Glycosylation on asparagines 61 and 62 has a big impact on secretion, protein uptake and localization of active form of cystatin F. Results also indicate that the transfer of the active form across membranes is not affected by possible O-glycosylation or phosphorylation on glycosylation sites. *The authors marked with an asterisk equally contributed to the work.

P-02.5-16
A comprehensive view on LINE-1 ORF1p granule composition

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Long Interspersed Nuclear Element 1 (LINE-1) remains the only autonomously active retrotransposon in human. Its transcription results in a bicistronic mRNA which encodes two proteins: ORF1p and ORF2p. After translation they preferentially interact in cis with their encoding mRNA and form a ribonucleoprotein particle – an important intermediate for retrotransposition. Besides the endonuclease and reverse transcriptase activities of ORF2p, ORF1p is essential for the process as well, but apart from its nucleic acid binding and chaperone activity its exact roles remain unclear. In cells, ORF1p is mostly compacted within cytoplasmic granules. We aimed to better characterise these granules and, additionally, to map the interactome of ORF1p in living cells in order to better understand the importance of the protein for retrotransposition. Using immunocytochemistry, we ascertained that cytoplasmic ORF1p granules differ from stress granules (SGs) as well as from processing bodies (P-bodies). After exposure of cells to different exogenous stressors, however, ORF1p clearly relocates to granules containing bona fide markers of SGs. P-bodies do not overlap with ORF1p granules neither in unstressed nor in stressed cells, although both are frequently in physical contact with one another, especially upon stress. In order to gain a comprehensive view on the composition of ORF1p granules, we used a novel proximity-based labelling method, i.e. biotin identification (BioID), with biotin ligase fused to the C-terminus of ORF1p. Unlike immunoprecipitation, the method enables identification of stable as well as weak and transient protein interaction partners in the environment of living cells. In conclusion, revelation of ORF1p protein network sheds light on known as well as novel potential activators and repressors of retrotransposition and paves the way towards a better understanding of the complexity of the process and its regulation.

P-02.5-17
An attempt to identify proteins interacting with p66Shc in the mitochondria associated membranes

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An adaptor protein p66Shc, participates not only in mitogenic signalling, but also affects cellular redox state and promotes cell death by increasing mitochondrial reactive oxygen species (ROS) production. Localization of p66Shc in the region of endoplasmic reticulum (ER) closely interacting with mitochondria (mitochondria associated membranes (MAM) fraction), has been previously suggested however the details were still under debate. The aim of the presented study is to identify proteins interacting with p66Shc in MAM in order to clarify the mechanism by which

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p66Shc influences cellular redox state. To investigate p66Shc interactions with other proteins under physiological conditions, protein complexes were co-immunoprecipitated (co-IP) from MAM fraction samples prepared from mice livers, with the use of five, various, specific, anti-SHCA antibodies and then subjected to mass spectrometry (MS) analysis. The MS analysis revealed 197 potentially interacting proteins among which over 30 are involved in the response to oxidative stress or related to mitochondrial bioenergetics. In our studies we were interested in the group of proteins of mitochondrial or ER origin found in MAM simultaneously related to oxidative stress and mitochondrial bioenergetics. Then we verified whether these selected proteins were present together with p66Shc in the protein complexes isolated from MAM using techniques like two dimensional gel electrophoresis and co-IP. None of the respiratory chain related proteins were confirmed to interact with p66Shc so far. Interestingly, such approach didn't confirm also the interaction of p66Shc with cytochrome c, what was previously proposed in order to explain the involvement of p66Shc in mitochondrial ROS production. Moreover, our data supported the reports showing that p66Shc is not detectable in pure mitochondrial fraction, but its presence has been confirmed in MAM, where it may interact with proteins annotated to other organelles.

P-02.5-18
The COMM-domain containing protein 1 interacts with the guanine-rich RNA sequence binding factor 1 and regulates its subcellular distribution

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Background: The guanine-rich RNA sequence binding factor 1 (GRSF1) is an RNA-binding protein of the hnRNP H/F family and has been implicated in different cell functions. GRSF1 can also be located in the mitochondria and plays an important role in mitochondrial RNA metabolism (Jourdain et al. 2013, Cell Metabolism, 17(3), 399–410). COMMD1 belongs to the family of COMM-domain containing proteins and has been implicated in different cellular processes such as copper homeostasis, the function of the epithelial sodium channel or cell proliferation. Recent studies suggest the possibility that regulation of the ubiquitin pathway may constitute the functional basis for the biological activity of COMMD1 (Maine et al. 2007, Cell and Mol Life Sciences, 64(15), 1997–2005). Methods: We employed the yeast two-hybrid system to search for GRSF1-binding proteins and carried out co-immunopreciptation experiments to confirm this protein-protein interaction in living cells. To further characterize this protein–protein interaction, we performed siRNA mediated knock-down in human cell lines followed by co-immunoprecipitation experiments and differential centrifugation experiments. Results: Using the yeast two-hybrid system we identified the COMMD1 protein as GRSF1 binding partner. It interacts with the alanine-rich domain of GRSF1 in reconstituted in vitro system but also in cells expressing the two proteins. Lack of COMMD1 has no effect on GRSF1 ubiquitination but reduces the mitochondrial GRSF1 content. Conclusion: These data indicate that COMMD1 specifically interacts with GRSF1 in mammalian cells. Lack of COMMD1 prevented mitochondrial import of this RNA-binding protein. General significance: This is the first report describing a protein-binding partner for GRSF1, which modifies the subcellular distribution pattern of this RNA-binding protein. (Dumoulin et al. 11/2020, Biochem Biophys Acta - General Subjects)

P-02.5-19
Nucleophosmin interactome in acute myeloid leukemia

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Nucleophosmin (NPM), one of the most abundant nucleolar phosphoproteins, is involved in initiation and/or development of acute myeloid leukemia (AML). AML-associated NPM1 gene mutations lead to altered aminoacid sequence at the C-terminal part of NPM and to aberrant cytoplasmic localization of the mutated protein (NPMmut). NPM serves as a chaperone, and majority of its interactions are mediated by its N-terminal oligomerization domain, although proteins interacting with regions near the NPM C-terminus were also reported. In any case, NPM broad interaction network is significantly affected by the mutation: whereas some interactions are disrupted, others are retained, the interacting proteins being dislocated along with the NPMmut. By combination of standard biochemical methods and time-resolved fluorescence techniques, we established a robust experimental system for analysis of NPM interactome in both cell lysates and live cells. We focused in detail on impact of NPM mutations on NPM interaction with tumor suppressor p53 and we analyzed an effect of nuclear-export-inhibitor, selinexor, on this complex. We confirmed the presence of p53 in cytoplasm of NPMmut co-transfected cells suggesting the NPM-p53 interaction is not disrupted by the AML-associated NPM mutation. N-terminal NPM oligomerization domain was also found to be non-essential for the interaction. These results were confirmed both in cell lysates by immunoprecipitation and in live cells by FLIM-FRET measurements. Another NPM variant, lacking N-domain and concurrently mutated at the C-terminus, caused extensive p53-cytoplasmic localization, but its interaction with p53 was substantially attenuated. We monitored time-course of selinexor-induced NPM/p53 nuclear relocalization and we found that individual proteins revert into the nucleus with different rates. These results indicate highly dynamic character of the NPM-p53 interaction. The work was supported by the Czech Science Foundation grant No. 19-04099S.

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P-02.5-20
Temperature-induced structural transitions in epidermal growth factor

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Human epidermal growth factor (EGF) consists of 53 amino acid (AA) residues, and it has a dynamic conformational structure. According to the known data, the amount of EGF AA residues in antiparallel β-sheets varies from 7 to 35%, while the amount of AA residues in α-helices varies from 0 to 23% depending on experimental conditions. Circular dichroism (CD) spectra for synthetic EGF with native AA sequence and EGF reduced by tris(2-carboxyethyl)phosphine were obtained on a Chirascan CD spectrometer at physiological pH during the heating from 20°C to 50°C. EGF has been dissolved in 0.01M phosphate buffer (pH 7.4). EGF concentration in obtained saturated solution was 495.3 μg/ml. Deconvolution of CD spectra was performed by BeStSel server. The amount of AA residues forming antiparallel β-sheets is significantly higher in reduced EGF than in native EGF (33% vs. 24%, $P = 0.012$), while the content of α-helices is significantly lower (16% vs. 20%, $P = 0.033$). Taken together, the EGF conformational state is almost identical at 20°C, 25°C, and 50°C, but at 35°C the highest amount of AA residues forming antiparallel β-sheets both in native and reduced EGF was observed (31% and 46%, respectively). In contrast, at 40°C the number of AA residues forming antiparallel β-sheets both in native and reduced EGF was the lowest (11% and 9%, respectively). The number of AA residues forming α-helices both in native and reduced EGF was minimal at 35°C (14% and 13%, respectively), and it was maximal at 40°C (24% for both). There is evidence that significant conformational changes occur in both native EGF and EGF with reduced disulfide bonds at the temperature between 35°C and 40°C. Based on known structural data, the EGF conformation observed at about 40°C is similar to PDB ID 1JL9: there are no β-strands, but just four residues making two beta bridges (Cys20 with Cys31 and Val34 with Tyr37). Exactly in such a relaxed state, EGF should be able to form a functional complex with its receptor.

P-02.5-21
Engineering and characterization of human 14-3-3 zeta with the controlled oligomer dynamics

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14-3-3 proteins coordinate numerous intracellular processes via binding to phosphopartners and also show phosphorylation-independent chaperone-like activity (CLA) by preventing aggregation of misfolded or denatured proteins. Although 14-3-3 proteins form dimers, phosphorylation of Ser58 in the interface induces partial dimer dissociation. It was proposed that, depending on the oligomeric state, 14-3-3 may play different roles in the cell. Here, we endow human 14-3-3 zeta with the engineered A16C and S58C mutations, which would fix the dimer interface upon oxidation and open new perspectives to study properties of 14-3-3 zeta depending on dimer dissociation. According to size-exclusion chromatography and differential scanning calorimetry, the A16C mutation preserves dimerization of recombinant human 14-3-3 zeta, whereas S58C mutation causes partial dimer destabilization. After finding conditions for an efficient 14-3-3 zeta A16C/S58C (=14-3-3Zcc) oxidation without using specific chemicals, we confirmed that such modification does not disturb 14-3-3Zcc interaction with phosphorylated human HSPB6, as compared with the wild-type 14-3-3 zeta (=14-3-3Zwt). Tryptophan fluorescence at a constant heating rate indicated that the thermal stability of the oxidized 14-3-3Zcc is 15 C° higher than that of the 14-3-3Zwt, confirming fixation of 14-3-3Zcc dimer by disulfide bridges and indirectly supporting the dissociative mechanism of 14-3-3 zeta denaturation. Finally, we assessed the CLA of 14-3-3Zcc on the thermally induced aggregation of myosin subfragment 1 (S1). Our preliminary data suggest that the oxidation of 14-3-3Zcc and fixation of its dimer interface decreases its CLA on aggregating S1, suggesting the functional role of the interface region in the anti-aggregation activity of 14-3-3 and warranting further studies using other protein substrates and different chaperone:substrate ratios. This work was supported by RSF grant no. 19-74-10031

P-02.5-22
Engineered disulfide in the orange carotenoid protein enables control of its structural dynamics associated with photoprotection function and carotenoid transfer

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The Orange Carotenoid Protein (OCP) plays a key role in the cyanobacterial photoprotection via the reversible photoactivation from the orange to the red form, binding to the light-harvesting complexes (phycobilisomes, PBs), and heat dissipation of the excess energy. Photoactivation entails significant conformational rearrangements of a compact dark-adapted state including the detachment of the N-terminal extension (NTE) from the C-terminal domain (CTD) and domain separation. The PBs binding and quenching ability of OCP is inactivated by the Fluorescence Recovery Protein (FRP) and, in principle, can be modulated by the natural C-terminal domain homolog (CTDH) through the light-controlled carotenoid arrest. OCP functioning seems to depend on domain separation and exposure of the CTD surface covered by NTE. Here, we devise an OCP variant with the NTE trapped on the CTD via an engineered disulfide (OCPcc). NTE trapping preserves OCP photoactivity but weakens functional interaction of OCP with FRP and PBs during photoactivation, which is completely restored upon reduction of the introduced

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disulfide. Interestingly, NTE trapping did not fully abolish but reduced the apparent efficiency of carotenoid transfer to CTDH. Non-interacting with the dark-adapted oxidized OCPcc, FRP binds dark-adapted reduced OCPcc nearly as efficiently as OCP devoid of the NTE, suggesting that the low-affinity FRP binding to dark-adapted OCP is realized via NTE displacement. The unexpected PBs fluorescence lifetime (~600 ps) in the presence of photoactivated oxidized OCPcc indicates that it binds to PBs in an orientation suboptimal for quenching PBs fluorescence. This approach shows the effective redox controlled uncoupling of the OCP spectral and functional photoactivation. Partially supported by RFBR grant no. 20-54-12018. Previously published in: Slonimskiy YB et al. (2020) BBA - Bioenerg. 148174.

P-02.5-23
Structure, function and localization of NME6 – a member of the NME/NDP kinase family

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NME6 is a member of the nucleoside diphosphate kinase (NDPK/NME/Nm23) family, enzymes that catalyze the transfer of gamma phosphate from ATP to other nucleoside diphosphates. The family has attracted considerable interest due to the involvement of its members in metastasis suppression. The family is divided into two groups. Group I (NME1–NME4) are highly conserved in their amino acid sequence and exhibit the NDPK activity, while Group II (NME5-NME9) members display less homology and seem to lack NDPK activity. Some of the Group II members (NME5–7) arose very early in the evolution of Metazoa, therefore, it is presumed that they participate in one or more basic cellular process. Since little is known about Group II members, we focused our studies on revealing the subcellular localization, quaternary structure and function of the Group II human NME6 protein. The expression of NME6 was screened in several human tumor cell lines by western blot, using specific anti-NME6 antibodies. All of them express significant amounts of NME6. Fluorescent reporter systems coupled with confocal microscopy as well as cell fractionation were used to assess the NME6 subcellular localization. NME6 colocalizes with mitochondria although it does not possess the mitochondrial signaling sequence. Crosslinking with glutaraldehyde as well as size exclusion chromatography revealed that the NME6 is present mostly in the monomeric, or, in a smaller portion, in a dimeric state. The NDP kinase activity was tested using a coupled pyruvate kinase-lactate dehydrogenase assay which confirmed that the NME6 does not possess traceable NDPK activity. Further studies will be focused on studying the precise submitochondrial localization of the protein, it's possible function in mitochondrial respiration and potential interacting partners.

P-02.5-24
Quantitative analysis of the dormant *Mycobacterium tuberculosis* proteome and prognostic value of some protein antigens for tuberculosis serodiagnosis

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Formation of dormant *Mycobacterium tuberculosis* (Mtb) cells is responsible for the phenomenon of latent tuberculosis. Serodiagnosis is an effective method for early detection of pathogen for many infection diseases. However, this method demonstrates low effectiveness for TB detection that could be due to incorrect antigens selected. We suggest to study the expression of protein antigens in dormant state of Mtb in vitro (5 mo old cells) obtained under gradual medium acidification which may reflect the real situation for the pathogen in case of latent TB. Analysis of the proteomic profile of active and dormant Mtb was carried out by LC-MS/MS. In comparison with active Mtb 130 proteins significantly increased in the proteome of dormant Mtb. Among of them isoniazid inducible protein IniB, the multiple antibiotic resistance regulator (MarR), universal stress protein Rv2623, toxin VapC46, sigma factor SigK, transcriptional regulator DevR, heat shock protein Hsp, trehalose-6-phosphate phosphatase OtsB1 were found. Obtained protein signature substantially differs from published result for Wayne model based on O2 limitation highlighting difference in two for dormant Mtb models. Under dormancy, the enzymes of biosynthesis of macromolecules (DNA, RNA, proteins, cell wall) and transporters are weakly expressed in Mtb. Three unique Mtb proteins from dormant Mtb proteome were selected and diagnostic effectiveness of these antigens (Rv0341; Rv1509 and Rv2018) was verified with serum of TB patients. According to immunoblotting and IFA, all three recombinant proteins showed the ability to bind to serum antibodies. However, only 30 to 40% of patients turned out to be positive according to this criterion with a specificity of 70 to 90%. As a result, new, previously unpublished, Mtb -specific proteins which may be associated with latent TB were found. This work was supported by the Russian Ministry of Science and Higher Education.

P-02.5-25
Effect of inactivation of uncharacterized protein Lmo0946 on expression of Hfq chaperone of *Listeria monocytogenes*

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Listeria monocytogenes (Lm) is a Gram-positive pathogen which is able to survive exposure to highly stress conditions. One of the bacterial proteins which plays a crucial role in response to adverse conditions is Hfq – the RNA chaperone. Hfq of Lm is involved in the ability to tolerate osmotic and ethanol stress as

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well as contributes to long-term survival under starvation and pathogenesis process. Recent research led us to identification of lmo0946 as an important gene in a stress response of Lm. Interestingly, Lmo0946 is small protein with unknown function, and the function of its homologs, found mainly in the Firmicutes phylum, has not been established yet. Based on the convergent phenotype of mutants in hfq and lmo0946 genes, it was hypothesized that effects of the lmo0946 inactivation may result from the influence on hfq expression. Therefore, the aim of the study was to determine the effect of inactivation of lmo0946 on transcription and translation of the hfq gene. At first transcriptional and translational fusions of the hfq promoter and lacZ reporter gene were constructed and introduced into wild-type Lm and lmo0946 mutant strain. The results of β-galactosidase assay revealed decrease in the reporter activities in lmo0946 mutant strain as compared to the wild-type strain, both in transcriptional and translational fusions in most of selected culture conditions. However, due to overall low level of reporter activities, further analysis was performed using direct methods, i.e. northern blot and western blot. The results of these studies revealed that the transcript and protein Hfq were present only in lmo0946 mutant strain, while in the wild-type strain were below the detection threshold. The obtained results imply that the lack of Lmo0946 affects expression of Hfq chaperone. Further studies are required to elucidate the exact nature of the observed phenomenon. This work was supported by a grant no. 2016/21/B/NZ6/00963 from the National Science Center, Poland.

P-02.5-26
Structural and physiological basis of salmonella YfdX-driven regulation of antibiotic tolerance and virulence

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Typhoid fever, caused by *Salmonella typhi*, exhibits severe symptoms: fever, hepatitis, lymph node necrosis, and intestinal perforation and bleeding. Antibiotics such as ciprofloxacin and ceftriaxone are now widely used for the treatment of this illness. In addition, newly available antibiotics with variable mechanism of action are in a continuous development. However, repeated exposure to antibiotics has resulted in steadily increasing of antibiotic-resistant *S. typhi*. Therefore, uncovering mechanisms behind antibiotic-resistance and handling the properties are attractive approaches to successful antimicrobial treatment against the disease. YfdX is a downstream regulator of the EvgAS two-component system, which induces transcription of genes involved in acid and multidrug resistance. Even though previous reports suggested a possibility that YfdX is associated with recognition of diverse antibiotics, its precise functional role and molecular properties remained to be elucidated. In this study, we investigated structural, biochemical, and physiological uncovered issues of YfdX. We determined the tetrameric crystal structure of S. Typhi YfdX, and identified a dimeric form as a smallest functional unit. An unexpected pH-dependent dynamic conversion of YfdX in solution between dimeric and tetrameric states was identified, which was further verified by biochemical analysis using structure-based mutant proteins. We also demonstrated that Salmonella YfdX induces tolerance of living bacteria to antibiotics such as penicillinG and carbenicillin, and it reduces bacterial virulence toward the Salmonella infection animal model, *Galleria mellonella*. Our studies therefore provide novel insights for determining association between YfdX and responses against environmental stresses, especially antibiotics, in Salmonella. *The authors marked with an asterisk equally contributed to the work.

P-02.5-27
Biological and technical variability of *Cronobacter sakazakii* protein identification method

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Cronobacter spp. are opportunistic food-borne pathogens. These Gram-negative bacteria could cause rare but life-threatening infections such as meningitis, necrotizing enterocolitis and sepsis, especially to immunosuppressed individuals and newborns with low birth weight. According to epidemiological studies, Cronobacter-related infections are caused mainly by specific sequence types (ST) within Cronobacter genus. *Cronobacter sakazakii* (ST4) used in this study is often associated with serious neonatal meningitis. Although several virulence factors have been described, the process of Cronobacter pathogenesis remains unclear and it would be useful to find a reliable method that enables to identify as many bacterial proteins as possible. In this study, we used the subfractionation method for isolation of outer membrane, inner membrane, periplasm and cytosol of *Cronobacter sakazakii* (ST4). Proteins of these fractions were purified with SDS-PAGE, digested with trypsin and then identified by mass spectrometry coupled with liquid chromatography (LC-ESI-Q-TOF MS). Essential steps of analysis including subfractionation, sample preparation, trypsin in gel digest and protein identification by mass spectrometry were performed three times. The results show biological and technical variability of this method. In total, more than 1300 proteins were identified, which is approximately 33 % of the predicted *Cronobacter sakazakii* proteome.

P-02.5-28
Photoactivation of novel fluorescent proteins from the sea salp *Thalia democratica*

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Photoactivatable fluorescent proteins exhibit fluorescence that can be modified by a light-induced chemical reaction. Upon photoactivation, some of these proteins become brighter, others change their fluorescence spectrum. Here we report two novel photoactivatable fluorescent proteins, named ThFP1 and ThFP2, from the salp *Thalia democratica*. Based on transcriptome analysis, two GFP-like proteins were identified. These proteins were cloned, expressed in *Escherichia coli* and purified by metal-affinity and gel-filtration chromatography. The electrophoretic mobility of the purified protein in SDS-PAGE corresponded to 26 kDa. Absorption spectra of purified proteins showed maxima at

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400 and 488 nm for ThFP1, and at 337 and 510 mm for ThFP-2. Fluorescence emission spectra of ThFP-1 peaked at 512 nm, while that of ThFP-2 peaked at 527 nm. ThFP1 and ThFP2 proteins were photoactivatable upon irradiation with 365 and 445 nm light, resulting in a significant increase of long-wavelength bands in the absorption spectra of the proteins. Both proteins were capable of reversible photo-switching, showing very similar activation barriers (22.36 and 23.97 kcal/mol for ThFP1 and ThFP2, respectively). Despite the close values of activation barriers at all tested temperatures the relaxation rate of ThFP1 was 1.2–1.5 times lower than that of ThFP2. ThFP2 photoswitching was shown to be a multistep process, while the ThFP1 curve was monophasic under the same conditions. Gel-filtration chromatography indicated the oligomeric status of both proteins, with ThFP2 showing a tendency to monomerise upon photoactivation. This work was supported by the President grant for leading scientific schools LS-2605.2020.4.

P-02.5-29
Effects of myopathy-causing mutations R91P and R245G in TPM3 gene on structure and functions of slow skeletal muscle tropomyosin, its homo- and heterodimers

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Tropomyosin (Tpm) is one of the major proteins of the sarcomere, which binds actin and troponin and regulates muscle contraction. Tpm3.12 (γ-Tpm) is the main Tpm isoform in slow skeletal muscle. Mutations in Tpm3.12 among other disorders are known to cause congenital fiber type disproportion (CFTD), which is characterised by hypotrophy of slow muscle fibers, muscle weakness, troubles with walking and breathing. Still, little is known yet about molecular mechanisms of this disorder. In present work, we chose two mutations that are known to be associated with CFTD: Arg91Pro and Arg245Gly and applied various methods to investigate how these mutations affect structure and functions of Tpm. Mutations R91P and R245G both in γ*γ*- and γ*γ-Tpm homodimers (with mutations either in both or in only one of two γ-chains) as well as in γ*β-Tpm heterodimers (with mutation in the γ-chain) significantly destabilised Tpm complexes with F-actin, whose stability was measured by the temperature-dependent decrease in light scattering. Using differential scanning calorimetry, we showed that mutation R91P strongly destabilises N-terminal part of Tpm molecule by shifting its thermal transition to a lower temperature, while mutation R245G destabilises the C-terminal part of the molecule where it is located. The experiments in an in vitro motility assay were performed using myosin and troponin extracted from rabbit slow skeletal muscle. The results showed that the most dramatic decline in the sliding speed of regulated thin filaments occurred in γ*γ*-homodimers and γ*β-heterodimers; moreover, in that case the cooperativity of dependence of sliding speed from Ca concentration decreased, which means the impairment of regulation. In conclusion, our results showed that these myopathic mutations may cause significant disruption of Tpm structure and function, that is important for establishing the molecular mechanisms involved in CFTD development. Supported by RSF grant 16-14-10199 to DL.

P-02.5-30
Study of NdCTR1 properties and its purification method

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Copper (Cu) is a vital trace element as its ions are cofactors for a number of essential enzymes and also directly participate in regulation and signalling. Despite this fact, free Cu ions are toxic as they induce ROS formation. Cu dyshomeostasis leads to development of the inherited and sporadic diseases, also Cu is necessary for tumor growth. Chelation therapy is a promising approach in treatment of mentioned disorders but nowadays there's a lack of natural chelators with no side effects. In this work we suggest the 55 a.a. extracellular N-terminal domain of human copper importer CTR1 (NdCTR1) as a safe natural Cu chelator, describe its purification procedure and partly its properties. Initially 67 a.a. NdCTR1 was expressed as a GST-fused protein. GST-NdCTR1 producing bacteria accumulated more Cu and Ag ions (Ag^+ ion is similar to Cu^+) than a control GST-synthesizing strain. Presence of GST-NdCTR1 in *E. coli* increased their resistance to mentioned ions as well as to Ag nanoparticles due to their chelation by NdCTR1 what was shown by SEC of lysates and GST-NdCTR1 immunoprecipitation with subsequent measurement of metals by AAS in obtained fractions. GST dimeric nature and NdCTR1 hydrophobic amino acid cluster (55-67 a.a.) led to GST-NdCTR1 accumulation in inclusion bodies without possibility of refolding after denaturation by chaotropes. To overcome solubility problem NdCTR1 was fused with GB1 protein which was soluble up to 1 mM. GB1-NdCTR1 was purified by IMAC on Cu-charged NTA-sepharose with subsequent SEC. GB1-NdCTR1 binds Cu and multimerizes upon binding as was shown by Cu accumulation in corresponding molecular weight fraction and spectrophotometry. Pure NdCTR1 was obtained after GB1-NdCTR1 proteolysis with thrombin and IMAC. NdCTR1 chelating properties are being researched at the moment and its use as a chelating agent in therapy of Cu-related diseases is discussed. The work was supported by RFBR grants 19-315-90129, 18-515-7811 and RSF grant 20-74-10087.

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P-02.5-31
Keratin-associated proteins in grey and pigmented hair: reaction to UV irradiation

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Low-molecular keratin-associated proteins (KAPs) in human-hair shaft are considered as an important component capable of interaction with structural water-insoluble keratin and with external factors (moisture, chemicals, UV light). Photo modification of KAPs can be detected using thiol content as was shown earlier [Fedorkova M. et al 2016]. In the current study we compared total amount of KAPs (as soluble proteins (SP, ug/mg) and thiol group (SH) content in these proteins (ug/mg) in grey and pigmented hair shafts of 15 volunteers aged 45–70 years, without hair coloring. A part of hair shafts in each sample were irradiated with UV-C light at an intensity of 1.5 mW/cm2 on the sample using the band of a mercury lamp peaked at 254 nm with rejection of remaining bands. Irradiated and control samples were homogenized and both SP and SH were assayed in the resulting supernatants. No significant difference was found between pigmented and grey hairs in control samples. After UV irradiation SP increased by 16% in pigmented hairs ($P < 0.05$) and by 37% in grey hairs ($P < 0.01$), and SH content increased by 44% and by 52%, respectively ($P < 0.01$). Thus, SP content in irradiated grey hair (221 ± 46 ug/mg) was higher than that in pigmented hair (179 ± 39 μg/mg), $P < 0.05$. The probable mechanism may involve photoinduced damage of S-S bonds in insoluble keratins and/or between these keratins and KAPs with release of soluble peptides and proteins. Obviously, a pigmented hair is protected with melanin unlike a grey one, which may explain difference in SP content after irradiation. It was also found that proximal segments of grey hairs (3–20 mm from hair bulb) were more sensitive to UV light than the distal (100–120 mm from the bulb) ones: an increase in SP and SH content by 81 and 57%, respectively, was obtained for the former, and an increase in SP and SH content by 46 and 34%, respectively, was obtained for the latter. *The authors marked with an asterisk equally contributed to the work.

P-02.5-32
Constitutive differential distribution, activity and expression of type 2 transglutaminase in cells derived from celiac patients and from healthy subjects

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Type 2 transglutaminase (TG2) has an important pathogenetic role in celiac disease (CD), an inflammatory intestinal disease caused by the ingestion of gliadin-containing cereals. TG2 deamidates specific glutamines of gliadin peptides in the intestinal mucosa, thus enhancing gliadin immunogenicity. A strong autoimmune response to TG2 also characterizes CD development. Recent studies have demonstrated the occurrence of some constitutive differences between cells from CD and control subjects, mainly regarding a general higher level of phosphorylation and an alteration of vesicular trafficking in CD cells. Moreover, we observed that anti-TG2 antibodies specifically derange, in skin control fibroblasts, the uptake of the α-gliadin peptide 31–43, which is responsible for the innate immune response. However, anti-TG2 antibodies fail to protect CD fibroblasts from 31–43 internalization and consequential effects. To define the contribute of TG2 to the constitutive different phenotype of celiac cells, we investigated TG2 subcellular distribution in skin fibroblasts from CD and control subjects and also analysed how 31–43 modulated TG2 expression and activity. We used confocal microscopy and differential centrifugation to study TG2 subcellular localization. A microplate colorimetric assay was employed to evaluate TG2 in situ activity, whereas TG2 expression was quantified by Western blot and PCR. We found that TG2 was more abundantly associated with cell membrane surface and early endosomal and autophagic compartments in CD fibroblasts than in control cells. We also observed that 31–43 activated TG2 more in control than in celiac cells and induced TG2 expression in celiac cells, but not in control ones. Differences in TG2 localization and in the way 31–43 modulates TG2 activity and expression in control and CD cells suggest that TG2 participates in defining the constitutive celiac cellular phenotype. Previously published in: Paolella G. et al. (2020) Int J Mol Sci 21, 1231

P-02.5-33
Molecular mechanisms of the inhibition of the Rho/ROCK/LIMK2/cofilin pathway by the SecPH domain of Neurofibromin, Nf1, the protein responsible for Neurofibromatosis type I

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Cell signalling transduction pathway crosstalk allows fine regulation of the fate of the cell. In a previous study, we have shown an interconnection between Ras and Rho signalling pathways via the interaction between the RasGAP Nf1 and the kinase LIMK2 downstream of Rho. We then demonstrated that the SecPH domain of Nf1 prevents LIMK2 activation by ROCK, its

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upstream regulating kinase (Vallee et al. (2012) PLoS One 17;7 (10):e47283). We went further into the characterization of this interconnection by determining the molecular mechanisms involved into this process. We identified the domains of each protagonist involved into this interconnection and showed that the SecPH domain of Nf1 as well as the KINASE domain of ROCK interact with the same two domains of LIMK2, its SP/PDZ and KINASE domains. We demonstrated that the SecPH domain of Nf1 abolishes the interaction between the KINASE domains of ROCK and LIMK2, preventing the phosphorylation and subsequently the activation of this latter by the precedent. Our findings bring new insights into the understanding of the regulation of the Rho/ROCK/LIMK2/cofilin pathway by the RasGAP Nf1, and could open new therapeutic perspectives for Neurofibromatosis type I treatment, as this regulation is impaired in this disease.

P-02.5-34
New insights into the regulation of actin cytoskeleton dynamics via the Rho/ROCK/LIMK2/cofilin signalling pathway: a novel mechanism of regulation of cofilin by LIMK2

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LIM kinase 1 (LIMK1) and LIM kinase 2 (LIMK2) are serine/threonine and tyrosine kinases. They play a crucial role in cytoskeleton dynamics as they independently regulate actin filament and microtubule remodelling. By phosphorylating and thus inhibiting cofilin, an actin-depolymerizing factor, LIM kinases prevent actin filament turnover. The molecular mechanism by which LIM kinases regulate microtubules remodelling is still unknown. Because of their key role in the regulation of cytoskeleton dynamics, LIM kinases have been shown to be involved in cancer development, metastasis, neurological diseases and viral infections, and have recently emerged as new and promising therapeutic targets. Three isoforms of LIMK2 are described in the literature: LIMK2a, LIMK2b and LIMK2-1. LIMK2-1 has a slightly shorter kinase domain and a supplementary Protein Phosphatase 1 (PP1) inhibitory domain in its C-terminal extremity compared to its two counterparts. Recently, we have shown that LIMK2-1 is unable to phosphorylate cofilin although it possesses the threonine 505, phosphorylated and activated by the upstream regulating kinase ROCK (Vallee et al., (2019), Biochemical Journal, 475:3745–3761). Our purpose is now to understand why LIMK2-1 does not phosphorylate cofilin, and therefore to determine the molecular requirements for cofilin phosphorylation by LIM kinases. We have shown that the full-length kinase domain is not sufficient for cofilin phosphorylation, while the C-terminal part of LIMK2a is indispensable for this process. Furthermore, the C-terminal part of LIMK2a is phosphorylated in its own. By site-directed mutagenesis, we pointed out a single amino acid of LIMK2a C-terminal extremity that is phosphorylated, and its phosphorylation is required for cofilin phosphorylation. We want to further characterise this process. Altogether, our data unravel the existence of a new mechanism of regulation of LIM kinases.

P-02.5-35
Impact of various poly(ADP-ribose)polymerase 1 level on DNA base excision repair status in human cells

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DNA repair systems exist to correct damage to the structure of DNA, thereby maintaining genome stability. Base excision repair (BER) is responsible for repair chemically modified bases, that are one of the most prevalent type of DNA lesions. It is considered that regulatory proteins are required for improving DNA repair efficiency and acceleration. Poly(ADP-ribose)polymerase 1 (PARP1) is a well-known protein involved in the repair processes regulation, including BER. Multiple studies have suggested that its function is being a "sensor" of damage and promote recruiting the complex for their removal by the ADP-ribose polymer synthesis, whereas others indicate that PARP1 can suppress activity of some key BER proteins. This work is aimed at studying the influence of various PARP1 level on the efficiency of BER steps and on expression of genes, encoding proteins involved in BER. For this purpose, we used mRNAs and the whole-cell extracts obtained from wild-type, PARP1 knockdown (via siRNA transfection) and PARP1 knockout (via CRISPR/Cas9) human cell lines: HEK-293T and HEK-293FT. RT-qPCR assay data has not shown significant changes in the expression at levels of mRNA, encoding BER proteins: PARP1, PARP2, UNG, APEX1, Polb, Lig3 in both PARP1 knockdown and knockout cells in comparison with wild-type ones. We have performed functional tests with protein extracts and ^{32}P-labeled DNA substrates mimicking BER intermediates and demonstrated that various PARP1 level in cell extracts has little impact on the efficiency of key BER steps: removal of uracil from DNA, abasic site processing, gap filling and ligation. Taking together, results of the work show that knockdown and knockout of PARP1 lead to negligible change in BER status as well as in the kinetics of the pathway. The data obtained is important for better understanding the real role of PARP1 in BER regulation. The research was supported by RSF grant № 19-14-00204.

P-02.5-36
The mechanism of SARS-CoV-2 coronavirus nucleocapsid protein interaction with human 14-3-3 proteins

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The coronavirus nucleocapsid protein (N) is a structural protein that regulates the transcription and replication of the viral genome and promotes the packaging of RNA into viral particles. The N protein has a complex domain structure and undergoes

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multipoint phosphorylation in the central unstructured region after entering the cell. According to the literature data, phosphorylation of homologous N protein from the SARS-CoV coronavirus leads to its retention in the cytoplasm due to direct binding with cellular regulatory proteins of the 14-3-3 family. Human 14-3-3 proteins are represented by seven isoforms that are highly expressed in tissues susceptible to SARS-CoV-2 infection. Through interaction with N 14-3-3 proteins may play an important role in the life cycle of the coronavirus. In this work, we dissected the molecular mechanism of the interaction between SARS-CoV-2 N and human 14-3-3 proteins. The phosphorylated form of N (pN) was obtained in a special system of co-expression with protein kinase A in *E. coli*, which led to phosphorylation of more than 20 sites in N. We have shown that only pN interacts with all isoforms of human 14-3-3 with micromolar affinity and the stoichiometry 2:2. Through the series of truncated mutants of N, it was shown that the presence of pS197 residue is necessary for the binding of 14-3-3 proteins. Utilizing the genetic code expansion allowing for the site-specific, translational incorporation of phosphoserine residues into proteins of interest we confirmed that S197 phosphorylation is sufficient for binding to 14-3-3 proteins. It is noteworthy that this 14-3-3-binding site is quite conserved among N proteins from various coronaviruses. So, the proposed molecular mechanism for the formation of the 14-3-3/pN complex could be universal and useful in the development of new therapeutic approaches for fighting coronavirus infections. Partially supported by RSF № 19-74-10031. *The authors marked with an asterisk equally contributed to the work.

P-02.5-37
New insight into relationship between stress granules and angiogenin/RNase 5

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Angiogenin (ANG) is a 14-kDa extracellular protein originally identified in tumor cells conditioned media, ever since studied in several organs and involved in physiological and pathological processes. Numerous evidences extended ANG biological activity from inducing angiogenesis to stimulating cell proliferation and more recently to promoting cell survival. Under stress conditions, ANG is accumulated in cytoplasmic compartments and modulates the production of tiRNAs, a novel class of small RNAs, that contribute to translational inhibition and recruitment of Stress Granules (SGs). To date, there are still limited experimental evidence relating to the role ANG in the epidermis, the outermost layer of human skin, continually exposed to external stressors. On the regard, our study is focused on clarifying ANG possible role in human keratinocytes (HaCaT cells) subjected to different stress stimuli. Our results clearly indicate that in HaCaT cells endogenous ANG is localized both in the nucleus and in the cytoplasm, on the contrary, in stressed HaCaT cells the protein dramatically changes its localization moving largely towards transient cytoplasmic SGs. Further tests indicate that recombinant ANG is able to greatly limit the expression of heat shock proteins in stressed cells, to attenuate the number and size of SGs, to positively alter cell cycle and intriguingly also to induce an increase of expression of endogenous ANG. As ANG is internalized and translocated to the nucleus after its binding to the cell surface receptor, additional experiments were carried out on a recombinant ANG variant in which nuclear translocation sequence between M30 to G34 was changed, in order to verify whether the inability to move to the nucleus actively alters cell response to stress conditions and/or its potential activity in promoting transcription. Collected results to date suggest that ANG could represent a promising protective strategy against skin damage skin damage.

P-02.5-38
The nucleus-like shell takes part in the regulation of the phiKZ bacteriophage genome transcription

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The giant phiKZ bacteriophage has a unique transcription program independent from bacterial DNA-dependent RNA-polymerase (RNAP). The two non-canonical multisubunit phage RNAPs transcribe the phage genome. The first one, called virion RNAP (vRNAP), is injected into the cell together with phage DNA and transcribes early genes. The second one, non-virion RNAP (nvRNAP), is produced in the bacterial cell during infection and transcribes late genes (previously published in: Yakunina M. et al. (2015) Nucleic Acids Res. 43, 10411–10420). Recently, it has been shown that during infection, the phiKZ bacteriophage forms a proteinaceous shell in the bacterial host cell (previously published in: Chaikeeratisak V. et al. (2017) Cell Rep 20, 1563–1571). The phage DNA is packed inside that nucleus-like shell (previously published in: Danilova Y.A. et al. (2020) Viruses 12, 1197, 1–16). In this work, we investigated the localization of both phage RNAPs in the *Pseudomonas aeruginosa* cell during infection using methods of immunology, fluorescence and electron microscopy. We found that the number of early transcripts decreases to the middle of the infection cycle, but the amount of vRNAP increases by two orders of magnitude at the same time. The vRNAP is demonstrated to localize outside the shell in the second part of the infection. Apparently, the vRNAPs are separated from phage DNA and could not bind to their promoters. NvRNAP is transferred into the shell where all phage DNA is located to perform the transcription of the late genes. It seems that the phage DNA compactization inside the shell is important for late transcription initiation by nvRNAP. Based on our data, we suppose that the nucleus-like shell plays a great role in phiKZ genome transcription regulation. The work was supported by the Russian Science Foundation grant № 19-74-10030.

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P-02.5-39
Biological function of tumor suppressor developmentally regulated GTP-binding protein 1 (DRG1) is conserved from sponges to humans

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Cancer is one of the most threatening, emerging and studied human diseases. Although the cancer research has advanced substantially and expanded our knowledge of this issue, we are still unsuccessful in eradication or conversion of cancer to a chronic disease. Therefore, we need new approaches focusing on the fundamental aspects of this disease that will answer basic questions of cancer origin and the mechanisms of spreading. Interestingly, comparative genomics studies have shown that most genes linked to human cancer emerged during the early evolution of Metazoa. Thus, basal multicellular animals, such as sponges (Porifera), may be an innovative model system for understanding the molecular mechanisms involved in cancer biology. One of the cancer-related genes/proteins that is evolutionary conserved from sponges to humans is a metastasis suppressor Developmentally regulated GTP-binding protein 1 (DRG1) which is stabilized by its interaction with Zinc finger CCCH-type containing 15 protein (ZC3H15, LEREPO4). Human DRG1 is necessary for normal cell growth and has a role as a microtubule-binding protein. The aim of this study was to evaluate the biological function of human DRG1 and its homolog from sponges, the simplest multicellular organisms. We over-expressed human DRG1 and its sponge homolog in breast cancer cell line MCF-7 and studied their biological features. In particular, we analyzed co-localization, as well as intra- and inter-species interaction of both sponge and human DRG1 with either LEREPO4 or alpha-tubulin. A tumor suppressor role of human DRG1 and its sponge homolog was compared by performing biological assays for cell apoptosis, migration and invasion. Our results on basic function of DRG1 will contribute in elucidating the origin of cancer and physiological function of genes linked with cancer.

P-02.5-40
The mitochondrial NME6, a member of the nucleoside diphosphate kinase family interacts with RCC1L (WBSCR16), a protein involved in coordination of the mitochondrial ribosome assembly

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NME6 is a member of the nucleoside diphosphate kinase (NDPK/NME/Nm23) family, a group of proteins catalyzing the transfer of gamma phosphate from NTPs to NDPs. The ping-pong reaction is dependent on its hexamer assembly in eukaryotes, and involves the synthesis of high energy intermediate via the phosphorylation of a specific histidine residue within the catalytic site. The family is divided in two groups: Group I (NME1-NME4) members are highly homologous among themselves and exhibit NDPK activity; Group II (NME5-NME9) members display less homology and seem to lack NDPK activity. Extensive research has been conducted on Group I members after the discovery of NME1's role in metastasis suppression, while Group II remained largely unexplored. Although little is known about Group II members, these evolutionary old genes are presumed to participate in one or more basic cellular processes, therefore, our studies focused on the human NME6 protein. Building on our previous findings on expression of endogenous NME6 isoforms and its subcellular localization in mitochondria, we aimed to resolve the precise sub-mitochondrial localization, understand the enzymatic properties, determine quaternary structure and to reveal interacting partners. Fractionation of mitochondria by the swelling/shrinking procedure was analyzed by western blot and showed a NME6 distribution pattern highly similar to proteins facing the matrix. Western blot analysis revealed a lack of NME6 histidine phosphorylation, an indispensable prerequisite for enzymatic activity. Immunoprecipitation experiments showed that NME6 does not interact either with Group I NME members, or with itself which would, also, be mandatory for its enzymatic activity. Proximity ligation assay followed by immunoprecipitation showed that NME6 physically interacts with RCC1L, a protein involved in coordination of the mitochondrial ribosome assembly. Together, these results provide precious clues for understanding the NME6 function.

P-02.5-41
Structure of a bacterial full-length type 2 IleRS reveals the C-terminal tRNA binding domain insertion dispensable for aminoacylation

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Isoleucyl-tRNA-synthetases (IleRS) are universally conserved enzymes that covalently couple isoleucine to its cognate $tRNA^{Ile}$ in a two-step aminoacylation reaction. These multidomain proteins consist of an aminoacylation domain, a proofreading domain and a C-terminal anticodon-binding domain involved in tRNA recognition. IleRSs cluster into two clades, IleRS1 and IleRS2, which differ in antibiotic resistance and the architecture of their C-terminal domain. The structure of the C-terminal anticodon-binding domain of IleRS1 is already known and entails three subdomains (SD): SD1 with helical bundle topology, SD2 consisting of four antiparallel β-sheets and SD3 that is a αβ-fold with a zinc-binding motif. [1] At the same time, the structure of the C-terminal domain of IleRS2 remained unknown as only structures of truncated enzymes were reported. Here, for the first time, we present the structure of full-length *Bacillus megaterium* IleRS2 with a completely resolved C-terminal domain at 2.3 Å resolution. The structure unveils that the C-terminal domain of IleRS2 consists of three subdomains analogously to IleRS1. SD1 and SD2 in IleRS2 align structurally well with the corresponding subdomains in IleRS1. In contrast, SD3 lacks the zinc-binding motif of IleRS1 SD3 and surprisingly, topologically resembles the SD2. Finally, the structure visualized a novel 75 amino acid long SD2 insertion, which is absent in IleRS1. We prepared a *B.*

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megaterium IleRS2 mutant with the SD2 insertion exchanged to a $[Gly_4Ser]_2$ loop. The mutant has relatively modest 5-fold decrease in aminoacylation rate as compared to the wild-type enzyme, which indicates that the SD2 insertion is important, but not essential for IleRS2 aminoacylation. The results thus open an intriguing question whether SD2 of IleRS2 has a role outside of translation. Reference: [1] F. L. Silvian, J. Wang, A. T. Steitz, Science. 285 (1999) 1074–1077.

P-02.5-42
Dimerization of cardiac sodium channel Nav1.5

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Not long ago, voltage-gated sodium channels were thought to function as monomers. However, this dogma was challenged by the recent studies that pointed to dimerization of neuronal Nav1.1. Nav1.2, Nav1.7 and cardiac Nav1.5 channels. Accordingly, functional cooperativity of the Nav dimers was emphasized for neuronal and cardiac diseases. By using immunoblotting analysis, we have detected both dimers and monomers of Nav1.5 in HEK293 cells transiently or stably expressing human hH1 variant. Furthermore, we have shown that these α-α dimers are present at the plasma membrane and that reducing agents are disrupting them into monomers. In our hands, Nav1.5 dimers were not affected by 14-3-3 proteins as suggested in previous reports. Surprisingly, we were able to detect only monomers of Nav1.5 in healthy mice heart tissues. It is unclear at this point, whether our research method lacks sensitivity or whether endogenous α-α interaction of Nav1.5 is preconditioned by some pathological cardiac state. Taken together, our results are bringing closer the understanding of the (patho)physiology of cardiac sodium channel on fundamental level.

P-02.5-43
Post-translational modification of STAT3 modulates its cellular distribution

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Signal Transducer and Activator of Transcription 3 (STAT3) is a pleiotropic transcription factor involved in major physiological and pathological processes, such as normal development, response to stress and cancer. Canonical STAT3 transcriptional activity depends on its homodimerization, Y705 phosphorylation and nuclear translocation. However, Y705-independent, non-canonical STAT3 functions have been also described at nuclear, mitochondrial and endoplasmic reticulum levels. Both canonical and non-canonical STAT3 pathways are regulated by additional post-translational modifications (PTMs). A Venus-STAT3 bimolecular fluorescence complementation (BiFC) assay was recently developed to study the role of PTMs on STAT3 homo-dimerization. Residues susceptible for PTMs were blocked by site-directed mutagenesis. STAT3-Knockout cells were transfected with pairs of Venus-STAT3 BiFC constructs bearing single or double mutations in a symmetric (same STAT3 proteoform) or asymmetric (two different STAT3 proteoforms) way. Cellular localization of STAT3 dimers was monitored by time-lapse fluorescence microscopy. After cytokine stimulation, symmetric wild-type STAT3 pairs accumulated in the nuclei within 10 min. This effect was abrogated in symmetric Y705F STAT3 pairs, while asymmetric Y705F/WT pairs responded normally. A second PTM-resistant mutation restored nuclear translocation of Y705F mutants. These results suggest that 1) only one of the STAT3 monomers need to be phosphorylated for the homodimers to accumulate in the nucleus, and 2) additional PTMs could be involved in the lack of nuclear translocation by Y705-unphosphorylated STAT3. These results are also consistent with our previous findings indicating that PTM asymmetry influences the behavior STAT3 homodimers, and contribute to a better understanding of the mechanisms underlying STAT3 signaling pathways.

P-02.5-44
Structural and dynamic properties of type A (I) lantibiotics determining their binding to lipid II

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Antibiotic resistance is one of the biggest public health challenges of our time and this is the motivation for finding new antibiotics. A class of bacteriocins — lantibiotics – are promising agents for drug discovery. Lantibiotics are ribosomally synthesized and post-translationally modified cationic antimicrobial peptides. The specific target of type A (I) lantibiotics is the pyrophosphate moiety (PPi) of lipid II. Nisin, gallidermin, and epidermin are the main peptides in this class of bacteriocins. In binding to the lipid II the main role is played by residues 1–12. The aim of the study was to explore the structural properties of these lantibiotics' fragments in the presence of dimethyl pyrophosphate ion (DMPPi) that mimics PPi of lipid II via molecular dynamics (MD) simulations in water. It was shown that $nisin_{1–12}$ forms 4–5 intermolecular hydrogen bonds (H-bonds) with DMPPi, while $gallidermin_{1–12}$ and $epidermin_{1–12}$ create 6–7 H-bonds, mainly via backbone NH groups of the ring A. This fact correlates with experimental data on higher antimicrobial activity of gallidermin and epidermin. For the studied antibiotics, we found a similar conformation, in which the largest number of intermolecular H-bonds was formed. In this conformation, the rings A and B are placed opposite each other, thus being stabilized by two intramolecular H-bonds in the case of nisin (Dha5-Dab8 and Dha5-Pro9) and one H-bond in the case of gallidermin/epidermin (Lys4-Dab8). In nisin, gallidermin, and epidermin, the MD-derived lifetime of the aforementioned state are ≈28%, ≈85%, and ≈88%, respectively. The significant difference between nisin and gallidermin/epidermin is the position of the NH group of 5th residue, which is turned towards DMPPi in the latter case. It has been shown that lantibiotics of type A (I) are able to form stable complexes of a similar structure with the PPi mimetic of lipid II, and the residue 5 affects the binding.

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P-02.5-45
MSC extracellular vesicles induce TGF-beta receptor type II clustering in myofibroblasts as a part of anti-fibrotic action

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Differentiation of fibroblasts to myofibroblasts is a central event in pro-fibrotic action of TGF-beta. Previously we showed that extracellular vesicles (EV) secreted by multipotent mesenchymal stromal cells (MSCs) abrogated this TGF-beta effect. Observed anti-fibrotic action of MSCs-derived EVs was mediated by the transfer of specific microRNAs, which caused down-regulation of genes associated with myofibroblast phenotype. We hypothesized that MSC-derived EVs could also directly affect TGF-beta signaling pathway in myofibroblasts. We tested this using in vitro model of TGF-beta-induced differentiation of human fibroblasts into myofibroblasts. Cells were treated by EVs isolated from the conditioned medium of human MSCs (ASC52telo, ATCC) for 1, 24 or 96 hours. The expression and spatial distribution of TGF-beta signaling pathway components were analyzed by ICC, Western blot and RT-PCR. TGF-beta caused the increased exposure of TGF-beta receptor type II (TGFbRII) to the cell surface. Unexpectedly, MSC-derived EVs did not suppress this, but rather slightly increased the expression of TGFbRII in myofibroblasts. MSC-derived EVs caused the preservation of the TGF-beta induced intracellular distribution of TGFbRII. Furthermore, MSC-derived EVs led to the formation of large receptor clusters in few days. This was not accompanied by the activation of SMAD 2/3 phosphorylation. These data suggests that EVs could directly transfer additional copies of TGFbRII, which form large non-signaling clusters, therefore sequestering functional TGF-beta receptors on myofibroblasts and preventing the activation of SMAD 2/3 dependent pro-fibrotic TGF-beta signaling in myofibroblasts. The study was supported by the Russian Foundation for Basic Research (RFBR #20-315-90120).

P-02.5-46
Interplay of Gic1 or Gic2 proteins with translation termination factors Sup35 and Sup45 in the yeast

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In the Saccharomyces cerevisiae yeast, the SUP35 and SUP45 genes encode eRF3 and eRF1 proteins, respectively. Mutations in these genes lead not only to omnipotent nonsense suppression, but also to a number of other pleiotropic effects. This may indicate that the translation termination factors are involved in multiple cellular processes besides of their main function – recognition of stop codons and termination of protein synthesis. Previously, we performed a screening for the genes that influence viability of the sup35 and sup45 nonsense-mutants; among such genes we identified the GIC1 and GIC2 paralogues. The corresponding proteins are involved in control of bud formation, cell size, and bud localization. Gic1 and Gic2 take part in regulation of actin cytoskeleton formation and control of cell cycle switch to G1. These proteins affect GTPase Cdc42, which has conservative CRIB (Cdc42/Rac-Interactive Binding) sequence for this interaction. In our previous work, interaction between Gic2 and Sup35 was shown using the yeast two-hybrid system, and a CRIB-like sequence was identified in the C-terminal part of Sup35. Additional copies of GIC1 and GIC2 affected the termination efficiency in the sup35 and sup45 mutants. In some strains where either GIC1 or GIC2 was overexpressed, we observed alterations in the level of one or both translation termination factors. We constructed the yeast strains with the disruptions of the chromosomal copies of either SUP35 or SUP45 harboring single and double deletions of GIC1 and GIC2. This allowed us to appreevaluate how the sup35 and sup45 mutations influence formation of the actin cytoskeleton and budding process in cells lacking the GIC1 and GIC2 genes. The work was supported by RSF grant 18-14-00050 "Genetic and epigenetic regulation of translation termination". Part of experimental work was done in the resource centre of SPBU "Centre for Molecular and Cell Technologies".

P-02.5-47
Analysis of human milk protein complexes containing biologically active proteins and peptides

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Milk is not only a unique and universal source of essential nutrients for newborns, but also contains a number of biologically active components necessary for the protection, growth and development of infants. Some functionally significant milk proteins and peptides can save their biological activity even in the aggressive environment of the gastrointestinal tract due to the formation of high-molecular protein complexes that can protect biologically active components from proteolysis while preserving their biological properties. We studied the presence and distribution of innate immunity proteins and peptides of human milk serum and high-molecular fractions of serum (more than 50 kDa) obtained by ultrafiltration. The studied preparations were characterized by a number of electrophoretic methods under denaturing and non-denaturing conditions in an alkaline, acidic and neutral environment and also by two-dimensional electrophoresis. Individual biologically active proteins and peptides (human neutrophil defensins HNP 1–3 and HNP 4, lactoferrin (LF), lactoperoxidase (LPO), myeloperoxidase (MPO)) were detected in the analyzed samples and also the main chromatographic fractions obtained by RP HPLC using Western blotting and Dot-ELISA. It has been shown that high-molecular and low-mobility proteins, such as LF, LPO and MPO, and low-molecular-weight peptides with higher electrophoretic mobility, HNP 1–3 and 4, are detected in the composition of high-molecular complexes in the studied preparations. Two different types of high molecular weight complexes of human milk have been

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isolated and characterized which are stable under aggressive conditions at pH 3 and high ionic strength (2M sodium chloride). Complex No.1 contains mainly LF and other biologically active proteins and peptides, such as HNP 1–3 and HNP 4, LPO, MPO and lysozyme, whereas protein complex No.2 contains mainly α-lactalbumin, as well as LF and HNP 1–3, and does not contain HNP 4, LPO, MPO and lysozyme. *The authors marked with an asterisk equally contributed to the work.

P-02.5-48
Dj1 proteoforms in breast cancer cells: the escape of metabolic epigenetic misregulation

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In cancer cells, the high glycolytic flux induces carbonyl stress, a damage that increases reactive carbonyl species making DNA, proteins and lipids more susceptible to glycation. Together with glucose, methylglyoxal (MGO), a byproduct of glycolysis, is considered the main glycating agent. MGO is highly diffusible, enters the nucleus and reacts with lysine- arginine-rich tails of histones. Glycation adducts on histones undergo oxidization and rearrange to form stable species known as advanced glycation end-products (AGEs). This modification alters nucleosomes stability and chromatin architecture deconstructing the histone code. Formation of AGEs has been associated with cancer and several age-related diseases. DJ1, a cancer-associated protein that protects cells from oxidative stress, has been described as a deglycase enzyme. In several human tumours, its expression, localization, oxidation, and phosphorylation were found altered. This work aims to explore the molecular mechanism that triggers the peculiar cellular compartmentalization and the specific post translational modifications (PTM) that influences the DJ1 dual role, in breast cancer cells. Using a proteomic approach, we identify on DJ1 a novel threonine phosphorylation, part of a putative Akt consensus. Interestingly we found that pharmacological modulation of Akt pathway induces a functional tuning of DJ1 proteoforms revealing that the pathway is critical for DJ1 protumorigenic abilities. In breast cancer cells, the overactivation of Akt signaling enhances DJ1-phosphorylation. Phosphorylated DJ1 increases its glyoxalase activity thus preventing glycation-induced histones misregulation. In this work we report the characterization of a novel proteoform of DJ1 accounting to the ability of cancer cells to counteract carbonyl stress. Dj1 Glyoxalase activity is crucial for the escape of metabolic induced epigenetic misregulation that otherwise could impair the malignant proliferative potential of cancer cells.

P-02.5-49
The Zn^{2+} -dependent change of Nucleobindin-2 structure

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Nucleobindin-2 (Nucb2) is a physiologically relevant protein, which participates in variety of biological processes i.e. carcinogenesis, food intake inhibition and sleep regulation. Additionally, broad expression level of Nucb2 has been found in central nervous system, adipose tissue, heart, pancreas and digestive system suggesting its potentially new roles in various metabolic functions. Metal ions such as Ca^{2+} and Zn^{2+} might modulate the structure of proteins to facilitate performing their functions. Nucb2 contains the C-terminus two EF-hands, responsible for Ca^{2+} binding and a putative Zn^{2+}-binding site at the N-terminus. Two homologs, Nucb2 from *Gallus gallus* (ggNucb2) and *Homo sapiens* (hsNucb2) was previously characterized as Ca^{2+}-binding intrinsically disordered proteins (IDPs). The aim of our study was to investigate if and in which way Nucb2s structure is modulated by Zn^{2+} ions. We prepared both homogeneous proteins in a four step procedure: ion metal affinity chromatography (IMAC), digestion of the His-Tag by HRV 3C protease, second IMAC and gel filtration. The hydrogen-deuterium exchange coupled with mass spectrometry (HDX-MS) showed that peptides at N-terminal half of Nucb2s, located in the near proximity of the putative Zn^{2+} binding site, are more solvent exposed in the presence of Zn^{2+} than in the absence of these ions, which suggests that this part of the protein is characterized by a significant flexibility. Simultaneously, the Trp fluorescence spectra revealed that Nucb2s undergo compaction upon Zn^{2+} addition. Surprisingly, two Trp residues of Nucb2s are located at C-terminal half of proteins. We concluded that the Zn^{2+} impacts both parts of Nucb2s, leading to the structural rearrangement of the entire protein molecule. The functional importance of these alternation should be further explained. Funding: This work was supported by the National Science Centre Grant (A. O.) 2018/29/B/NZ1/02574.

P-02.5-50
Conformational changes of nesfatin-1 in the presence of Zn(II) ions

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Human nesfatin-1 is a small 82-amino acid (9.8 kDa) hormone peptide that is a product of proteolytical cleavage of N-terminus of Nucleobindin-2. Nesfatin-1 is comprised of three domains: N-terminal (N23), middle part (M30) and C-terminal (C29). Nesfatin-1 is thought to exert its biological functions, especially its anorexigenic properties through, the N23 domain. Nucleobindin-2/nesfatin-1 is engaged in the regulation of many important physiological processes, e.g. energy homeostasis, reproduction, epilepsy, anxiety, depression, regulation of circadian rhythm and carcinogenesis. Multifunctionality is a characteristic feature of intrinsically disordered proteins (IDPs) thus nesfatin-1 might be a member of this family as indicated by in silico analysis. Structural studies of nesfatin-1 are therefore essential to establish the relationship between its structure and function. Herein we utilized recombinant nesfatin-1 from Homo sapiens expressed in Bl21(DE3)pLysS E. coli cells. Intrinsic disorder of nesfatin-1 in the absence of ions was proven by circular dichroism spectroscopy (CD). Further CD experiments revealed a strong concentration-dependent increase in the α-helical content of nesfatin-1 under Zn(II) treatment. Sedimentation-velocity analytical ultracentrifugation confirmed structural changes of nesfatin-1 and compaction of its elongated structure in the presence of Zn(II)

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ions. We also observed a propensity of nesfatin-1 for oligomerization. Above findings suggest that nesfatin-1 may be engaged in Zn(II) homeostasis. Furthermore, our data demonstrating context-dependent structural flexibility of nesfatin-1 might help to elucidate multifunctionality of this peptide at the molecular level. This work was supported by the National Science Center grant (AO) 2018/29/B/NZ1/02574.

P-02.5-51
Quality-controlled lipid-based protein sorting into selective endoplasmic reticulum exit sites by glycan remodeling

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GPI-anchored proteins (GPI-APs) exit the endoplasmic reticulum (ER) through a specialized export pathway in the yeast *Saccharomyces cerevisiae*. We have recently shown that very long acyl chain (C26) ceramides present in the ER membrane drives clustering and sorting of GPI-APs into selective ER exit sites (ERES). Here we found that this lipid-based ER sorting also involves the C26 ceramide as lipid moiety of GPI-APs, which is incorporated into the GPI anchor through a lipid remodeling process after protein attachment in the ER. Moreover, we also show that the presence of the C26 ceramide in the GPI anchor is monitored by the remodeling of the GPI-glycan. Therefore, our study reveals a novel quality control system that ensures lipid-based sorting of GPI-APs into selective ERES for differential ER export. *The authors marked with an asterisk equally contributed to the work.

P-02.5-52
Cytosolic localization and *in vitro* assembly of human *de novo* thymidylate synthesis complex

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Cancer cells reprogramme one-carbon metabolism (OCM) to support enhanced growth and proliferation, in this contest Serine hydroxymethyltransferase (SHMT) is a pivotal enzyme. SHMT mainly exists in three isoforms: two localized in the cytosol (SHMT1/SHMT2 α) and one (SHMT2) in the mitochondria. SHMT1 undergoes to a nuclear localization during the S-phase of the cell cycle to sustain de novo dTMP synthesis [1]. The de novo thymidylate synthesis is a crucial pathway for normal and cancer cells. Deoxythymidine monophosphate (dTMP) is synthesized by the combined action of three enzymes: serine hydroxymethyltransferase (SHMT), dihydrofolate reductase (DHFR) and thymidylate synthase (TYMS), the latter two targets of widely used chemotherapeutics such as antifolates and 5-fluorouracil. It had been suggested that these three proteins assemble in the nucleus into the thymidylate synthesis complex (dTMP-SC) [1]. We have recently understood the intracellular dynamics of dTMP synthesis complex in lung cancer cells by in situ proximity ligation assay, showing that it is also detected in the cytoplasm. This result strongly indicates that the role of the dTMP-SC assembly may go beyond dTMP synthesis. We have also successfully assembled the dTMP synthesis complex in vitro, employing tetrameric SHMT1 and a bifunctional chimeric enzyme comprising human TYMS and DHFR by using a different array of techniques. Moreover, we have demonstrated that the SHMT1 tetrameric state is required for efficient complex assembly, indicating that this aggregation state is evolutionary selected in eukaryotes to optimize protein-protein interactions. Lastly, we have set-up an activity assay of the complete thymidylate cycle in vitro, which may provide a useful tool to develop drugs targeting the entire complex instead of the individual components. [1] Anderson, D. and Stover, P. (2009). SHMT1 and SHMT2 Are Functionally Redundant in Nuclear De novo Thymidylate Biosynthesis. PLoS ONE, 4(6), e5839.

P-02.5-53
β-Lactoglobulin covalent modification by phycocyanobilin: effect on protein's techno-functional and IgE binding properties

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β-Lactoglobulin (BLG) is the major milk allergen with many techno-functional properties desirable for the food industry. Our previous study demonstrated that phycocyanobilin (PCB), the bioactive pigment of Spirulina platensis with many health-promoting effects, covalently binds to BLG at physiological pH via free cysteine residue. To help produce hypoallergenic food, this study explored the possibility of reducing the allergenicity, while at the same time improving the techno-functional properties of BLG. Antioxidant properties, heat-induced changes, pepsin and pancreatin digestibility and IgE binding properties of BLG-PCB covalent adduct were investigated and compared to unmodified protein. BLG-PCB possesses enhanced antioxidative properties, while also being protected by PCB against free-radical induced oxidation. Although thermally as stable as unmodified protein, BLG-PCB is less susceptible to heat-induced oligomerization and aggregation under moderate heat treatment (63°C, 30 min). The surface hydrophobicity of BLG-PCB is lower than that of BLG and, contrary to unmodified BLG, it does not change upon heating. The heating of BLG-PCB decreases its β-sheet content, making it less prone to the formation of amyloid-like structures. Covalently modified protein is more resistant to pepsin and pancreatin digestion in comparison to unmodified protein. Enzyme-linked immunosorbent assay indicated that covalent modification by PCB is effective in reducing the IgE-binding capacity of BLG. Taken together, these results indicate that BLG covalent modification by PCB improves BLG's techno-functional properties,

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without increasing its IgE binding abilities, thus making it a useful ingredient in the food industry.

P-02.5-54
Formation and dynamics of focal adhesions during cell spreading

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Focal adhesions are linked to contractile actomyosin bundles, and assembled/disassembled in migrating cells. Therefore, cells generate contractile force through the focal adhesions, and this force is essential for the formation and maintenance of focal adhesions. However, the underlying mechanisms by which actomyosin tension regulates the formation and dynamics of focal adhesions are unclear. To elucidate this mechanism, we studied the formation and dynamics of focal adhesions during cell spreading using total internal reflection fluorescence microscopy. Within 0.5 h after cell adhesion, dot-like focal complexes were formed at the tips of filopodia and at the leading edge of lamellipodia. At 1 h after cell adhesion, focal complexes elongated and matured into focal adhesions with the formation of actomyosin bundles. At 4 h after cell adhesion, focal adhesions were located at the ends of organized stress fibers in well-spread cells. In the presence of myosin-II inhibitor (-)-Blebbistatin or Rho-kinase inhibitor Y-27632, dot-like focal complexes were formed, but less elongated focal adhesions. These results clarify actomyosin-dependent contractile force is essential for the anisotropic elongation of focal adhesions.

Neurobiochemistry

P-03.1-01
Subacute effects on mice central nervous system of dimethyl isophthalate (DMIP)

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Phthalates are a family of chemical compounds primarily used to make PVC flexible. They are used in many products in homes, hospitals, cars, and businesses. Phthalates are known as toxic substances and they can affect the central nervous system. DMIP is used as a perfume fixative and as a plasticizer to make polyester resins. There is no information on DMIP toxicity. The aim of the study was to determine the histopathological effects and oxidative stress-inducing potential in the brain tissue by subacute exposure of DMIP. In the study, animals were orally fed with 150, 300, 600 mg/kg/day DMIP for 5 consecutive days per week for 28 days (OECD Guideline 407). At the end of the study, no significant changes were observed in body weight gains, absolute and relative organ weights of DMIP treated mice compared with the control. SOD activities in the tissues at the150 and 300 mg/kg DMIP treatment groups were significantly higher than the control ($P < 0.05$). The degrees of lipid peroxidation changed dose-dependently. In GST activities, there is no significant difference in the groups. However, AChE activities significantly decreased dose-dependently in all exposure groups. Moreover, DMIP caused dose-dependent histological changes such as an expansion of capillary vessels in the brain cortex and medullar areas. Also, excessive vacuolization in stromal areas were observed. Pyknotic nucleus and atrophic degenerate cells were observed in most of the granular and pyramidal cells in the brain cortex and in the highest dose, astrocytic infiltration concentrating on the pia mater is noteworthy. These results suggested that DMIP exposure induces oxidative stress in the brain and exposure of DMIP during a long period of time could lead to serious brain damage. In conclusion, DMIP has been shown to have neurotoxic effect on brain tissue. Acknowledgments: Ege University Local Ethical Committee of Animal Experiment (31.08.2016, 2016-073). Funding: Ege University Project No: 2017/FEN/027

P-03.1-02
Effect of glucosylceramide accumulation on the neuronal homeostasis: a new neuronal *in vitro* model of Gaucher disease

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Gaucher disease (GD) is a lysosomal storage disorder due to an impairment of the lysosomal β-glucocerebrosidase (GCase) activity with consequent accumulation of glucosylceramide (GlcCer). Nowadays it is clear that a common feature of all the different phenotypes of GD is the onset of neuronal degeneration; nevertheless, the molecular mechanism underlying the relation between GBA mutations and the onset of neuronal damage in GD remains unclear so far. To figure out which is the possible molecular mechanism linking GCase loss of function with the onset of neuronal damage, we developed an *in vitro* model of the neuronal form of GD represented by iPSCs-derived dopaminergic neurons, obtained from healthy subjects' fibroblasts and treated for 7, 13 and 29 days with conduritol B epoxide (CBE), a specific inhibitor of GCase. In CBE-treated neurons we found a progressive and time-dependent accumulation of GlcCer. Upon reaching a threshold of GlcCer accumulation, CBE-treated neurons showed: i) a significant neuronal damage as demonstrated by the reduction of the main neuronal markers such as Tau, Synapsin, MAP2 beta3-tubulin, and PSD95, ii) increased volume of intracellular acidic organelles and augmented lysosomal biogenesis, iii) impairment of the lysosomal sphingolipid catabolism, iv) block of the autophagic flow in term of augmented LC3IIB and p62, and v) as occurs in several other lysosomal storage disorders, the secondary accumulation of not catabolized glycosphingolipids. In addition, we found that the accumulated GlcCer is not just confined to the lysosome but affects also the plasma membrane. In conclusion, this *in vitro* model helps to investigate the onset of cell damage induced by GlcCer accumulation and lysosomal dysfunction. The obtained results let to speculate on the existence of a mechanism involving the plasma membrane in the onset of neuronal degeneration occurring in the brain pathology of GD.

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P-03.1-03
New functional cAMP compartment in neuroprotection organized by AKAP6

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Neuronal survival and axon growth are known to be regulated by cAMP signaling. Last decades of research showed that cAMP signaling in neurons is highly compartmentalized, however besides dendritic and synaptic regulation, only few functional subcellular compartments in terms of molecular architecture have been identified so far. cAMP signaling is largely orchestrated by multimeric complexes called A-kinase anchoring proteins (AKAPs) that locally target PKA and other signaling molecules to specific compartments. These complexes are thought to serve as nodal points for signaling integration for pro-survival and pro-regenerative upstream stimuli. By using new PKA sensors for live-cell FRET imaging and molecular tools to specifically alter local cAMP level, we demonstrated that perinuclear compartment organized by AKAP6 is necessary for hippocampal neurons outgrowth and survival. Displacement of AKAP6-associated phosphodiesterase 4D3 (PDE4D3) with competing peptide (4D3-mCherry) significantly enhanced local cAMP elevations and promoted neuronal extension in the absence of any additional stimuli. Contrary, increasing PDE4D3 hydrolytic activity by targeting constitutively active enzyme to this compartment dramatically reduced length of axons and neuronal survival. In addition, in vivo delivery of 4D3-mCherry using AAV2 increased retinal ganglion cell survival following optic nerve injury. Our findings provide a demonstration of a new, functionally distinct neuronal compartment that regulate cAMP-dependent neuroprotection and axon growth and may be therapeutically targeted with AAV-based gene therapy. Supported by National Science Center grant no. 2019/33/B/NZ4/00587

P-03.1-04
Cotinine and 6-hydroxy-L-nicotine attenuates memory deficits and reduce anxiety and oxidative stress in a zebrafish (*Danio rerio*) model of Alzheimer's Disease

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Alzheimer's Disease (AD) is a progressive neurodegenerative disorder which affects almost 47 million people worldwide representing thus the most common form of dementia. AD is characterized by progressive cognitive decline and mood changes, accompanied by a loss of cholinergic neurons. Zebrafish (*Danio rerio*) has been successfully used to simulate AD pathology. In the central nervous system, nicotinic acetylcholine receptors (nAChRs) are involved in higher brain functions, such as memory, cognition and learning. There is considerable interest in modulating nAChRs to treat nervous system disorders, such as AD. Nicotine is a well-known agonist of nAChRs and was reported to improve memory, learning and attention, but the therapeutic use in AD was limited by its cardiovascular and addictive side-effects. Thereby, we focused on two structural related nicotine derivatives, namely cotinine (COT) and 6-hydroxy-L-nicotine (6HLN), that previously showed to improve cognition without exhibiting nicotine's side-effects. We evaluated the impact of COT and 6HLN on memory impairment, anxiety and oxidative stress in a zebrafish model of AD induced by scopolamine (SCOP). For this, COT and 6HLN were acutely administered by immersion to zebrafish that were treated with SCOP before testing. Anxiety was measured using the novel tank diving test (NTT) and memory performances were assessed by Y-maze and novel object recognition test (NOR). The oxidative stress was measured from brain samples. We have shown that 6HLN and COT improve memory performances in Y-maze and NOR tasks and reduce the anxiety level in NTT. Moreover, our data showed that these compounds reduce SCOP-induced oxidative stress. These findings support the premise that COT and 6HLN could be used as therapeutic agents in AD. *The authors marked with an asterisk equally contributed to the work.

P-03.1-05
Donepezil exerts cytotoxic effect on glioma cells

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Donepezil is a highly selective reversible inhibitor of acetylcholinesterase (AChE) that has been used to treat Alzheimer's disease (AD) due to its neuroprotective effects. There are a few studies that show its cytotoxic effect on cancer cells, but the mode of its action is poorly understood. In the present study, we investigated the mechanisms of potential cytotoxic effect of donepezil on C6 (rat glioma) and U251 (human glioma) cell lines. The viability rate of C6 and U251 cell lines was determined with the Cristal violet and MTT assay after 24h. Morphological changes were followed using light microscopy. Production of the reactive oxygen species (ROS), caspases activity, externalization of phosphatidylserine and the presence of the acid cytoplasmic vesicles were measured by Flow cytometry using specific fluorochromes (DHR, apostat, annexin-propidium iodide, and Acridine orange, respectively). In order to silence autophagy, U251 cells were transfected shRNA targeting human LC3II and AMPKα 1/2 genes. Donepezil decreased viability of both cell lines in dose dependent maner. When applied in its IC50 concentration donepezil triggered oxidative stress which has led to the caspase activation and the increased number of double-positive Ann/PI cells indicating the induction of apoptosis. In addition, donepezil induced autophagy since it increased the presence of the acid cytoplasmic vesicles (quantified as an increase of the orange-FL3/FL1 fluorescence, compared to the control). However, the production of ROS was decreased in AMPK and LC3II knockout cells, pointing out that oxidative stress triggers autophagy in U251 cell line. Based on the given results, it could be concluded that donepezil exerts its cytotoxic effect by inducing oxidative stress that causes both, apoptotic and autophagic cell death of C6 and U251 cells.

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P-03.1-06
Decrease of amino acid level in mice brain by L-lysine α-oxidase

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L-Lysine is an essential amino acid for humans. In recent years, several new effects of this amino acid have been discovered: the regulatory action of this amino acid and its protective effect on non-enzymatic glycosylation of proteins. Impaired L-lysine metabolism in the brain is associated with such diseases as glutaric aciduria type I and pyridoxine-dependent epilepsy. The specific pathway of L-lysine metabolism in brain is the oxidative deamination of α-amino group by L-amino acid oxidase. A similar enzyme was found in microscopic fungi of the genus Trichoderma. The aim of the work was to investigate the possibility of L-lysine α-oxidase from *Trichoderma* cf. *aureoviride* Rifai VKMF-4268D penetration through the blood-brain barrier and its effect on amino acids in brain. LO was injected into the tail vein of mice and determined in brain by enzyme immunoassay. Polyamines (PA) and amino acids were determined by HPLC. It is noteworthy that LO (two subunits of 60 kDa) crossed the blood-brain barrier in 15 min. The content of LO (87.2 ± 10.6 ng/g of tissue) was significantly lower than in the liver (675.9 ± 199.9 ng/g), but remained at the detectable level for 24 h. L-Lysine content in brain after 1 h was 69,1 % and reached minimum - 35,2 % in 6 hours and did not fully recover after 24 h. Since LO affects to a lesser extent structurally similar amino acids, the dynamics of their changes was studied. After 1 h, the content of ornithine and arginine was 80,4 % and 63 %, respectively, and even after 24 hours did not become normal (95,5 % and 76 %). Since ornithine is a precursor of PA, it was determined that their concentrations dropped down significantly. So, it is possible to conclude that LO may be in perspective investigated as a drug against serious brain diseases. The publication was prepared with the support of the "RUDN University Program 5-100"

P-03.1-07
Dissecting the biochemical background of galactose-induced transient hippocampal reductive shift

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We have previously shown that oral galactose treatment prevents and rescues cognitive decline in the streptozotocin-induced rat model of sporadic Alzheimer's disease. In contrast, chronic parenteral galactose administration causes oxidative stress in rodents and is therefore used as a model of accelerated aging. Interestingly, our results from the acute oral galactose treatment experiments suggest galactose can both increase oxidative stress and potentiate protective mechanisms, but the nature of this phenomena and its tissue specificity remain unknown. Male Wistar rats were treated with D-galactose (200mg/kg) by orogastric gavage and sacrificed 30, 60, or 120 minutes after the treatment. Our novel method was used for spatial reductive analysis. 1,2,3-Trihydroxybenzene autooxidation, carbonato-cobaltate (III) complex formation, redox couple ORP, TBARS assay, and DTNB thiol quantification were used to determine redox parameters. Galactokinase and c-fos were measured by western blot. Total and reduced NADP were estimated colorimetrically. Hippocampal and plasma samples were analyzed. The principal component analysis was used for multivariate exploration. Acute oral galactose treatment induces a robust activation of the hippocampal NADP system accompanied by a time-dependent increase in reduction potential. Oxidative stress parameters suggest the observed effect might be a consequence of hypercompensatory response to oxidative noxis. Galactose treatment increased hippocampal galactokinase, but decreased c-fos expression. Acute oral galactose induces transient hippocampal reductive shift at least partially by fueling the nicotinamide adenine dinucleotide phosphate system. The obtained results suggest hormetic stimulation of antioxidant defense system as one potential mechanism responsible for its neuroprotective properties.

P-03.1-08
ApoE genotype in Alzheimer's disease determination

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Alzheimer's disease (AD) is a progressive, dementing disorder, usually of middle or late life. It is the most common neurodegenerative disease and represents frequent cause of dementia accounting for roughly half of all cases. A clinical diagnosis of probable AD can be made with confidence if there is a typical insidious onset of dementia with progression and if there are no other systemic or brain diseases that could account for the progressive memory and other cognitive deficits. More than 20 genes affecting the risk of developing Alzheimer's disease have been identified. The strongest association with AD has the apolipoprotein E (APOE) gene. The most common alleles are Apo E4, E3 and E2 in heterozygous or homozygous states. The strongest genetic risk factor for AD is the homozygous ApoE E4/E4. The aim of our study was to identify for the first time the frequency of ApoE alleles in the Slovene population of patients with dementia that have been treated at Center for Cognitive Impairments at the Department of Neourology, University Medical Centre in Ljubljana. The cohort included over 500 patients with dementia in 64 healthy volunteer controls. We isolated DNA from venous blood and determined ApoE genotypes by RT-qPCR on LightCycler (Roche), based on hybridization probes. Initial analyses show that the genotype ApoE E3/E3 was the

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most common homozygous allele in the Slovene population and genotype ApoE E4/E4 was the least common. The statistical analyses to correlate a particular ApoE genotype and biochemical blood markers with the clinical diagnosis are in progress. The results will provide a new insight in clinical diagnostic value of ApoE genotype for AD in the Slovenian population.

P-03.1-09
Effects of long-term chemotherapy on elderly Wistar rats in experimental model *in vivo*

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Conventional anti-glioblastoma chemotherapy includes temozolomide (TMZ) and dexamethasone (DXM) to prevent brain oedema, but patients often suffer from the side effects on the nervous system, and the molecular mechanisms of them are poorly understood. Major components of brain tissue extracellular matrix are chondroitin sulfate proteoglycans (CSPGs) which are involved in the development of various brain pathologies. The aim of this work was to study the effect of long-term administration TMZ and/or DXM on behavior of experimental animals and the expression of the CSPGs in the brain tissue. TMZ (150 mg/m2) and/or DXM (2,5 mg/kg) were administrated to elderly Wistar rats for 4 months. To investigate the general locomotor activity and anxiety-related behavior, open field and the elevated plus maze tests were used. Expression of the main CSPGs (Cspg4/Ng2, brevican, aggrecan, versican, neurocan, biglycan, Cspg5, phosphacan) was studied using RT-PCR. TMZ administration resulted in a decrease of horizontal locomotor activity, increase anxiety levels of rats and reduced expression of aggrecan by 7,8-fold ($P < 0,05$) in rat brain tissue. DXM treatment led to an increase in total locomotor activity and down-regulation of aggrecan (35-fold) and neurocan (1,8-fold, $P < 0.05$) mRNA levels. The combined treatment TMZ and DXM increased the vertical locomotor activity and decreased aggrecan transcription level (19,5-fold, $P < 0.05$). Taken together, obtained results demonstrate that long-term TMZ administration reduces the general locomotor activity of adult rats and induced anxiety-related behavior. Downregulation of aggrecan and neurocan expression upon TMZ treatment might be involved into these changes in the behavior characteristics of the experimental animals. This study was funded by the Russian Science Foundation, grant number 19-75-00051. AVS was supported by a scholarship of Russian Federation President for young scientists (SP-1816.2019.4).

P-03.1-10
How halogen substitutions steer pharmacological properties of a molecule – development of antidotes for organophosphorus poisoning

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Organophosphorus compounds (OPs) are used as pesticides or misused as chemical warfare nerve agents because they irreversibly inhibit acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) that leads to the accumulation of acetylcholine in the synapses of central and peripheral nervous system and to a life-threatening cholinergic crisis. The reactivation of inhibited cholinesterases by oximes in combination with antimuscarinic and anticonvulsive drugs is crucial in the treatment of OP poisoning. Oximes used in medical practice have a permanently charged pyridinium ring and cross the blood-brain barrier (BBB) poorly. However, their BBB penetration rate could be improved by the addition of a halogen substituent that increases lipophilicity. Structures of efficient bispyridinium oxime reactivators that differ in the linker between the rings (propane, butane, butene)[1] were the basis for the synthesis of analogues with chlorine[2,3] or fluorine atoms. Using biochemical techniques, we tested the analogues and observed specific patterns in their interaction with AChE and BChE (pharmacological targets) and in antioxidative potential of oximes (possible supplementary pharmacological mechanism) based on the linker and substituents. Moreover, the structural differences of oximes were reflected in their cytotoxicity on neuroblastoma cell line. Our results highlighted di-chlorinated bispyridinium oxime with propane linker as the best candidate for antidotal treatment after OP poisoning due to its high reactivation potential and no cytotoxic effects at pharmacologically relevant concentrations. Acknowledgement: Supported by the Croatian Science Foundation (HrZZ-IP-2018-01-7683, HrZZ-UIP-2017-05-7260). [1]Kuca et al. JEIMC, 2003, 18 (6), 529–35. [2]Zorbaz et al., JMC 2018, 61(23), 10753–66. [3]Zorbaz et al., CBI, 2019, 307, 16–20.

P-03.1-11
Characterization of the interaction between Shank-PDZ and GKAP-GH1 domains

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Post-synaptic density (PSD) scaffolding proteins showcase high structural variability and a large number of interactions where structural dynamics play a crucial role. While participating in the formation and maintaining the organization of the PSD, they also contribute to the regulation of signaling and modulating glutamatergic response. Shank proteins are a family of scaffolding proteins abundant in the PSD with a significant role in establishing PSD morphology and bridging the various glutamate

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receptors with the cytoskeleton. Members of the protein family have a characteristic domain composition, consisting of globular domains – including a PDZ domain – connected by disordered regions. Dynamic behaviour of these globular domains is yet to be characterized and disordered segments are suspected to also regulate interaction dynamics. PDZ domains of Shank proteins have multiple interaction partners, binding the C-terminal motif of GKAP, another scaffold, with a notably higher affinity than its other binding partners. Recombinant protein constructs corresponding to the PDZ domains (rat Shank1 and Shank3 PDZ, the latter also including a disordered segment towards the N-terminal) and GKAP GH1 C-terminal domains were produced in E. coli and purified using a protocol of subsequent chromatography steps (IMAC, IEC and SEC columns). NMR and ECD measurements were performed to assess proper folding of each construct and native PAGE and IMAC pull-down assay verified both PDZ-GH1 interactions. Preliminary ITC measurements were performed and Kd values were determined but require further experiments to verify. A double isotope-labelled (^{15}N and ^{13}C) Shank1 PDZ construct was produced and 3D NMR measurements were performed, resulting in spectra sufficient for structure determination, and a near complete backbone assignment was obtained. NMR titration experiments with full-length GH1 revealed the dynamic behaviour of the PDZ domain.

P-03.1-12
Intracellular distribution of CPEB protein Orb2 in brain neurons of *Drosophila melanogaster* depends on 3'UTR of Orb2-coding mRNA

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CPEB proteins take part in translation activation mediated by cytoplasmic polyadenylation. CPEB protein Orb2 from *D. melanogaster* is expressed in central neuros system of adult flies. Compromised Orb2 expression leads to impairment in learning and long-term memory formation. Presumably, 3'UTR of Orb2 mRNA participates in protein translation via autoregulatory processes. To study this mechanism, we have deleted the most part of 3'UTR of Orb2 gene bearing multiple CPE-sequences with help of CRISPR/Cas9 system, resulting allele was designated as Orb2R. Immunofluorescence staining of brain of Orb2R flies using antibodies against Orb2 protein displayed a drastic reduction of its level in neuron outgrowths compared to wild type, while Orb2 level in cell soma was the same. Synaptosome fraction prepared from Orb2R fly brain extracts contained 5 times lower Orb2 protein amount than wild type had, although total Orb2 protein level was 2 times lower than in wild type, probably due to decrease of mRNA stability. Obtained results allow us to suggest the following model of Orb2 expression regulation: Orb2 protein, being translated in cell body, binds its own mRNA and transfers it to synapses in complex with a transport protein. Earlier studies have shown the ability of Orb2 to facilitate dendritic transport of several mRNAs, Orb2 also can bind its own mRNA via CPE-sequences. 3'UTR deletion leads to decrease of Orb2 protein level only in synapses, that is why it is important for mRNA transport and on-sight translation. In synapses translation from localized Orb2 mRNA starts, what causes predominant localization of Orb2 protein in synapses. This model is also supported with previously obtained data which have shown the prevalence of Orb2 protein localization in neurons outgrowths. Here we have shown the new role of autoregulation process in Orb2 expression depending on its intracellular localization. This work was supported by Russian Science Foundation grant 18-74-10051.

P-03.1-13
Dynamics of the salivary cortisol level and some autonomic nervous system indexes under the biofeedback controlling motor-cognitive task at healthy volunteers

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The research is aimed at studying a 4-day course of biofeedback (BFB) training influence on saliva cortisol level and autonomic nervous system functional state of 25 20–22-year-old healthy male volunteers. During the sessions, all subject alternately, both hands, for 60 seconds using the force applied to the power joystick placed on the stabilometric platform, was shifting the mark on the computer screen. The mark shifting corresponded to the force applied to the joystick, and the trajectory depended on objects randomly appearing on the screen with which the controlled mark must be combined. Before and after trainings background physiological values (saliva cortisol level, heart rate variability) were recorded. The concentration of salivary cortisol was studied using an Anthos 2010 microplate photometer with filters (400–750 nm) and ADAP + (Biochrom Ltd, UK) on Salivary Cortisol Elisa SLV-2930 diagnostic kits (DRG, USA). The functional state of the volunteers' autonomic nervous system was monitored using by of heart rate variability (HRV) method using the complex "Omega-M" ("Dynamics", St. Petersburg "). During the of the biofeedback controlling motor-cognitive task course on subjects was recording decrease of cortisol concentration from the third day of the study (by 18.37 $P < 0.05$). On the 4th day, the maximum decrease in this indicator values of by 36.41% (3.34 ± 0.1 m.mol/l) was recorded. The data obtained are confirmed by subjects' heart rate variability increase (on the 5th day of the study, the values of total power of heart rate spectrum increased by 22.6% $P < 0.05$, the stress index decreased by 27.9% $P < 0.05$). Preliminary results of this study indicate, that the BFB training result can be associated to the salivary cortisol level decrease and their autonomic nervous system functional state changes. Supported by the V.I. Vernadsky Crimean Federal University grant for young scientists № AAAA-A20-120012090164-8.

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P-03.1-14
A novel anticonvulsant drug prototype with high inhibition effect on ERK1/2 pathway

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An effective treatment for epilepsy is one of the most important social and medical problems of our time. Up to 30% of cases of epilepsy are characterized by pronounced pharmacoresistance, which requires a search for a new drug. GIZH-298 is a new 4-benzoilpiridine oxime derivative which prevents convulsions and protects 100% of experimental animals against death (90% death in the control group) in a dose-dependent manner in the maximal electroshock seizure test in mice. This compound exhibits higher protection than valproic acid, routinely used anticonvulsant drug. Moreover, GIZH-298 has a pronounced anticonvulsant effect in the chronic cobalt and D,L-homocysteine thiolactone induced epilepsy model which generates seizure activity in various brain structures in rats with a predominant effect in the cortex and hypothalamus. The protection is realized in the decrease of motor manifestations and in elimination of EEG discharges as well as in protection against the death of treated animals. Using the maximal electroshock model in mice, we showed that administration of GIZH-298 at a dose of 60 mg/kg significantly reduces the phosphorylation of extracellular signal-regulated kinases (ERK1/2) and synapsin I, which plays an important role in seizure. This ERK1/2 inhibition effect of GIZH-298 was comparable to that of valproic acid. Exploring neuroblastoma SH-SY5Y cell culture we demonstrated that GIZH-298 also reduces ERK1/2 phosphorylation level in a dose-dependent manner in vitro. The data obtained suggest that the mechanism of GIZH-298 protective effect is based on a decrease of the ERK1/2 phosphorylation by direct inhibition of the upstream components of the ERK1/2 cascade. The obtained results enable to conclude that GIZH-298 can be recommended for further study as a promising anticonvulsant drug in humans. The work was supported by the grant of Russian Science Foundation #19-14-00167

P-03.1-15
Proteins binding RNA transcripts from C9orf72 gene mutation

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Mutation in C9orf72 gene is the most common genetic cause of two fast progressing and incurable neurodegenerative disorders – amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). The disorders are very close on genetic and neuropathological spectrum, while symptomatically different. In ALS motor neurons are affected, which leads to muscle atrophy, while FTD is presented by degeneration of frontal and temporal cortex. C9orf72 mutation is presented as increased number of hexanucleotide repeats of G4C2 sequence in gene non-coding region. Healthy individuals have up to 23 repeats, while in patients several hundred or several thousand repeats are present. Three mechanisms of action are proposed for the mutation. First, presence of extended repeats can cause haploinsufficiency of C9orf72 protein. Second, hexanucleotide repeats are transcribed to RNA in sense (G4C2) and antisense (C4G2) direction, both transcripts form mostly nuclear RNA foci in spinal cord and brain neurons of C9 ALS/FTD patients and are proposed to interact with RNA binding proteins important for normal cell functioning. Third, non-canonical translation of RNA repeats produces proteins with dipeptide repeats (DPRs), which are also to be toxic for the cell. The aim of our research is to identify the proteins binding to sense and antisense RNA transcripts from the C9orf72 gene mutation and define their role in the development and progression of ALS and FTD. For identification of the proteins, we have set up RNA pull-down assay using long, biologically relevant RNA constructs in combination with mass spectrometry. For identification of interaction protein-RNA proximity ligation assay was set up. We have found proteins involved in various processes important for cell survival and normal function to be interacting with sense and antisense RNA repeats. We will present our latest findings on these interactions and their importance for disease development and progression.

P-03.1-16
Analysis of Vti1a and Vti1b double deficiency in neuronal cells

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During embryogenesis crucial steps of the neuronal development are strictly regulated by intra and extracellular signals therefore endosomal transport has emerged as a modifier of signaling. SNARE proteins play a central role in these trafficking processes. Vti proteins belong to the Qb-SNARE family and are involved in the neuronal development during the embryonal stage. They are conserved from yeast to humans. A double deficiency of the mouse homologues Vti1a and Vti1b was created in previous work. A single knock-out of one of the Vti genes is not lethal whereas the lack of both proteins leads to a perinatal death due to neuronal cell death and defects in neurite outgrowth. In this work, we present investigations into neuronal cells of double knock-out (DKO) mouse neurons via immunocytochemistry and other techniques to show differences in neurite morphology and postsynaptic densities. Recently, we were able to present that the Golgi morphology in DKO neurons is changed compared to double heterozygous (DHET) littermates. Previously published in: Emperador-Melero J et al. (2018) Nat Commun 9, 3421. In this work we could go further into details of these differences. To deepen the understanding of the molecular mechanisms that underlie the genetic defects we analyzed a variety of signaling pathways.

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P-03.1-17
Deciphering the antidepressant effect of ketamine by evaluating its action on astrocytes

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Ketamine (KM), an anaesthetic and psychotomimetic drug, exerts rapid potent and long-lasting antidepressant effect. The cellular and molecular mechanisms of this action are incompletely understood. Besides neurons, KM also affects the function of astroglia. We therefore examined the effect of (sub) anaesthetic doses of KM on fusion pore activity of secretory vesicles, mobility of vesicles carrying the inward rectifying potassium channel (Kir4.1) and stimulus-evoked calcium signaling in cultured rat astrocytes. High-resolution patch-clamp membrane capacitance measurements were used to determine the fusion pore activity of secretory vesicles. The spontaneous mobility and plasmalemmal localization of Kir4.1-EGFP in pKir4.1-EGFP-transfected astrocytes, labeled by the membrane styril dye FM4-64, was determined by confocal microscopy. The effect of KM on ATP-evoked calcium (Ca^{2+}) response was examined by measuring the Fluo-4 fluorescence in KM-treated and non-treated astrocytes. KM-evoked increase in vesicle bursting activity was well correlated with a decrease in irreversible vesicle fission from the plasmalemma (R = 0.93 for increasing KM incubation time and R = 0.99 for increasing [KM]). 30 min KM treatment sufficed to decrease the directional mobility of Kir4.1-positive vesicles. The apparent surface localization of Kir4.1 at astrocyte plasmalemma decreased from 56% in non-treated controls to 43% ($P < 0.05$) and 33% ($P < 0.05$) in astrocytes treated with 2.5 and 25 μM KM, respectively. The ATP-evoked peak Ca^{2+} responses were diminished in KM-treated astrocytes with ATP mobilizing ~3.3-fold less Ca^{2+} than in non-treated controls ($P < 0.001$). Distinct but not mutually exclusive mechanisms of KM action may synergistically evoke changes in synaptic functional plasticity, resulting in sustained strengthening of excitatory synapses, required for antidepressant effects. Previously published in: Stenovec M et al. (2020) Neurochem Res. 45, 109–121

P-03.1-18
NMR-based structural characterization of the post-synaptic density scaffold protein GKAP

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NMR Scaffold proteins of the post-synaptic density (PSD) have been shown to have a high relevance in different neuronal disorders, as they participate in the regulation and modulation of protein-level processes of learning and memory. Detailed structural description of these proteins might lead to the better understanding of their interactions and mechanism of action, and also their contribution to the associated diseases. GKAP is one of the essential scaffold proteins in the PSD, interacting with the GK domain of PSD-95, the dynein light chain (DLC2) motor protein, and the PDZ domain of Shank. Most of GKAP is predicted to be disordered, and its structure is unknown, except for the GH1 domain on the C terminal. Our aim is to explore the structural ensembles and dynamics that characterize the disordered segments of GKAP: the GK binding region, the dynein binding segment, and the first 300 residues with unknown function or interaction partners. To do so, we are performing protein–protein interaction assays (among others ITC and ECD spectroscopy), and triple resonance 3D NMR measurements on 13C- 15N-labeled protein constructs alone and titrated with their interaction partners. The obtained chemical shifts will serve as input for generating ensemble-based structural models of GKAP, which will contribute to a more detailed description of PSD organization. Keywords: GKAP, protein structure, post-synaptic density.

P-03.1-19
TNF-induced astrocyte reactivity and augmented β3 integrin levels are regulated by Rab endocytic pathways

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The pro-inflammatory environment generated by brain injury induces "Gliosis", a process where astrocytes undergo hypertrophy, migration and increased expression of proteins including glial fibrillar acidic protein (GFAP), syndecan-4, and ß3 integrin. Increased levels of αVβ3 integrin in reactive astrocytes promote cell migration through association with the neuronal protein Thy-1. The mechanisms implicated in the up regulation of αVβ3 integrin in astrocytes under pro-inflammatory conditions remain unclear. We performed a comparative meta-analysis of gene expression profiles from four different datasets (GSEs) of non-reactive versus reactive astrocytes. The hierarchical clustering comparisons were performed using the MultiExperiment Viewer program, and affected signaling pathways were examined with the Kyoto Encyclopedia of Genes and Genomes. DITNC1 astrocytes were treated with the proinflammatory cytokine TNF for 48 h to induce reactivity in vitro. β3 integrin levels were evaluated by western blotting. GTPase activities were measured using pull-down assays. Thy-1-induced astrocyte migration was evaluated in transwell assays. The bioinformatic analysis revealed alterations in Rab endocytic pathways in reactive astrocytes. TNF treatment changed the expression of proteins associated with the appearance of reactivity markers, such as GFAP, and increased surface levels of β3 integrin in the DITNC1 cells. This same treatment altered Rab and Rac1 GTP loading. Thy-1-induced astrocyte migration was also associated with the changes observed in Rab protein levels. These studies aid in understanding the molecular mechanisms involving Rab endocytic pathways in astrocyte reactivity, an important process observed in pathological conditions, such as neurodegenerative diseases like Amyotrophic Lateral Sclerosis and following brain injury. Study financed by FONDECYT 1200836 (LL), 1170925, 1210644

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P-03.1-20
Regulation of aerobic glycolysis by agonists selective for the canonical L-lactate receptor HCAR1

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Astrocytes provide energy to support neurons, which involves astroglial aerobic glycolysis, where the metabolite pyruvate is converted to L-lactate by lactate dehydrogenase (Dienel GA (2012) ASN Neuro 4(5); Vardjan N et al., (2018) Front Mol Neurosci 11:148). We are interested to understand the regulation of astroglial aerobic glycolysis by L-lactate, which is not only fuel but also likely represents an extracellular signal. L-lactate signaling may occur through several mechanisms, including the activation of the L-lactate-sensitive receptors, such as the G_i-protein coupled receptors HCAR1 or the yet unidentified plasma membrane receptor(s). It is known that extracellular L-lactate in astrocytes activates adenylyl cyclase, elevates cytosolic cAMP and accelerates aerobic glycolysis. Interestingly, by using selective agonists for HCAR1, even in the absence of this receptor, in astrocytes from HCAR1-knockout (KO) mice, elevation in cytosolic cAMP was still recorded. This indicates that in addition to the HCAR1, these agonists activate also a yet unidentified L-lactate receptor-like mechanism, which is also present in undifferentiated 3T3-L1 cells (Vardjan N et al., (2018) Front Mol Neurosci 11:148). To identify the new L-lactate receptor we determined potential candidates by using bioinformatic analyses and molecular dynamics. Furthermore, by using the CRISPR/Cas9, 3T3-KO cell lines were made. We used a FRET (fluorescence resonance energy transfer) nanosensor to measure the elevation of the intercellular L-lactate, upon stimulation of the knocked-out cells with different concentrations of selective agonists. On the basis of our preliminary results, we noticed differences in dose-dependent responses and responsiveness between wildtype and KO cells. This research will help us to better understand the dynamics of the mechanisms of GPCR-mediated signaling pathways in astrocytes and in 3T3 cells.

P-03.1-21
Conjugates of fluorescent proteins and animal toxins: molecular probes for ion channel visualization

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The problem of ion channel detection and visualization seems to be relevant in the light of the high diversity of these proteins in different cells and tissues. At the present time, expression profile of ion channels is almost exclusively studied at the mRNA level (without information on expression at the functional protein level), and the detection and visualization of proteins is performed with antibodies (the most common limitations of their use include low discrimination between close channel isoforms, usage of intracellular epitopes, and high price). In our consideration, application of molecular probes based on natural polypeptide ligands that act on ion channels could serve a powerful approach to detect these target proteins. We have designed and produced dozen chimeric proteins which are comprised of several essential parts (modules). One of the modules is natural toxin serves for selective channel recognition, and other is presented by fluorescent protein for effective detection using fluorescent assays. Modules are separated by short amino acid linker to prevent interference between the module functions. We conjugated a number of known polypeptide ligands of different ion channels (acetylcholine receptors, potassium, sodium, and calcium channel) with several fluorescent proteins varying by spectral properties. Such toolkit of chimeric proteins could be further applied for ion channel detection on single cell membrane, visualization of ion channels in cell culture, and staining tissues slices. Also, these fusion proteins could be used as reporter instruments for observation of ion channel expression level during some diseases. Finally, toxins modified by fluorescent proteins are chip and effective compounds for the screening systems in pharmacological researches. This work was supported by the Russian Foundation for Basic Research [grant number 20-34-70031].

P-03.1-22
Human three-finger neuromodulator Lynx2 down-regulates cognition-related processes

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Human endogenous three-finger modulators of nicotinic acetylcholine receptors (nAChRs) are proteins from the Ly6/uPAR family with wide functional diversity and can play various roles in the essential processes. These proteins demonstrate structural similarity and share high structural homology with snake α-neurotoxins. Here we investigated structural and functional properties of Lynx2, one of the poorly studied members of the Ly6/uPAR family. It is known that Lynx2 is a membrane-tethered by GPI anchor protein, binds specifically to α4β2-nAChRs and is connected with anxiety-related behavior. The recombinant analog of water-soluble variant of human Lynx2 was produced in *E. coli* in a form of the cytoplasmic inclusion bodies with subsequent renaturation. The structure and dynamics of Lynx2 were studied by NMR in aqueous solution. Lynx2 adopts the "three-finger" fold, having a compact beta-structural core with three protruding extended loops. Magnetic beads coupled with recombinant Lynx2 specifically extracted from the total rat brain homogenate the α4, α6, and β2 subunits of nAChR and α5 and γ2 (but not β2) subunits of the GABAA-R. Analysis of the Lynx2 binding to the α1, α3, and α7 nAChR subunits did not allow to make an unambiguous conclusion about the interaction of Lynx2 with these subunits. Recombinant Lynx2 inhibited the long-term potentiation (LTP) modeling synaptic plasticity in mouse hippocampal slices. The similarity of α-Bgtx (potent selective inhibitor of a7-nAChR) and Lynx2 effects suggests that the suppression of LTP by Lynx2 can be associated with negative modulation of α7-nAChR. Treatment by recombinant Lynx2 also demonstrated

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down-regulation of the spine number and mRNA expression of α7, α4 and β2 nAChR subunits, synaptophysin, and synapsin 1 in rat hippocampal neurons. Altogether these data point to the negative Lynx2 influence on cognition-related processes. Study was supported by the Russian Science Foundation (Project № 19-74-20176).

P-03.1-23
Baicalin and Rhoifolin prevents scopolamine-induced memory impairment and brain oxidative stress in zebrafish (*Danio rerio*)

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Alzheimer's disease (AD) is a multifactorial progressive and irreversible neurodegenerative disorder. AD is the most common form of dementia affecting about 10% of people over the age of 65. The disease is characterized by the massive loss of neurons and synaptic connections in the cortex and hippocampus, leading to memory difficulties, anxiety, and depression. For the simulation of AD disease, *Danio rerio* fish had been used successfully. AD is associated with a significant problem in cholinergic transmission, so there is a growing interest in the discovery of different agents with an inhibitory role on acetylcholinesterase (AChE). Numerous studies show that certain natural flavonoids significantly improve cognitive abilities and may have an inhibitory effect on AChE. We focused on two natural flavonoids, namely Rhoifoline (Rho) and Baicalin (Bac), which are described in previous studies as having beneficial effects on cognitive processes, with no adverse reactions. Substances were administered by immersion of zebrafish once daily for 16 days. To induce anxiety and memory impairment, scopolamine was administered. Anxiety was measured using the Novel Tank Diving Test (NTT), and memory was assessed by the Novel Object Recognition Test (NOR) and Y-maze test. We also evaluated the impact of the two flavonoids on the oxidative state of this animal model. Our data show that Rho and Bac improved spatial memory in the Y-maze tasks and also improved the recognition memory of the zebrafish in the NOR test and reduced anxiety levels in NTT. Moreover, our data indicate that these compounds reduced the level of oxidative stress caused by the administration of scopolamine and increased the activities specific to catalase and glutathione peroxidase, which suggests the antioxidant profile. Our results argue that Rho and Bac can restore memory degradation and induce neuroprotective effects in dementia animals. Therefore, they could be used as therapeutic agents in AD.

P-03.1-24
Specific serotonin uptake as a functional indicator of the developmental potential of follicles in the mouse ovary

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Serotonin is a universal signaling agent involved in a number of processes outside the nervous system. One of its crucial functions is the regulation of a female reproductive system. In particular, serotonin is involved in the regulation of functional activity of the mammalian ovary, oogenesis, and steroid hormone synthesis. Due to low levels of serotonin synthesis in the ovary, the main mechanism for its accumulation is the uptake of maternal serotonin from the extracellular environment, presumably from the bloodstream. We have shown that the mechanism of serotonin uptake is active in oocytes of growing follicles, and is supplied by the specific serotonin membrane transporter SERT. Accumulation could be inhibited by a selective serotonin reuptake inhibitor (fluoxetine), but not by a non-selective OCT/PMAT-mediated polyspecific biogenic amine transport inhibitor (decinium-22). The fact that the activity of serotonin uptake by the oocytes of the growing follicles differ is of particular interest. About half of the oocytes show the pronounced activity of the serotonin uptake mechanism, while the rest do not accumulate serotonin at all. At the same time, both these groups relate to the same stage of folliculogenesis. The correlation of SERT activity with some indicators of the morpho-functional state of the components of the ovarian follicle: follicular morphology, the proliferation of follicular cells, and chromatin conformation in the germinal vesicles of oocytes were also analyzed. It has been shown that the activity of SERT in oocytes is a functional indicator of the developmental potential of ovarian follicles. The research was done using equipment of the Core Centrum of Institute of Developmental Biology RAS at the expense of a grant from the Russian Foundation for Basic Research (20-04-00303) and a grant from the President of the Russian Federation (MK-931.2020.4).

P-03.1-25
Serotonin uptake into human cord blood platelets: modulation by maternal metabolic state

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Serotonin (5-HT) plays a crucial role in fetal brain development by regulating the outgrowth of its own neurons and the maturation of their target regions. In adults, active levels of 5-HT in the blood are tightly controlled by the high-affinity uptake of 5-HT into platelets. The aim of the present study was to examine the presence of an analogous 5-HT uptake system in the fetal circulation and investigate its potential modulation by maternal gestational diabetes mellitus (GDM). The study was performed on mother-newborn pairs recruited at the Clinical Hospital Centre Zagreb, Croatia, as a part of our ongoing birth cohort study PlaNS (Placental and Neonatal Serotonin). Cord blood samples were collected via umbilical venipuncture immediately after baby birth. Platelet rich plasma (PRP) was isolated by centrifugation and 5-HT uptake kinetics was studied using a radiotracer-based assay. Mean platelet volumes in whole cord blood and PRP samples were highly correlated, demonstrating that population of platelets isolated in PRP represented well platelets in the whole cord blood samples. Neonatal platelets showed efficient, time- and temperature-dependent 5-HT uptake, with initial rates of specific 5-HT transport saturable over the high-affinity range of 5-HT concentrations (0.1 to 2.0 μM). In all subjects tested, values of Michaelis affinity constant (Km) and maximal transport velocity (Vmax) were characteristic of the uptake-1 (high-affinity / low-capacity) transport mechanism, and comparable to those in adult platelets. Further, 5-HT transport into cord blood platelets

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was compromised by maternal GDM, due to decreased substrate affinity (increased Km value). In conclusion, data provide the first demonstration of a functional system for 5-HT uptake in human neonatal platelets and suggest that it could represent a potential mechanism mediating the influence of GDM on fetal brain development. The study was funded by the Croatian Science Foundation (IP-2018-01-6547).

P-03.1-26
Clobetasol promotes neuromuscular plasticity in a mouse model of motoneuronal loss

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Neuronal loss represents the consequence of direct or indirect insults to neurons and the major factor mediating persistent disability. Sonic hedgehog (Shh) is a signalling protein regulating cell fate and differentiation and has been indicated as a critical factor in developing and maintenance of the central nervous system (CNS), thus holding great potential in promoting CNS repair and regeneration. Recent evidences support a neuroprotective and regenerative role of the Shh-signalling activator clobetasol, able to target and to activate the effector of Shh pathway smoothened. Herein we aimed at studying behavioural, neuromuscular and metabolic restorative effects of clobetasol in a mouse model of spinal motoneuronal depletion induced by cholera toxin-B conjugated to saporin. We found that clobetasol ameliorates behavioural impairment and muscle denervation restoring Shh-signalling on resident glial cells and neurons and fostering spinal plasticity. These evidences were coupled with a reduced reactive astrogliosis and pro-inflammatory microglial phenotype in the affected spinal tract. We then analyzed the effects on muscular trophism and metabolism, finding that clobetasol increases the mean myofiber area and mitochondrial fitness, recovering ATP/ADP ratio and energy charge potential at 6 weeks post-spinal motoneuronal ablation. Our results suggest that clobetasol supports compensatory processes and represents an exploitable approach for denervating and degenerative disorders, increasing neuromuscular plasticity upon motoneuronal depletion. *The authors marked with an asterisk equally contributed to the work.

P-03.1-27
T3 thyroid hormone regulates the actin cytoskeleton dynamics during hypoxia through avb3 integrin in differentiated PC-12 cell

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Abstract: Integrins mediate a lot of cellular responses to extracellular signals. Hypoxia induces the F actin accumulation and actin filament rigidness, that initiate the neurodegeneration and reduce the plasticity during hypoxia. It was hypothesized that thyroid hormones via avb3 integrin maintain the actin filament rearrangement ability during hypoxia and increase the viability of neuronal cells. We investigated the action of 3,5,3′-triiodo-L-thyronine (T3) and L-thyroxine (T4), and anti-avb3-integrin antibody (for integrin inhibition) on the actin cytoskeleton dynamics and analysed the possible integrin-mediated downstream signalling pathway under hypoxic conditions in differentiated pheochromocytoma (PC-12) cells. It was found that T3 deprivation alters the G/F actin ratio reducing the actin filament rearrangement ability during hypoxia. Whereas the presence of thyroid hormones maintains the dynamic ability of actin filament inducing the partial activation of Fyn through avb3-integrin that and alters G/F actin ratio via Rac1/NADPHoxidase/cofilin-1 pathway and survive the neuronal cells during hypoxia. We propose that presence of T3 during hypoxia maintains the neuronal actin filament dynamics via avb3 integrin and this way increases the viability of the cells during hypoxia and elevates the recovery ability of cells after hypoxia. Keywords: Integrin; cofilin-1; actin filament; Fyn; hypoxia; PC-12. Significance of the Study. To our knowledge, these observations may be important for understanding the effects of thyroid hormone in the hypoxia-induced alterations and avb3 integrin participation in the recovery ability of neurons due to increasing the G actin pools, alteration in G/F ratio and actin filament dynamics during hypoxia. Acknowledgments: This research is supported by the Shota Rustaveli National Science Foundation of Georgia SRNSF Georgia grant #: PHDF-19-751. The author would like to thank the World Federation of Scientist.

P-03.1-28
Transcriptome of the audiogenic rat strain and identification of possible audiogenic epilepsy-associated genes

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Audiogenic epilepsy (AE), developing in rodent strains in response to loud sound, is widely used as a model of generalized convulsive epilepsy. The molecular mechanisms that determine the expression of AE are currently not well understood. In the present work we compared transcriptomes from the corpora quadrigemina (CQ), the crucial epileptogenic zone of rats with different AE proneness. Rats of three strains were used: Krushinsky-Molodkina (KM) strain (100% AE-prone); "0" strain, selected from F2 KMxWistar hybrids for lack of AE and Wistar outbred rats (with no AE proneness). It is shown that gene expression profile of the KM strain has a number of characteristic differences from those of Wistar and "0" strains. In particular, KM has increased expression of a few genes involved in positive regulation of the MAPK signaling cascade and apoptotic processes. It was shown previously that the activity of the MAPK cascade is associated with generation of serizures, and that KM has increased apoptosis level in the CQ. The next characteristic difference between the KM strain from Wistar and "0"

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is a multiple increase in the expression level of the Ttr gene, and a significant decrease in the expression of the Msh3 gene involved in the DNA mismatch repair system and in a number of the oxidative phosphorylation system genes. These data are consistent with those indicating a lower level of ATP production in KM brain mitochondria. Our data confirm the complex multigenic nature of AE inheritance in rodents. A comparison with the data obtained from other models of AE suggests that the convulsive phenotype may develop in different ways in independently obtained audiogenic rat strains. On the other hand, changes in the expression of the same genes may occur also in another rodent species, apparently leading to the development of the AE phenotype. The work was supported by the grant of Russian Science Foundation #191400167

P-03.1-29
Phase separation of synapsin, alpha-synuclein and synaptic vesicles

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Neurotransmission relies on the tight spatial and temporal regulation of the synaptic vesicle (SV) cycle. Nerve terminals contain hundreds of SVs that form tight clusters. Recent compelling evidence indicates that SV clusters at the nerve terminal are assembled as liquid condensates in which one component of the phase are SVs and the other synapsin 1, a highly abundant synaptic protein. Another major family of disordered proteins at the presynapse includes synucleins, most notably α-synuclein. The precise physiological role of α-synuclein in synaptic physiology remains elusive, albeit its role has been implicated in nearly all steps of the SV cycle. To determine the effect of α-synuclein on the synapsin phase, we employ the reconstitution approach using natively purified SVs from rat brains and the heterologous cell system to generate synapsin condensates. Our data indicate that synapsin condensates recruit α-synuclein, and while enriched into these synapsin condensates, α-synuclein still maintains its high mobility. The presence of SVs enhances the rate of synapsin/α-synuclein condensation, suggesting that SVs act as catalyzers for the formation of synapsin condensates. Notably, at physiological salt and protein concentrations, α-synuclein alone is not able to cluster isolated SVs. Excess of α-synuclein attenuates the kinetics of synapsin/SV condensate formation, indicating that the molar ratio between synapsin and α-synuclein is essential in assembling the functional condensates of SVs. Understanding the molecular mechanism of α-synuclein interactions at the nerve terminals is crucial for clarifying the pathogenesis of synucleinopathies, where α-synuclein, synaptic proteins and lipid organelles all accumulate as insoluble intracellular inclusions.

P-03.1-30
Biochemical properties of human D-3-phosphoglycerate dehydrogenase

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L-Serine plays a key role in the metabolism of eukaryotic cells and in several physiological functions. Moreover, L-serine is the precursor of glycine and D-serine, two coagonists of NMDA receptors. Human D-3-phosphoglycerate dehydrogenase (hPHGDH, EC 1.1.1.95) catalyzes the reversible transformation of D-3-phosphoglycerate (3PG, generated by glycolysis) into 3-phosphohydroxypyruvate (PHP) using NAD+ as cofactor. This is the first and the rate-determining step in the "phosphorylated pathway", which regulates the de novo biosynthesis of L-serine [1,2]. Here, we performed a detailed biochemical characterization of recombinant hPHGDH. The effect of pH, temperature, ligands, and ions on hPHGDH activity was investigated. We clarified the substrate specificity by establishing the kinetic parameters in the forward reaction for 3PG and alternative carboxylic acids: 3PG is the best substrate. In the reverse direction, we found that PHP and α-ketoglutarate are efficiently reduced by hPHGDH, indicating that in vivo α-ketoglutarate could compete with PHP, producing the oncometabolite D-2-hydroxyglutarate. Indeed, hPHGDH can maintain an intrinsic NAD+/NADH recycle by using α-ketoglutarate. Notably, PHP, L-serine, glycine and D-serine were not able to inhibit hPHGDH activity. Moreover, we highlighted the presence in solution of different conformations and/or oligomeric states of hPHGDH through the investigation of NADH and phosphate binding. The clarification of the biochemical properties of hPHGDH will allow a better understanding of the effects of mutations related to pathological states and help identify new approaches to modulate L-serine levels with the final aim to reduce cancer progression and treat neurological disorders. This project was founded by "PRIN-2017 – Dissecting serine metabolism in the brain". References: [1] Murtas G et al., (2020). Cell Mol Life Sci; 77(24):5131–5148. [2] Grant GA., (2018). Front Mol Biosci; 5:110.

P-03.1-31
The effect of 6 weeks of unilateral strength training on signaling response in contralateral leg to strength exercise session

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Strength training of one limb increases muscle strength in both trained and non-trained (contralateral) limbs due to cross-transfer of neural adaptation. The aim of present study was to examine the effect of cross-transfer on the activation of anabolic signaling after a single strength exercise in the contralateral limb. Eight recreationally active healthy young males (18–25 years old) participated in the study. The participants performed progressive strength training program during 6 weeks (3 times per week, 3–5 sets of one leg press with 65–85% of 1RM load). Before (for trained leg) and after strength training (for contralateral leg),

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strength exercise sessions (4 sets of one leg press with 65% of 1RM load) were carried out; before and 2 h after the exercise sessions biopsies from m. vastus lateralis were taken. Six weeks of training increased the 1RM for the trained leg by 15% and for the contralateral leg by 10%. The volume of m. quadriceps femoris of the trained leg was increased by 7.9% and was not changed in the contralateral leg (−0.8%). Work volumes performed during the strength exercise sessions were comparable for trained and contralateral untrained leg (60[60–65] and 62[57–64] repetitions, respectively), post-exercise blood lactate levels also did not differ (7.5[6.5–8.6] mM and 7.5[6.3–8.7] mM, respectively). Comparable activation of anabolic signaling (an increase in the phosphorylation levels of $p70S6k^{Thr389}$, $rpS6^{Ser235/236}$, $Erk1/2^{Thr202/Tyr204}$ and decrease of phosphorylation level of $eEF2^{Thr56}$) was observed 2 h after both exercise sessions: pre training and after training for trained and untrained legs accordingly. Collectively, these findings show that the cross-transfer of neural adaptation in the contralateral leg did not lead to alteration of the anabolic signaling response induced by a strength exercise session. This work was supported by the RFBR grant №20-315-70034.

P-03.1-32
Dissecting the role of serine in human-induced pluripotent stem cell derived-astrocytes

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Tau and beta-amyloid (Aβ) pathology, metabolism alteration, inflammation and neurodegenaration are common features in Alzheimer's Disease (AD). Among all the mediators of neurodegeneration, astrocytes, which normally support neuronal metabolism, may activate and proliferate around Aβ plaques. Astrocytes are responsible for the synthesis of L-serine, the precursor of D-serine. This latter is the main co-agonist of NMDA receptors (NMDARs), which are involved in synaptic transmission and brain plasticity. Recently, L-serine/D-serine pathway dysregulation has been associated to NMDAR-mediated synaptic defects in AD. To assess the role of serine and its related metabolites in AD, we performed untargeted metabolomic analysis by LC- and GC-MS, which was then integrated with transcriptomic and proteomic analysis, in a model of human-induced pluripotent stem cell derived-astrocytes (hiPSC-astrocytes). hiPSC-astrocytes were differentiated from human neural stem cells (NSCs), maintained in long-term cultures and analyzed at different time-points (2, 15, 30, 40, 56 days). Our results indicate that hiPSC-astrocytes became mature after 30 days of differentiation, showing increased glial fibrillary acidic protein (GFAP) levels and reduced SOX2 levels, indicative of loss of stemness. Moreover, metabolomic analysis revealed that several pathways were affected during differentiation. Interestingly, pathway enrichment analysis showed that the serine, glycine and threonine metabolism was among the most enriched pathways. However, most metabolites of the serine pathway have a tendency to decrease at longer time points during differentiation (40 and 56 days), indicating an alteration of astrocyte metabolism. Our results suggest that hiPSC-astrocytes are a promising tool to investigate metabolic alterations in AD pathophysiology. This project was founded by "PRIN-2017 - Dissecting serine metabolism in the brain".

P-03.1-33
Exosomes from hypoxia-activated microglia deliver death message to neuronal cells through STAT1

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Microglia, the resident immune cells of central nervous system, plays a central role in brain homeostasis as well as in the development of neurodegenerative diseases. In case of sustained neuroinflammation, like in cerebrovascular dysfunction and hypoxic ischemic conditions, microglial activation leads to the hyperactivation of several signaling pathways in a self-perpetuating cycle of neurotoxicity and neuronal loss. Recently, we demonstrated the involvement of the transcription factor STAT1 in M1 microglial activation under hypoxia highlighting its active role in neuronal cell death (previously published in: Boriero D et al. (2020) FEBS J. https://doi.org/10.1111/febs.15577). Herein, we suggest that this death message, from microglia to neurons, is delivered by exosomes, extracellular vesicles known to be involved in cell-to-cell communication. Exosomes were harvested by polymeric precipitation from media conditioned by murine microglia BV2 cells cultured under hypoxia and normoxia in serum-free media. The isolated material was characterized by Western Blot (WB), Transmission Electron Microscopy and Nanoparticle Tracking Analysis to assess the presence of specific exosome markers, exosome morphology, quantity and size distribution. Immunofluorescence microscopy was performed to state the presence of STAT1 inside the exosomes. The neurotoxic effect of exosomes was evaluated following administration of specific of exosomes to human neuroblastoma SH-SY5Y cells cultured under hypoxia. WB and immunofluorescence confirm the presence of STAT1 in BV2 derived exosomes. The administration of hypoxia BV2 exosomes induces a significant decrease in SH-SY5Y cell viability compared to cells treated with normoxia BV2 exosomes. These data highlight the importance of exosomes in the crosstalk between hypoxia activated microglia and neurons and suggest a key role of STAT1 in the onset and progression of neurodegenerative diseases.

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P-03.1-34
Effect of social isolation on the brain injury following focal cerebral ischemia in mice and proteomic approach for identifying possible cellular pathways

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Social isolation has become a significant part of our everyday life during the Covid-19 pandemic, resulting in drastic effects on physiological wellbeing. It was shown that loneliness or psychosocial stress such as social isolation, predict the onset of depression, as well as increased stroke-related morbidity and mortality. However, the mechanisms through which social isolation influences the disease outcomes are largely unknown. In this study, we aimed to evaluate the effects of early-life social isolation on the brain injury, and protein expression profile after focal cerebral ischemia. For this purpose, post-weaning male mice were randomly divided into socially isolated (SI) or pair-housed (PH) cage conditions. SI mice were placed in individual cages while PH mice stayed together throughout the experiment. After 8 weeks of housing, focal cerebral ischemia was induced on half of the SI and PH mice (n=7). Remaining animals were kept as non-ischemic groups. In the acute phase of brain injury, neuronal survival and apoptosis were evaluated. In all groups, proteomics analysis was done. Here, we showed that, ischemic SI group mice had a significantly greater number of apoptotic cells and decreased neuronal survival compared to ischemic PH mice ($P < 0.01$), while there were no significant differences in the macroscopic brain structures between non-ischemic groups (n=7). Moreover, 55 proteins were identified as significantly changed in the striatal region between non-ischemic SI and PH groups by proteomics analysis, and 5 proteins were identified as significantly changed in the same region between ischemic groups. The results obtained in this study demonstrate that social isolation dramatically increased the extent of damage in a mouse ischemia model and altered the levels of proteins which might be responsible for the elevated brain injury. Further investigations are required to elucidate the role of the proteins that are altered in response to injury in social isolation.

P-03.1-35
Cytoplasmic coupling and translocation of protein-functionalized magnetic nanoparticles into neurite tips of human dopaminergic cells

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In the mammalian central nervous system neuronal regeneration is limited by extracellular inhibitory molecules but local axonal growth restrictions could be overcome by counteracting intracellular signaling mechanisms. Expression of tagged RAS pathway stimulating proteins, i.e. permanently active Harvey-RAS protein HRAS (H-RAS^{V12}) or RAS-activating catalytic domain of the guanine nucleotide exchange factor SOS1 (SOS1cat), were used to promote neurite growth in secondary dopaminergic cells (1). *In vitro* and in living cells we show binding of γ-Fe_2O_3@SiO_2 nanoparticles to H-RAS^{V12} or SOS1cat proteins by applying fluorescence correlation spectroscopy and multiangle light scattering (2). By using an external magnet, we achieve controlled translocation of the SOS1cat-coupled nanoparticles from the cytoplasm into the neurite tip where endogenous H-RAS protein is accumulated. For scaling-up from single cells, we show the cytoplasmic delivery of the nanoparticles into large numbers of secondary cells without changing their cellular response to nerve growth factor. We consider these results as an initial step to explore tools for refining cell replacement therapies based on transplanted human induced dopaminergic neurons containing functionalized magnetic nanoparticles in models of Parkinson's disease. (1) Raudzus F., Schöneborn H., Neumann S., Secret E., Michel A., Fresnais J., Brylski O., Ménager C., Siaugue J.-M., Heumann R. Magnetic spatiotemporal control of SOS1 coupled nanoparticles for guided neurite growth in dopaminergic single cells. Sci. Rep. (2020) 10: 22452. (2) Schöneborn H., Raudzus F., Secret E., Otten N., Michel A., Fresnais J., Ménager C., Siaugue J.-M., Zaehres H., Dietzel I.D. and Heumann R. Novel Tools towards Magnetic Guidance of Neurite Growth: (I) Guidance of Magnetic Nanoparticles into Neurite Extensions of Induced Human Neurons and In Vitro Functionalization with RAS Regulating Proteins. J. Funct. Biomater. (2019) 10(3): 3

P-03.1-36
Human brain phosphoserine aminotransferase: biochemical characterization of the wild-type enzyme and its pathogenic D100A variant

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In the human brain, L-Ser is mainly synthesized from the glycolytic precursor 3-phosphoglycerate (3-PG) by the three-step "phosphorylated pathway". Phosphoglycerate dehydrogenase

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oxidizes 3-PG to 3-phosphohydroxypyruvate (3-PHP) that is subsequently transaminated by phosphoserine aminotransferase (PSAT) to O-phosphoserine (OPS). Finally, OPS is converted to L-Ser by phosphoserine phosphatase (1). Enzyme variants with impaired function cause serious neuronal diseases, due to the pivotal role of L-Ser in the CNS [1]. PSAT is a pyridoxal 5'-phosphate (PLP)-dependent enzyme that catalyzes the reversible transamination of 3-PHP using L-Glu as the amino donor [2]. Here, we present the first functional characterization of human PSAT and of its pathogenic variant D100A, originally identified in two siblings, who showed low concentrations of Ser in plasma and cerebrospinal fluid [3]. The human enzyme expressed in E. coli is correctly folded and exhibits a Tm of 62°C. The absorption spectrum of PSAT in the visible region shows two peaks centered at 344 and 408 nm, which correspond to the unprotonated and protonated forms of the cofactor, respectively. Kinetic parameters were determined for both the forward and the reverse reactions, confirming that the forward reaction proceeds with higher catalytic efficiency (5.9×10^6 $M^{-1}s^{-1}$ vs 3×10^5 $M^{-1}s^{-1}$). Both reactions show substrate inhibition. At variance with published data (3) we did not find any relevant effect of the D100A substitution on the fold, stability and kinetic parameters of the enzyme and we propose that this pathogenic variant might indirectly affect serine production by an as yet unidentified mechanism. This project was founded by "PRIN-2017 – Dissecting serine metabolism in the brain". References: [1] Tabatabaie L et al, Mol Genet Metab. 2010, 256. [2] Basurko MJ et al., IUBMB Life. 1999, 525. [3] Hart CE et al, Am J Hum Genet. 2007, 931.

P-03.1-37
Investigation of astrocyte activation, neuronal damage, neuronal regeneration and neuroinflammation in multiple sclerosis and neuromyelitis optica spectrum disorders

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In neuroimmunology, biomarkers have been determined as having a pivotal role. In our study, we aimed to evaluate NfL, GFAP, Osteopontin, IL-6, GAP-43 in different subgroups of Multiple Sclerosis (MS), Aquaporin-4(AQP4) seropositive Neuromyelitis Optica Spectrum Disorders (NMOSD), Myelin Oligodendrocyte Glycoprotein (MOG)-Ab-positive (MOGAD). All biomarkers were evaluated both in paired serum and cerebrospinal fluid (CSF). Serum (n = 96) and available CSF (n = 20) (paired with serum, only MS subtypes) samples have used this cross-sectional study. For measuring NfL and GFAP concentrations in sera and CSF samples, we used ultrasensitive Single Molecule Array (SIMOA) technology and assays on the Quanterix SIMOA HD-X ANALYZER.IL-6 measurements were performed on the Roche Cobas e-411 analyzer with the electrochemiluminescence method. Osteopontin and GAP-43 levels determined with the ELISA assays. Clinical features and radiological data at sampling were collected for each case. Serum values of NfL and GFAP were strongly positively correlated with CSF values ($P = 0.001$, $r = 0.686$; $P = 0.00$, $r = 0.594$). There was also a strong positive correlation between serum NfL and GFAP levels ($P = 0.004$, $r = 0.612$). Besides, there was a strong negative correlation between NfL serum values and GAP-43 serum values ($P = 0.013$, $r = -0.665$). Additionally, there was a strong positive correlation between IL-6 CSF values and CSF osteopontin values ($P = 0.04$, $r = 0.566$). The sGAP-43 levels of the patients in the SPMS group were higher than the NMOSD (2.42 ± 0.57) and MOGAD (2.63 ± 1.07) groups ($P = 0.012$, $P = 0.033$). NfL and GFAP are certainly promising biomarkers in MS patients. Additionally, GAP-43 is among candidate biomarkers to show regeneration capacity in these patients. Our study is the first that SIMOA technology has been used in Turkey.

P-03.1-38
ADAM10 hyperactivation acts on piccolo to deplete synaptic vesicle stores in Huntington's disease

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Synaptic dysfunction and cognitive decline in Huntington's disease (HD) involve hyperactive A disintegrin and metalloproteinase domain-containing protein 10 (ADAM10). To identify the molecular mechanisms through which ADAM10 is associated with synaptic dysfunction in HD, we performed an immunoaffinity purification-mass spectrometry (IP-MS) study of endogenous ADAM10 in the brains of wild-type and HD mice. In the normal brain, proteins implicated in synapse organization, synaptic plasticity, and vesicle and organelles trafficking interact with ADAM10, suggesting that it may act as a hub protein at the excitatory synapse. Importantly, the ADAM10 interactome is enriched in presynaptic proteins and ADAM10 coimmunoprecipitates with piccolo (PCLO), a key player in the recycling and maintenance of synaptic vesicles (SVs). In contrast, reduced ADAM10/PCLO immunoprecipitation occurs in the HD brain, with decreased density of SVs in the reserve and docked pool at the HD presynaptic terminal. Conditional heterozygous deletion of ADAM10 in the forebrain of HD mice reduces active ADAM10 to wild-type level, and normalizes ADAM10/PCLO complex formation and SVs density and distribution. The results indicate that presynaptic ADAM10 and PCLO are a relevant component of HD pathogenesis.

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P-03.1-39
Immunoproteasomes participate in long-term potentiation in murine hippocampus

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Ubiquitin-proteasome system performs hydrolysis of a majority of intracellular proteins. Proteasomes are central elements of the UPS and several different forms of proteasomes are known. Broad specificity inhibitor Bortezomib that targets several proteasome forms is used to treat cancer, although its application is limited due to the strong side effects including neurotoxicity. Inhibitors specific to a particular proteasome form were found to be less neurotoxic and may be considered as a promising alternative. Broad specificity proteasome inhibitors, MG132, lactacystin and epoxomicin were shown to influence long-term potentiation (LTP) in hippocampus; however, the effects of the proteasome form-specific inhibitors on the LTP are unknown. Here we addressed the effect of immunoproteasome-specific inhibitor ONX-0914 on the LTP in murine hippocampus slices. A significantly decreased LTP upon ONX-0914 administration was observed. Alongside low levels of immunoproteasome genes expression and minimal amounts of immunoproteasome subunits were revealed in hippocampus. The data were previously published in Maltsev A et al. (2021) J Neuroimmune Pharmacol. Jan 6. doi:10.1007/s11481-020-09973-0. Our data indicate unexpected side effects of next generation proteasome inhibitor and suggests possible relevance of immunoproteasomes for the synaptic plasticity in the CNS. The study was supported by the Russian Science Foundation grant #18-74-10095.

P-03.1-40
Study of the role of voltage gated sodium channels in amyotrophic lateral sclerosis pathogenesis

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Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that affects motoneurons (MNs) and leads to patient paralysis and death. Among the most recent hypotheses, an increasing wealth of evidence suggests that neuron excitability defects may play an important role in ALS development. However, the molecular bases of such defects are currently unknown. Our previous studies, in a zebrafish model of ALS expressing the G93R-mutant form of superoxide dismutase 1 (Sod1)(Benedetti et al. (2016) Sci Rep. 15;6:24515) pointed to the alteration of the persistent sodium current in spinal motor/inter-neurons. Interestingly, we showed that this alteration was associated to neuronal hyperexcitability and aberrant behaviors in zebrafish embryos during early developmental phases. To better investigate the role of Voltage Gated Sodium Channels (VGSCs) function in MNs differentiation and ALS onset/progression, we took advantage of the NSC34 in-vitro model, a hybridoma which is often used to study MNs pathologies. Thus, we employed two different NSC34 lines, the first stably expressing the human mutant SOD1G93A enzyme, the second in which the vesicle-associated membrane protein-associated protein (VAPB) is silenced. Using biochemical approaches, we evaluated changes of VGSCs expression and functionality highlighting an impaired VGSCs expression in both experimental paradigms. Importantly, we pinpointed a strong correlation between VGSCs protein levels and neuronal differentiation: the lower the VGSCs expression, the shorter the neurite extension. Moreover, pharmacological studies on NSC34 untrasfected cells highlighted that the inhibition of VGSCs also leads to defects in neuronal maturation, suggesting that VGSCs activity might play an important role in the acquisition of a mature neuronal phenotype. In conclusion, this study indicates that VGSCs play an important role in both maintenance of neuron electrical phenotype as well as in the proper development and shaping of MNs.

P-03.1-41
Salivary proteome changes in response to acute stress in students performing an exam simulation with and without olfactory stimulation

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The autonomic nervous system (ANS) plays a crucial role both in acute and chronic psychological (mental) stress eliciting changes in many local and systemic physiological and biochemical processes. Salivary secretion is regulated by ANS. In this study we explored salivary proteome changes produced in 34 students by a test stress, which simulated an "oral exam". Students underwent a relaxation phase followed by the stress test, during which electrocardiogram was recorded. To evaluate the effect of the olfactory stimulus, 16 students experienced a pleasant odor diffused throughout the whole session in the room. Saliva samples were collected after the relaxation (T0) and the stress test (T1). State anxiety was also evaluated at T0 and T1. Salivary proteins were separated by two-dimensional electrophoresis and the T1 protein pattern was compared to the T0 one. Spots differentially expressed were trypsin digested and identified by LC-MS/MS. Western blot analysis was used to validate results. Anxiety scores and heart rate changes indicated that the fake exam induced anxiety. Significant changes of a-amylase, polymeric immunoglobulin receptor, and IgA secretion were observed after stress test in both conditions. Moreover, the presence of a pleasant odor reduced the acute social stress also affecting the salivary proteome changes. Therefore, saliva proteomic analysis was useful to evaluate the rapid responses associated to test anxiety.

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P-03.1-42
Ion mobility mass spectrometry reveals novel and rare ganglioside structures in human cerebrospinal fluid

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Unlike the brain biopsies, cerebrospinal fluid (CSF) has a higher availability for research. Besides, its permanent contact with the brain, its continuous renewal, and the shed of gangliosides (GG), known as important biomarkers of the brain pathophysiology, from brain to CSF, determined lately the development of protocols for the investigation of GGs and discovery of central nervous system (CNS) biomarkers expressed in CSF. Recently, ion mobility separation mass spectrometry (IMS MS) implemented by us in the investigation of human CSF gangliosidome [Sarbu M et al. (2020) Biochimie 170, 36–48], highlighted the incidence of a similar GG profile in the CSF to that in the human brain. Based on these findings, we continued now exploring in more detail the structure of some unusual glycoforms detected in the normal lumbar CSF. The extracted GG sample of 5 pmol/μL in methanol was infused into a Synapt G2s and the signal was acquired in the negative ion mode at 1.6kV ESI voltage. The structural investigation of the species at m/z 1019.004, assigned as GalNAc-GD1(d18:1/18:0), was achieved by collision-induced dissociation (CID) using collision energies of 40 eV. The hard evidence provided by IMS of only one mobility feature together with the fragment ions generated for both the glycan core and ceramide moiety allowed, among the six possible structures, the identification of the isomer present in the CSF, namely GalNAc-GD1c(d18:1/18:0), having both sialic acids and GalNAc attached to the external galactose unit. Further, the structural confirmation of GD3(d18:1/18:0) and GD2(d18:1/18:0), exhibiting shorter carbohydrate chain, a feature of CSF, was also achieved. The valuable information acquired here indicates the efficacy of IMS MS technique for detection and structure elucidation of glycolipid species in biological fluids and opens new perspectives for clinical research on human CSF for discovery of GG species associated to neurodegenerative diseases and brain tumors.

Receptor–ligand interactions

P-03.2-01
Effects of α7-nicotinic acetylcholine receptor antagonists on cancer cells overexpressing these receptors

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Lung cancer still represents a major problem in healthcare and is statistically the second most common cancer in both men and women, as well as the leading cause of cancer death, according to the American Cancer Society. In some cancer types, including lung cancer, elevated levels of nicotinic acetylcholine receptors (nAChRs) expression have been reported. It has been observed that binding of nicotine and its derivatives to specific subtypes of nAChRs (mainly α7 and α9) increased intracellular Ca^{2+} levels. These substances also activate signalling pathways that can trigger uncontrolled cell division, prevent apoptosis, cause angiogenesis and thus support tumour growth and metastasis. On the contrary, antagonists of these receptors have shown opposite effects, suggesting potential usefulness in cancer therapy. To explore this possibility, the main goal of our study is to analyse signalling pathways of several naturally occurring α7-nAChRs antagonists, such as snake venom secreted phospholipases A_2 ($sPLA_2$s), α-conotoxins and 3-alkylpyridinium polymers (polyAPS) on α7-nAChRs-overexpressing cancer cell lines. In the initial phase of our study, we prepared recombinant snake venom $sPLA_2$ ammodytoxin A and its human orthologues, group V and X $sPLA_2$s. Since it has been previously reported that $sPLA_2$s are able to suppress ACh-elicited ion currents also independently of their enzymatic activity, we prepared in E. coli also enzymatically inactive mutants of these three proteins. Secreted PLA_2 molecules were tested for their ability to modulate the activity of nAChRs and for their effects on cancer cells. The preliminary results are presented here and critically discussed.

P-03.2-02
Characterization of short neuropeptide F (sNPF) and its specific receptors in the hard tick *Ixodes ricinus*

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Neuropeptides are by far the largest and most diverse group of signalling molecules in multicellular organisms. They are acting as neurotransmitters, neuromodulators and are involved in the regulation of a diverse array of biological processes including reproduction, growth, circadian clock, ecdysis, feeding activity and metabolism. However, we have an insufficient information about their identity, function, and expression level in pathogen-carrying vectors like the hard ticks Ixodidae. In this study, we focused on sNPF signalling in I. ricinus. The gene coding for

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sNPF was cloned, and the identified nucleotide and peptide sequences used for development of the specific hybridization probe and peptide-specific antiserum. *In situ* hybridization and fluorescent immunohistochemistry disclosed cells producing sNPF in the central nerve system and in peripheral tissues like gut, and salivary glands. Homology-based search for sNPF receptors revealed two putative G protein-coupled receptors (sNPFR1 and sNPFR2) in the *I. ricinus* genome. Ligand binding assay showed that both receptors interacted specifically with sNPF. High expression levels of sNPFR1 and sNPFR2 were observed at the end of blood feeding in salivary glands and gut using quantitative PCR. All these findings suggest that sNPF and its receptors control feeding and digestion of blood in I. ricinus.

P-03.2-03
Adamantane based derivatives as reversible inhibitors of human AChE and BChE

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Acetylcholinesterase (AChE) and butyrylcholinesterase (BChE) are two related enzymes responsible to control the level of neurotransmitters. Acetylcholine (ACh) is removed from cholinergic synapse by enzymatic hydrolysis with AChE, which leads to termination of impulse transmission. Both, AChE and BChE, are identified as targets in the treatment of neurodegenerative disorders, such as Alzheimer's disease (AD). The most used approach in finding new drugs for the treatment of AD is the inhibition of AChE. We synthesized a series of 4-aminoquinoline derivatives of adamantane with different linkers between two subunits, based on the highly active and selective cholinesterase inhibitor with adamantane.[1] Compounds were tested as inhibitors of human AChE and BChE, with acetylthiocholine (ATCh) as the substrate for enzyme activity measurements. AChE and BChE activity toward ATCh decreased in the presence of all tested compounds. Inhibition potency of tested compounds expressed as enzyme-inhibitor complex dissociation constant (K_i), ranged from 0.1 to 4.2 μM for AChE and from 0.1 to 7.8 μM for BChE. Changing the length of the linker, structural isomerization and their conformation freedom, we influenced the inhibition potency of compounds, as well as their BChE / AChE selectivity. Increasing of steric demand of molecule resulted in a decrease of inhibitory activity. The compound with n-octyl alkyl chain between quinoline and adamantyl fragments has the lowest K_i values which are approximately 8 and 39 times lower for AChE and BChE respectively than previously tested derivative. [1] Docking simulations showed that adamantyl fragment achieved additional interactions with amino acids residues indicating that adamantane scaffold is important for inhibition of cholinesterase activity, aside from aminoquinoline pharmacophore. [1] Previously published in: Bosak A et al. (2019) Chem -Biol Interact 308, 101–109.

P-03.2-04
Novel fluorescent BODIPY probe for photoaffinity labeling

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Photoaffinity labeling (PAL) has become a popular tool in proteomics and drug discovery [1]. PAL probes act via UV-triggered free radicals generation followed by covalent bonding with a target molecule. Benzophenone (BP) derivatives are among such probes structures [2]. Often PAL probes include a reporter tag allowing better detection of labeled molecules, e.g. using fluorescence. Today boron-dipyrromethenes (BODIPYs) have become widely used as fluorophores due to their photostability and quantum yields. Both BODIPY and phenyl groups have planar cyclic conjugated structure, so BODIPYphenylketones (BPKs) can be considered as potential fluorescent analogs of BP-based PAL probes. However, there is only one research where BPK photoactivity has been mentioned [3]. Thus, we have synthesized a "minimalistic" BPK (1) by acylation of 8-methylBODIPY. Such acylation of a BODIPY been constructed on unsubstituted pyrroles hasn't been described yet. The structure is characterized by NMR, ESI-MS, UV-vis spectrometry and fluorimetry. From quantum-mechanical calculations (PBE, ma-def2-SVP) the HOMO/LUMO gap for 1 (1.78 eV) is smaller than for BP (2.88 eV) assuming that photoactivation of 1 can be achieved by less energetic light than BP. The compound was found to give an adduct with cytochrome P450 CYP7B1 after 365 nm-UV exposition according to monitoring of the fluorescent band with the parent protein mobility on SDS-PAGE. Molecular docking demonstrated affine binding of 1 with a set of human P450s (free binding energies up to -15,1 kcal/mol). Compound 1 is a potentially new photoactive fluorescent ligand. Thus, 1 and other BPKs can be developed as facile and powerful proteomic tools to reveal new structure-function features of biomolecules. Grants BRFFI X19PM-062/X19PM-062-1 – RFFI 19-54-04009 Bel_mol_a. References: [1] Yip MSG et al. (2013) Nat Chem Biol 9, 715–720. [2] Smith E et al. (2015) Future Med Chem 7, 159–183. [3] Murale DP et al. (2015) Chem Commun 51, 6643–6646.

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P-03.2-05
Structural basis of antiviral activity of a protective antibody against tick-borne encephalitis virus and structure-guided rational design of this antibody

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Currently, there is no specific therapeutics for the treatment of tick-borne encephalitis (TBE), while the vaccination rate in most European countries is still insufficient. To fill this gap, an antibody-based therapeutic drug for post-exposure prophylaxis and treatment of TBE are under development. In this study we determined a structure of the antibody-antigen complex for one of the protective antibodies against glycoprotein E (glycoE) of TBE virus (TBEV) with a resolution of 2.6 Å. It was found that the antibody recognizes an epitope located on the lateral ridge of the D3 domain of glycoE, one of the most promising regions of its surface. This epitope is highly accessible, and up to 120 antibody molecules can simultaneously bind to the same virion. There are some other anti-TBEV antibodies directed to this region, and the structures for two of them are known (pdb 5O6V and 6J5G). We found that the orientation of the studied antibody with respect to glycoE molecule is different compared to them. We suggest that this may affect the mechanism of antiviral action of these antibodies. It was shown by molecular modeling that the studied antibody is able to block infection not only by blocking attachment of virus to the cell surface, but also during intra-endosomal stages of infection via cross-linking glycoE molecules. As a result, this allows the antibody not only to block the infection with high efficiency, but also minimizes the ability of this antibody to cause an antibody-dependent enhancement of infection, which makes this antibody safer for therapeutic use. These findings are in a good agreement with the results of in vivo experiments performed earlier. Based of structural information we also generated some mutant antibody variants which possess 2–3 times higher affinity to recombinant D3 domains of glycoE belonging to Siberian and European subtypes of TBEV. The research was supported by the Russian Science Foundation (project # 19-74-00107).

P-03.2-06
A biophysical approach to the characterization of the glycosylation-dependent interaction of human NK cell receptors with Galectin-1

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CD69, a C-type lectin-like receptor of the CLEC2 family, is an early activation marker constitutively expressed by several subsets of immune cells. Although its precise role in immunity remains to be fully elucidated, compelling evidence has been shown of CD69 as a regulator of homing and lymphocyte migration. The recent identification of human Galectin-1 as a novel binding partner for CD69, and in consequence, of the involvement of the receptor-ligand pair in the regulation of Th17 cell differentiation, further supports the participation of CD69 in the modulation of inflammatory responses. Despite the progress made in the identification and characterization of Galectin-1 as a ligand for CD69, a thorough understanding of the biophysical and structural mechanisms of the complex formation are still lacking. Using a combination of biophysical approaches, we investigated the interaction of CD69 with Galectin-1 with respect to its dependency of an N-glycan moiety present on the surface of the receptor. The interaction was confirmed as N-glycan dependent. Unexpectedly, Galectin-1 was additionally shown to interact with similar or stronger affinity to other members of the CLEC2 family (e.g., human LLT1, KACL receptors), revealing that the interaction is not as specific as initially assumed. These findings suggest the possibility of a structurally conserved interaction between NK cell receptors of the CLEC2 family and human Galectin-1. Comprehensive characterization of the interaction in a structural and biophysical manner is pivotal for a better understanding of the precise role of Galectin-1 in the context of NK cells and of its possible implications in physiological and pathological circumstances. This study was supported by the Czech Science Foundation (18-10687S), the Ministry of Education, Youth and Sports of the Czech Republic (LTC17065 in frame of the COST Action CA15126), and by the Charles University (SVV260427/2019).

P-03.2-07
EpCAM's hydrophobic pocket and proximal regions affect its regulated intramembrane proteolysis

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EpCAM is a type 1 transmembrane glycoprotein expressed at low levels in intestinal epithelia and at high levels in a variety of stem cells. Additionally, high levels of expression have also been observed in a variety of carcinomas. Due to the latter characteristic, EpCAM has been proposed as a target in treating certain types of cancer. Indeed, therapeutic antibodies have been developed and are in use. However, our understanding of EpCAM's involvement in cellular processes leaves much to be desired. One of these processes is proliferative signalling via regulated intramembrane proteolysis (RIP). The extracellular part of EpCAM (EpEX) can be shed by the metalloprotease TACE (TNFα converting enzyme) and the remaining membrane-bound part can be further processed by γ-secretase. The resulting intracellular part of EpCAM (EpICD) forms a protein complex with FHL2, β-catenin and LEF-1, which increases the expression of proliferative and pluripotency factors. After the structure of EpEX had been experimentally determined, a hydrophobic pocket bound with the detergent decyl-β-D-maltopyranoside, a crystallization additive, was observed. We hypothesised that this pocket could play a role in regulating RIP via its potential ligand-binding capabilities. First, we designed and prepared mutant forms of EpCAM with an obstructed pocket and confirmed pocket closure using molecular dynamics. These mutant

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forms were more prone to RIP as observed in in cellula experiments using HEK293 and EpCAM$^{-/-}$ colon carcinoma HCT8 cells. Additionally, molecular docking was employed to explore the ligand-binding capability of the hydrophobic pocket. We used fatty acids of different lengths and their maltoside derivates as ligands to observe a minimum in binding energy around C16, which corresponds to the length of a palmitoyl group. However, binding of fluorescent lipids in vitro was unsuccessful. Overall, our data show that RIP is affected by the structural state of EpCAM's hydrophobic pocket.

P-03.2-08
Sigma-1 receptor ligand trifluoperazine attenuates Ca^{2+} responses induced by immunomodulators glutoxim and molixan in macrophages

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Sigma-1 receptors - unique ubiquitous multitasking endoplasmic reticulum-resident chaperones - have unique history, structure and pharmacological properties. The ligands of these receptors are compounds with different chemical structure and pharmacological activity: antidepressants, neuroleptics, analgesics, antitussives, and anticonvulsants. When functioning as chaperones, sigma-1 receptors interact with target proteins and modulate a variety of processes, including Ca^{2+} signalling in cells. Earlier, we have shown that immunomodulators glutoxim (disodium salt of oxidized glutathione with d-metal at nanoconcentration, PHARMA VAM, St. Petersburg) and molixan (complex of glutoxim with nucleoside inosine) cause intracellular Ca^{2+} concentration ($[Ca^{2+}]i$) increase due to Ca^{2+} mobilization from thapsigargin-sensitive Ca^{2+} stores and subsequent store-dependent Ca^{2+} entry in rat peritoneal macrophages. To elucidate the involvement of sigma-1 receptors in the effect of glutoxim and molixan on $[Ca^{2+}]i$ in macrophages we used sigma-1 receptor antagonist phenothiazine neuroleptic trifluoperazine, widely used for treatment of schizophrenia. Using Fura-2AM microfluorimetry, we have found that macrophage preincubation with 2 μg/ml trifluoperazine for 15 min before 100 μg/ml glutoxim addition leads to a significant suppression of both Ca^{2+} mobilization from the stores and subsequent Ca^{2+} entry into the cell, induced by glutoxim. Similar results were obtained when studying the effect of trifluoperazine on Ca^{2+} responses induced by molixan. Thus, we have demonstrated that sigma-1 receptor antagonist trifluoperazine inhibits both phases of the Ca^{2+} response induced by glutoxim or molixan, which indicates the involvement of sigma-1 receptors in complex signalling cascade triggered by these immunomodulators in macrophages. Our results also indicate that it is inadvisable to use glutoxim or molixan in combination with antipsychotic trifluoperazine in clinical practice.

P-03.2-09
Characterization of interaction between extracellular domains of EpCAM in EGFR

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EGFR in EpCAM are transmembrane glycoproteins, which are both overexpressed in various carcinomas, where they participate in promotion and progression of cancer. Recent studies have shown that EGFR and EpCAM interact with each other, where an extracellular domain of EpCAM (EpEX) acts as an EGFR ligand. EpEX is structurally different from other EGFR ligands, because it does not contain an EGF-like domain, which participates in a classical ligand binding to EGFR. Our aim was to characterize the EGFR and EpCAM complex with four different methods which can be used in the analysis of protein interactions: size exclusion chromatography (SEC), crosslinking, small-angle X-ray scattering (SAXS) and surface plasmon resonance (SPR). Protein samples needed for analysis – extracellular region of EGFR and several EpEX variants – were prepared using insect cell expression system. The crosslinking experiment confirmed the presence of the complex. Its results suggest that EpEX binds to EGFR either as a monomer or a dimer. However, we were unable to detect the presence of the complex with SEC, SAXS or SPR measurements. Since other studies which used EGFR and EpEX expressed in mammalian cells have successfully shown the presence of the complex even at very low concentrations, our hypothesis is that posttranslational modifications, which are different in mammalian and insect cells, have critical role in binding of EpEX to EGFR.

P-03.2-10
Tetrameric structure of cystathionine β-synthase domain-containing pyrophosphatase

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A quarter of prokaryotic Family II inorganic pyrophosphatases (PPases) contain a regulatory insert comprised of two cystathionine β-synthase (CBS) domains and one DRTGG domain in addition to the two catalytic domains that form canonical Family II PPases. The CBS domain-containing PPases (CBS-PPases) are allosterically activated or inhibited by adenine mono- and dinucleotides that cooperatively bind to the CBS domains with a nanomolar to micromolar affinity. Here we use chemical crosslinking and analytical ultracentrifugation to show that CBS-PPases from *Desulfitobacterium hafniense* and four other bacterial species are active as 200–250-kDa homotetramers. Most other reported PPases are homohexamers of approximately 20-kDa subunits (prokaryotic Family I), homodimers of 32–70-kDa subunits (eukaryotic Family I, prokaryotic canonical Family II, and cation-transporting membrane PPases), or monomers (Family III). CBS-PPase is thus unique among different PPases by the type of its oligomeric structure. The deletion variants of the CBS-PPases from *D. hafniense* and *Clostridium perfringens* containing only catalytic or regulatory (CBS and DTRGG) domains are dimeric. Co^{2+} depletion by incubation with EDTA converts CBS-PPase into inactive tetrameric and dimeric forms. Adenine

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nucleotides stabilize the tetrameric form of CBS-PPase. Canonical Family II PPase from *Streptococcus gordonii*, which has only catalytic domains in its structure, is homodimeric but dissociates to monomers in the presence of EDTA or 2-propanol. The structure of CBS-PPase tetramer was modelled from the structures of dimeric catalytic and regulatory parts. These findings signify the role of the unique oligomeric structure of CBS-PPase in its multifaced regulation. This work was supported by the Russian Science Foundation grant No. 19-14-00063. Previously published in: Anashkin VA et al. (2019) Biochem Biophys Res Comm 517, 266–271; Anashkin VA et al. (2020) Arch Biochem Biophys 692, 108537. *The authors marked with an asterisk equally contributed to the work.

P-03.2-11
Devising heterodimeric 14-3-3 chimeras with different phosphopeptides for structural studies of ternary 14-3-3 complexes

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Dimeric 14-3-3 proteins are phosphoprotein binding modules implicated in the regulation of multiple cellular processes and the development of various disorders. Though 14-3-3 interacts with hundreds of phosphoprotein clients, only a few 14-3-3/client complexes have been structurally characterized. Given that phosphosites are mostly located within structurally disordered regions, the protein/phosphopeptide binding approach to characterize pivotal 14-3-3/phosphoprotein interfaces works well when these interactions are strong, however transient interactions of biological relevance are challenging. To overcome these disadvantages of the traditional co-crystallization of 14-3-3 dimers with synthetic phosphopeptides, we have recently introduced the chimeric approach based on tethering phosphorylatable segments of partner proteins to the C-termini of 14-3-3, which secures binding stoichiometry, and improves crystallizability and peptide occupancy in the structures. Exploiting the unique heterodimerization preference of some 14-3-3 isoforms, here we advance this approach by proposing that tethering of two phosphopeptides to 14-3-3 paralogs preferring to heterodimerize would allow streamlined purification of 'ternary' complexes mediated by 14-3-3 and their structure determination. As proof-of-principle, we design and co-express His-tagged 14-3-3ε with untagged 14-3-3ζ, each tethered to a different phosphopeptide of the proapoptotic BAD protein. We show that careful adjustments of the promoter strength enable convenient co-expression and purification of the correctly assembled hetero-oligomeric chimera that is stoichiometrically phosphorylated and dimeric based on size-exclusion chromatography. Preliminary screening confirms that crystallization-promoting engineering of the constructs permits productive crystallization of the purified material, suggesting general applicability. Future improvements are also discussed. Partially supported by RSF grant no. 19-74-10031.

P-03.2-12
New somatostatin analogue interacts with receptors of experimental tumour models

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In N.N. Blokhin National Cancer Research Center we have synthesised somatostatin analogue (SSA) demonstrated significant antitumor activity on experimental tumours of animals [Previously published in: Shprakh ZS et al. (2014) Pharm Chem J 48, 159-62, Shprakh ZS et al. Exp Clin Pharmacol 82, 8, 27–31]. It is considered that direct antitumor activity of SSA realises via binding with somatostatin receptors (SSTR) on the cell surface. Created SSA has an amino acid sequence (Cys-Phe-D-Trp-Lys-Thr) responsible for affinity with SSTR. This study was performed to estimate the expression of somatostatin receptors in tumours of experimental animals. Immunohistochemistry method with primary antibodies to different receptor types (SSTR1-5) was used. The semiquantitative estimation has shown the most expressed positive immunoreactivity in the model of mice breast adenocarcinoma AC755. 80% of antigen-positive cells were discovered in 100% of the tumour samples. It was found that after the treatment with synthesised SSA receptor status of AC755 has changed: positive expression decreasing for SSTR5 on ninth and fourteenth days after the procedure and SSTR1-2 after fourteen days of treatment. From the other hand, SSTR3-4 positive expression increased after nine days of treatment. At that SSTR1-5 status in the control animal group didn't change. The results demonstrate that experimental tumour model AC755 is relevant to research somatostatin analogues antitumour activity and synthesised substance has a different degree of affinity to SSTR1-5. The work was funded by the Russian Ministry of Industry and Trade (Grant Number 11411.0008700.13.082).

P-03.2-13
Effect of sulfasalazine on insulin- and PDGF-stimulated glucose uptake in cultured skeletal muscle cells

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Type 2 diabetes (T2D) represents one of the most important public health challenges. Skeletal muscle is a major site of insulin-stimulated glucose uptake and therefore an important target tissue for treatment of insulin resistance and hyperglycemia in T2D. Sulfasalazine (SSZ), a conjugate of aminosalicylate and sulfapyridine, is used for treatment of chronic inflammatory bowel disease and inflammatory rheumatic diseases. Salicylic acid and some of its derivatives ameliorate glucose homeostasis in T2D, suggesting SSZ may exert similar metabolic effects. Consistent with this idea, clinical observations suggest that SSZ may increase risk of hypoglycemia in patients with rheumatic diseases and T2D, while animal studies showed that SSZ may block

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development of diabetic neuropathy and retinopathy. Hence we examined whether SSZ modulates insulin signaling in cultured rat L6 skeletal muscle cells (standard *in vitro* skeletal muscle model). Activation of insulin signaling pathway was assessed by measuring phosphorylation of serine/threonine kinase Akt at S473 and its 160 kDa substrate AS160 at S588. We also studied the effect of platelet-derived growth factor (PDGF), which activates the Akt-AS160 pathway independently of insulin receptor. Glucose uptake was estimated by measuring uptake of [3H]-2-deoxy-glucose. Phosphorylation of AS160 relieves suppression of GLUT4 translocation to the plasma membrane, thus leading to an increase in glucose uptake. We found that SSZ tends to suppress insulin- and PDGF-stimulated Akt phosphorylation. SSZ markedly suppressed phosphorylation of AS160 in the presence/absence of insulin or PDGF. SSZ inhibited insulin- and PDGF-stimulated glucose uptake. Collectively, our results do not support our hypothesis that SSZ improves insulin action and stimulates glucose uptake in skeletal muscle cells. Since SSZ blocked insulin and PDGF action, our results suggest that SSZ reduces glucose uptake by suppressing PI3-kinase downstream of insulin receptor.

P-03.2-14
On the role of dimerization in allosteric regulation of mammalian ALOX15

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Mammalian ALOX15's are non-heme iron containing lipid peroxidising enzymes that undergo allosteric regulation, but the molecular basis for this effect is still not well understood. Several studies on mammalian ALOXs have suggested allosteric sites for ligand and/or inhibitor binding that are different from the catalytic binding pocket. Alternatively, a mechanism that may involve an inter-molecular crosstalk between the two monomers within an ALOX15 dimer has been also postulated. In our current work on allosteric regulation in ALOX15 catalysis we found that the side chain of highly conserved Gln596 may be not only important for the structural performance of the enzyme but also fine-tunes the reaction specificity ALOX15-catalyzed arachidonic acid oxygenation. This residue is located at the end of flexible helix 18 and, hence, does not contribute directly to the shape and the form of the substrate binding cavity. In turn, it has been involved into the network of intermolecular contacts between helices 2 and 18 of individual ALOX15 monomers, thus, supporting dimeric mechanism of allosteric enzyme regulation. To prove our working hypothesis and/or to identify alternative binding sites that may be involved into allosteric regulation we created novel photoreactive ALOX15 probes by introducing a crosslinking moiety to the structures of known molecular ALOX15 effectors (inhibitors and activators). For this purpose N-(3-(1H-indol-2-yl)phenyl)sulfamoyl)carbamic acid (1), (E)-1-(7-benzylidene-3-phenyl-3,3a,4,5,6,7-hexahydro-2H-indazol-2-yl)-2-(piperazin-1-yl) ethan-1-one (2) and (E)-3-(2-(8-hydroxyoct-1-yn-1-yl)phenyl) acrylic acid (3) were modified by the introduction of tetrafluoroazide group using either esterification, or N- / O- alkylation reactions. RFBR (19-04-00082 and 19-54-12002) and DFG (KU 961/14-1) supported the experimental part of this work.

P-03.2-15
New insights into molecular mechanism of signaling by endocrine FGFs

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The three endocrine fibroblast growth factors (FGFs) — FGF19, FGF21, and FGF23 — are circulating hormones that regulate critical metabolic processes in liver, adipocytes, hypothalamus, and kidney. Endocrine FGFs mediate their cellular responses by binding to complexes composed of an FGF receptor (FGFR) and either an α-Klotho or β-Klotho receptor. Structural analyses of ligand-occupied Klotho extracellular domains have provided important insights concerning mechanisms underlying the binding specificities of FGF21 and FGF23 to β-Klotho or α-Klotho, respectively. They have also demonstrated that Klotho proteins function as primary high-affinity receptors while FGFRs function as the catalytic subunits that mediate intracellular signaling. We have described the crystal structure of the C-terminal tail of FGF19 (FGF19 CT) bound to β-Klotho and demonstrated that FGF19 CT and FGF21 CT bind to the same site on β-Klotho, via a multiturn Asp-Pro motif to Klotho domain 1 and via a sugar-mimicking Ser-Pro-Ser motif to the pseudoglycoside hydrolase region of Klotho domain 2. We have analyzed wild type endocrine FGFs and a variety of their mutant and chimeric forms, studying their binding affinities to Klotho and activation of signaling in the cells expressing Klotho together with FGFR1c or FGFR4. These experiments as well as detailed comparison of the structures of free and ligand-occupied β-Klotho to the structure of ligand-occupied α-Klotho reveal a general mechanism for recognition of endocrine FGFs by Klotho proteins and regulatory interactions with FGFRs that control their pleiotropic cellular responses. Kuzina, E. S., Ung, P. M., Mohanty, J., Tome, F., Choi, J., Pardon, E., Steyaert, J., Lax, I., Schlessinger, A., Schlessinger, J., and Lee, S. (2019) Structures of ligand occupied β-klotho complexes reveal molecular mechanism underlying endocrine FGF specificity and activity. PNAS, 2019 Apr 16;116 (16):7819–7824

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P-03.2-16
The interaction of CD40 receptor on B cells with different forms of recombinant CD40L

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In germinal centers of lymph nodes B cells undergo stimulation and differentiation. In order to mimic these conditions for B cells' activation in vitro feeder cells expressing CD40L and exogenous IL-21 were used. To optimize stimulation system, we tried different forms of CD40L molecule and CD40L/IL-21 ratios. The interaction of CD40L with its receptor, CD40, was evaluated using induction of CD95 on Daudi cells and proliferation of primary B cells. We generated several feeder cell lines stably transfected with CD40L, all of which had approximately the same stimulating activity. The most attractive stimulation system is the feeder-free one; however, monomeric CD40L does not activate cells well. To increase the activity of soluble CD40L we produced several multimeric forms of CD40L, in which the TNF homologous region of human CD40L were fused to the C-terminus either collagen like domain from mouse adiponectin (Adipo-40L) or Fc fragment of human IgG1 followed by trimerization isoleucine zipper (Fc-ILZ-40L). SDS-PAGE showed that generated hybrid proteins formed hexamers. Treatment of Daudi cells with soluble Fc-ILZ-40L minimally induced CD95, while Adipo-40L was highly active. Fc-ILZ-40L in combination with IL-21 induced primary B cells proliferation. Membrane-anchored CD40L could be a more potent agonist than the soluble form of this protein. To this end, we evaluated stimulation activity of larger extracellular vesicles, derived from supernatant of feeder cells and UV induced apoptotic bodies. Both preparations showed marked increase of CD95 on Daudi cells. Our ultimate goal is development of feeder-free B lymphocyte stimulation system that will allow B cells' cloning, evaluating the specificity of secreted antibodies and generating new human monoclonal antibodies. The work was supported by the Russian Science Foundation grant 19-15-00331.

P-03.2-17
Modulation of TrkB activation by zinc/brain-derived neurotrophic factor complex: implication for neurotrophic activity

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Brain-derived neurotrophic factor (BDNF) is a neurotrophin, essential for neuronal development and synaptic plasticity. Similarly, Zinc (Zn^{2+}) plays a pivotal role in the regulation of several neuronal processes. Dysregulation of BDNF signalling, as well as zinc dyshomeostasis, are involved in different neurodegenerative disorders. Interestingly, it has been reported the ability of zinc to promote the transactivation of TrkB, the high-affinity BDNF receptor. However, the dynamics of zinc-mediated regulation of BDNF is still unclear. This aspect was first investigated using a new small BDNF peptide-mimetic (D-BDNF). D-BDNF was effective in promoting TrkB receptor phosphorylation and dimerization demonstrating its potential neurotrophic activity. Then, the ability of the Zn-D-BDNF complex to modify the differentiation of a neuronal cell model (SH-SY5Y) was evaluated by morphometric analysis. The peptide-mimetic increased the neuronal differentiation in accordance with its ability to increase the expression of GAP43 and LAMC1. Interestingly, zinc produced a drastic reduction of D-BDNF activity on TrkB , probably a significant conformational change induced by the complexation of the small-size peptide with the metal. Similarly, zinc was able to form a complex with BDNF modifying its neurotrophic activity. The binding of Zinc to the BDNF decreased the TrkB phosphorylation; accordingly, the activation downstream signals, such as ERK, was reduced. The binding also modified the length of emitted neurite, as well as the percentage of differentiated cells. Overall, the obtained results shed light on a new aspect of zinc regulation of fundamental processes of the central nervous system such as neuronal differentiation. Furthermore, the promising activity of the peptide-mimetic opens the way for the development of new potential therapeutic agents for different neurodegenerative pathologies. *The authors marked with an asterisk equally contributed to the work.

P-03.2-18
Effects of rFSH on expression dynamics of GDNF, SIRT-1, and LIN28A in cultured Sertoli cells of azoospermic men

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Sertoli cells have a pivotal role for germ cell development in response to endocrine and paracrine stimulation. Glial cell line-derived neurotrophic factor (GDNF) is essential for spermatogonial stem cell (SSC) renewal and the role of GDNF in the testis SSC niche has also been established. SIRT-1 governs spermatogenesis through a male germ cell-autonomous role and is necessary in male germ cells for normal spermatogenesis. LIN28A is a key determinant of cell fate signals in multiple stem cell lineages. In this study, we aimed to investigate GDNF, SIRT-1, and LIN28A gene expressions of Sertoli cells in the etiology of azoospermia and the effect of in vitro recombinant FSH (rFSH) treatment on impaired gene expressions. Primary Sertoli cell cultures from nonobstructive (NOA, n=5) and obstructive azoospermic (OA, n = 5; control group) males were prepared from micro-TESE samples and gene expressions were evaluated by qRT-PCR before and after rFSH (10 IU/mL) supplementation. Against to OA, in NOA cases a decrease of 0.8-, 0.6- and 0.003-fold in LIN28A ($P > 0.05$), SIRT-1 ($P < 0.05$) and GDNF ($P < 0.05$) gene expressions were found, respectively. The LIN28A and GDNF ($P < 0.05$) expressions showed a significant increase after the rFSH treatment up to 0.9- and 0.3-folds, respectively, while decreasing to 0.5-fold in SIRT-1 expression ($P < 0.05$). Our results suggest that defects in GDNF, SIRT-1 and LIN28A expressions in Sertoli cells may have relevance to NOA. FSH treatment may have positive effects on Sertoli cells of NOA patients via changing the expressions of certain genes and

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restoring to levels in normal Sertoli cell population. Therefore, GDNF, SIRT-1 and LIN28A expression levels can be considered as a potential marker for evaluation of azoospermic men and rFSH may be an effective agent in the treatment of azoospermia by regulating this pathway.

P-03.2-19
Insulin and IGF-1 receptors transmembrane domain dimers: structure prediction and possible role in activation

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The receptor tyrosine kinase (RTK) superfamily comprises many different cell-surface receptors having similar membrane organization and function with signal transduction occuring in the dimeric state. Insulin receptor (IR) and type 1 insulin-like growth factor receptor (IGF-1R) differ from other RTKs being constitutively homodimeric transmembrane glycoproteins, and molecular mechanisms of their activation still remain elusive. Current hypothesis suggests ligand-triggered structural changes in the extracellular domain followed by transmembrane (TM) domains closure and dimerization leading to kinase activity in intracellular segments of the receptor. Using experimental data as constraints, we proposed several atomistic models of dimeric states of IR and IGF-1R TM domains. Molecular dynamics simulations of IR ectodomain revealed noticeable collective movements potentially responsible for closure of its C-termini corresponding to spatial approaching of the following TM helices. Also, we demonstrated that the juxtamembrane part of the IR does not impose strong restrictions on the positioning of TM helices. Finally, we utilized two independent structure prediction methods to generate a series of TM dimer conformations followed by cluster analysis and dimerization free energy estimation to select the best dimer models. Biological relevance of the later was further tested via comparison of the hydrophobic organization of TM helices for both wild-type receptors and two their mutants. Based on these data, the role of several TM segments from other RTKs in activation of IR and IGF-1R was explained. The elaborated models can be used for rational design of new factors modulating insulin signaling. The work was prepared within the framework of the HSE University Basic Research Program and funded by the Russian Foundation for Basic Research grants 18-54-15007 (MD simulations) and 18-04-01289 (NMR and biophysical experiments).

P-03.2-20
Nutmeg essential oil constituents are potent Cx43 gap junction inhibitors

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Essential oils from plants are potential source of molecules having cardiotropic, anticancer, anti-inflammatory, and others activities. However, most of their effects lack mechanistic explanations and structure–activity relationship testing. Also, there are few data on their action on intercellular communication that plays an important role in physiological and pathological processes such as homeostasis, embryogenesis, development, tumorigenesis. Gap junction (GJ) channels that provide direct electric and metabolic intercellular communication are formed of two apposing hemichannels in the contiguous cells. Each hemichannel is composed of six connexin proteins. The family of connexin genes consists of 21 members in the human genome. Cx43 is the most widely expressed connexin. In the present study, we: 1) extracted the nutmeg essential oil (NEO) and characterized its composition; 2) performed molecular docking of NEO constituents to Cx43 that is endogenously expressed in Novikoff rat hepatoma cells; 3) examined the effect of NEO and its selected constituents on Cx43 GJ conductance and gating in pairs of Novikoff cells; and 4) verified whether the obtained effects of NEO on GJs correlate with its effects on Novikoff cell viability, proliferation and colony formation capability. Our results demonstrate that NEO itself and its three constituents – monoterpenes sabinene and α-pinene and sesquiterpene α-copaene – can dock to as well as rapidly and reversibly inhibit Cx43 GJ channel conductance by a slow-gating mechanism acting with high potency through more than one active binding site on Cx43 subunits. In addition, late NEO effects manifest in down-regulation of total Cx43 expression and a decrease in the number of functional GJ channels. Finally, NEO can reduce Novikoff hepatoma cell viability, proliferation, and colony formation capability but by a communication-independent mechanism.

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P-03.2-21
Structural requirements for the interaction of the enzyme nicotinamide phosphoribosyltransferase with Toll-like receptor 4

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An interesting feature of the enzyme nicotinamide phosphoribosyltransferase (NAMPT) is that, behind its catalytic role in the intracellular biosynthesis of NAD, it is secreted into the extracellular space where it behaves as a cytokine, triggering an inflammatory response. Indeed, the level of the circulating protein markedly increases in patients with cancer and inflammatory disorders. Such a cytokine-like function is independent of the enzymatic activity and is reported to be mediated by direct binding of the protein to Toll-like receptor-4 (TLR4). In this work, we have used an integrated approach, combining bioinformatics, functional and structural analyses, to shed light on the interaction between the enzyme and the receptor at the molecular level. Starting from the evidence that, despite a high degree of structural conservation between human NAMPT and its bacterial ortholog, the bacterial protein is not capable of triggering the inflammatory response, we have mapped regions likely involved in the signalling function. Next, we have validated the bioinformatic analysis by assessing whether mutated proteins retained the ability to physically interact with biosensor tethered-TLR4 and trigger the inflammatory response in cultured macrophages. Our data identified two regions involved in TLR4 binding, thus paving the way to the selective targeting of the secreted enzyme's function.

P-03.2-22
The biological activity of *Bacillus thuringiensis* Cry37Aa protein is impaired by biotinylation

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The entomopathogenic bacterium *Bacillus thuringiensis* (Bt) produces diverse groups of insecticidal proteins toxic to many insects. The well-known crystal proteins (Cry) produced during the stationary phase of bacterial growth, are frequently employed in the management of insect pests whether formulated in Bt-based bio-insecticides or expressed in plants (Bt crops). *Cylas puncticollis* (Boheman), is one of the most important constraints on sweet potato production in Africa. The Cry23Aa/Cry37Aa proteins, believed as binary toxin, have been toxic against *Cylas puncticollis*. In the present study, the biological activity of Cry37Aa has been investigated in terms of toxicity and binding to midgut receptors of *C. puncticollis* larvae. Additionally, the proteolytic processing and the interaction between two components in solution have been studied. Although the bioassay results showed that Cry37Aa is toxic independently to Cry23Aa, an interaction between both components has been observed based on the size-exclusion chromatography results. Remarkably, proteolytic processing occurs for the Cry23Aa but not for the Cry37Aa following trypsin or *C. puncticollis* midgut proteases treatments. Binding assays showed that while biotin labelled Cry23Aa binds specifically to *C. puncticollis* brush border membrane vesicles (BBMV), no binding was detected for the biotinylated Cry37Aa protein. The interference of biotin labelling in the Cry37Aa binding ability has been explored based on the biological activity of this protein against *C. puncticollis* larvae. The bioassay results clearly showed that the biotin labelling of Cry37Aa drastically reduced the toxic activity of the protein. Altogether, our results point out that biotinylation of Cry37Aa abolished the insecticidal activity and probably the specific binding of this protein to C. puncticollis BBMV. *The authors marked with an asterisk equally contributed to the work.

P-03.2-23
Crosstalk between sphingosine-1-phosphate and endocannabinoid signalling

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Sphingosine-1-phosphate (S1P) and endocannabinoid (eCB) systems regulate the same intracellular signalling pathways involved in many biological processes through G protein-coupled receptors (GPCRs). In particular, S1P binds to five different GPCRs (S1PR1–5), whereas eCBs (N-arachidonoyl ethanolamine (AEA) and 2-arachidonoyl glycerol (2-AG) trigger type 1 and 2 (CB_1 and CB_2) cannabinoid receptors and GPR55, in addition to the transient receptor potential cation channel subfamily V member 1 (TRPV1) (1). S1P receptors show 20% sequence identity with CB_1 and CB_2 (2). Of note, interactions between S1P and eCBs have been documented in rat coronary artery reactivity (3). Here, the molecular effects of S1P on distinct elements of the eCB system have been investigated in murine myoblast C2C12 cells, by using quantitative-Real Time-Polymerase Chain Reaction (q-RT-PCR) and Western blot analysis. Moreover, the modulation of the mitochondrial membrane potential upon cell treatment with S1P and/or eCBs and antagonists to eCB-binding receptors was assessed by using the JC-1 fluorescent dye. q-RT-PCR analysis showed a significant increase in GPR55 and TRPV1 mRNA expression, and Western blot confirmed the increase in TRPV1 and evidenced a decrease in CB_2 protein expression. A significant depolarization of the mitochondrial membrane potential was found upon S1P treatment, an effect that was significantly reverted by AEA and 2-AG through CB_1, CB_2, GPR55 and (in the case of 2-AG) TRPV1. A preliminary docking analysis suggested that CB_1 antagonism by S1P could be due to binding to the N-terminal allosteric pocket of the receptor. In conclusion,

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S1P modulates the expression of some eCB-binding receptors at gene and protein level, and thus it may interfere with mitochondrial membrane potential regulation. [1]. Maccarrone M et al (2014) Nature Rev Neurosci 15, 786–801, [2]. Sanchez T et al. (2004) J Cell Biochem 92, 913–922, [3]. Mair KM et al. (2010) Br J Pharmacol 161, 176–192. *The authors marked with an asterisk equally contributed to the work.

P-03.2-24
Hot spots for partnership at the surface of the cell-adhesion molecule neuroligin versus the enzyme acetylcholinesterase, two cousins in the same structural superfamily

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The cell-adhesion molecules, neuroligins, and the hydrolytic enzymes, cholinesterases, are cousins in the alpha/beta-hydrolase fold superfamily of proteins. Their ectodomains enclose a conserved central core, where in the enzymes the catalytic center resides, while specific properties for partner recognition, versus substrate selectivity or regulation of catalysis, are dictated by core decoration with additional motifs of variable structures/positions. Autism-linked mutations in the neuroligin ectodomain, leading to intracellular retention and deficiency in cell-membrane presentation, can be transferred to cholinesterases and induce the same effect. Despite their distinctive modes of action, the neuroligin and cholinesterase ectodomains share surface binding patches and determinants for forming homodimers and accommodating respective partners. Several of these regions have been mapped through structural/mutational studies - others have been proposed based on biochemical or molecular docking data. We will review the partnership versatility of the neuroligin ectodomain associated with molecular flexibility and alternative binding sites. Based on a neuroligin/cholinesterase comparison, we will also propose regions with distinctive locations and properties for homo- or heterologous partner association at the neuroligin surface. By showing how specific or overlapped these interfaces are, we can emphasize their specificity versus potentially multifunctional character, and the diversity of the functional adaptations of the common structural fold. Bourne Y, Marchot P (2017) Hot spots for protein partnership at the surface of cholinesterases and related alpha/beta hydrolase fold proteins or domains - a structural perspective. Molecules 23, 35. Comoletti D, Trobiani L, Chatonnet A, Bourne Y, Marchot P (2021) Comparative mapping of selected structural determinants on the extracellular domains of cholinesterase-like cell-adhesion molecules (Review). Neuropharmacology 184, 108381. *The authors marked with an asterisk equally contributed to the work.

P-03.2-25
Ouabain suppresses IL-6 signalling in cultured skeletal muscle cells

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Cardiotonic steroids (CTS), which were used for centuries for treatment of heart failure, are thought to be endogenous adrenocortical hormones that play a role in regulation of cardiovascular system, salt and water homeostasis, metabolism, and immune system. CTS exert their biological effects by binding to the Na+-K+ pump, a heterodimeric (α/β) P-type ATPase, which also functions as a signal transducer. CTS bind to the catalytic α-subunit of the Na^+-K^+ pump, thus inhibiting its transport activity as well as modulating intracellular signalling. Exercise increases plasma levels of endogenous CTS, indicating their involvement in adaptation to exercise. Contracting skeletal muscles secrete interleukin-6 (IL-6), which promotes muscle and systemic adaptations to exercise by activating the JAK/STAT3 pathway via the IL-6Rα/gp130 receptor complex. Here we determined whether ouabain, which is considered to be a major endogenous CTS, modulates the IL-6/JAK/STAT3 pathway in skeletal muscle cells. Activity of the JAK/STAT3 pathway was assessed by measuring phosphorylation (Tyr705) of STAT3 in the presence or absence of IL-6. We found that ouabain (2.5–50 nM) markedly suppressed basal and IL-6-stimulated phosphorylation of STAT3 in human skeletal muscle cells. While 2.5 and 10 nM ouabain did not alter total STAT3 expression, 50 nM ouabain markedly suppressed it. Tocilizumab, a monoclonal antibody, which blocks IL-6 action by binding to IL-6Rα, reduced IL-6-stimulated phosphorylation of STAT3, but did not affect abundance of total STAT3. In contrast to human skeletal muscle cells, ouabain did not alter phosphorylation or expression of STAT3 in rat L6 skeletal muscle cells, which express ouabain-resistant α1-subunit of the Na^+-K^+ pump. Taken together, our results suggest that ouabain suppresses IL-6 signalling in human skeletal muscle cells by inhibiting phosphorylation and expression of STAT3. Further, they highlight a role for Na^+-K^+ pump in modulation of IL-6 action.

P-03.2-26
Effect of purine synthesis inhibitors on AMP-activated protein kinase and insulin action in skeletal muscle cells

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Skeletal muscles play a major role in whole-body glucose homeostasis. Reduced uptake of glucose by skeletal muscles due to insulin resistance can lead to increase in blood glucose concentration and eventually to type 2 diabetes. One very promising target

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for the treatment of insulin resistance and diabetes is AMP-activated protein kinase (AMPK). Activation of AMPK can reduce insulin resistance and stimulate insulin-independent glucose uptake in skeletal muscle. AMPK can be activated by antirheumatic drug methotrexate (MTX). MTX inhibits purine synthesis enzyme ATIC, which converts ZMP to IMP, leading to accumulation of ZMP. ZMP binds to and activates AMPK in the same way as the main endogenous AMPK activator AMP. Moreover, by inhibiting purine synthesis, MTX can lower ATP level, which inhibits AMPK, thus sensitizing AMPK for activation with AMP and ZMP. We explored whether other drugs that inhibit purine synthesis, such as mycophenolate mofetil (MMF), mercaptopurine (MP), trimethoprim (TMP) and trimetrexate (TMX), also activate AMPK and increase glucose uptake in skeletal muscle cells. In addition, we examined the effect of these drugs on insulin signaling and insulin-stimulated glucose uptake in skeletal muscle cells. MP activated AMPK and increased basal glucose uptake on its own while MMF and TMX promoted activation of AMPK with AICAR (pharmacologic precursor of ZMP) and increased AICAR-induced glucose uptake. MP markedly decreased insulin signaling while other drugs had no or smaller effects on insulin signaling than MP. Although MP attenuated insulin signaling, cells treated with MP and insulin showed higher glucose uptake than cells treated with insulin alone, most likely due to stimulation of basal glucose uptake by MP. In summary, our results show that not only MTX but also several other drugs that inhibit purine synthesis affect AMPK, basal glucose uptake and/or insulin action in skeletal muscle cells.

P-03.2-27
Insight on the role of pleiotrophin and its receptor PTPRZ1 in bone formation and remodeling

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Bone remodeling is important for bone development, homeostasis, and pathologies. Pleiotrophin (PTN) is a heparin-binding growth factor with potential biological activities related to bone remodeling. The PTN receptor PTPRZ1 is also related to bone pathologies, but its implication in osteoblast functions has not been studied. The aim of the present study was to elucidate the role of PTN and PTPRZ1 in the differentiation of osteoblasts and in fracture healing using in vitro and in vivo models. Aged $Ptn^{-/-}$ mice have lower bone quality and develop osteoporosis. In vitro, PTN decreased pre-osteoblasts proliferation and migration but induced their differentiation, similarly to a PTN peptide that corresponds to the PTN domain responsible for interaction with VEGFR2. An insight on the potential role of PTPRZ1 in bone formation came from studies using calvaria osteoblasts from $Ptprz1^{+/+}$ and $Ptprz1^{-/-}$ mice, related to differentiation and osteoblastic markers expression in vitro. In $Ptprz1^{-/-}$ mice *in vivo*, maturation of the bones and vertebrae was obtained at a smaller age compared to $Ptprz1^{+/+}$ mice, but there were no differences in the quality of the bone at higher ages or in intramembranous ossification during fracture healing in the drill-hole model *in vivo*. Deletion of PTN or PTPRZ1 results in osteoarthritis in vivo. These data suggest that PTN and PTPRZ1 are implicated in bone remodeling and warrant further studies to elucidate mechanistic aspects and identify PTN peptides that may be useful for the design of novel therapeutics. Acknowledgements: This research is co-financed by Greece and the European Union (European Social Fund- ESF) through the Operational Programme «Human Resources Development, Education and Lifelong Learning 2014–2020» in the context of the project "PLEIOBONE" (MIS 5047169). The $Ptn^{-/-}$ and $Ptprz1^{-/-}$ mice were a generous gift from Drs. H. Himburg and J.P. Chute, Division of Hematology/Oncology, Broad Stem Cell Research Center, UCLA, USA.

Membranes

P-03.3-01
Effect of propolis and some of its compounds on the model lipid membranes

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The pharmacological potential of propolis has been quite well investigated, but much less is known about the mode of its action, including the impact on the model lipid membranes. In our studies we have investigated the impact of the chosen compounds found in propolis (epigallocatechin gallate, chrysin …), and propolis itself, on the model lipid membranes composed from dipalmitoyl-phosphatidylcholine (DPPC). To investigate the effect of propolis and the small compounds on the membranes structural integrity we used the combination of differential scanning calorimetry (DSC) and fluorescence spectroscopy. To visualize the effect of incorporation of the small molecules into the lipid membrane the transmission electron microscopy was used. To determine the antioxidant effect of propolis on the model membranes, the proliposomes consist from commercial phospholipids (phospholipon 90G) was used. The findings so far are in favor of the hypothesis, that the mixture of different compounds (propolis) has a greater impact on the membrane integrity than the isolated small compounds by themselves. This is especially pronounced from the DSC results, when even very small quantities of propolis have a huge impact on changing the DPPC transition temperature and enthalpy of the transition. The individual small compounds need to be present in higher quantities to have a similar effect. It is therefore speculated that the mixture of molecules in propolis display strong synergistic effects, and thus effect the membranes more than a single molecule can, even in higher compound to lipid molar ratio. In the future research we will try to focus on the determination of mode of binding of chosen compounds to lipid bilayer and to identify their potential use in combination with the antibiotics for the treatment of the bacterial infections.

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P-03.3-02
Characterization of the binding of Rabphilin-3A to membranes

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Rabphilin 3A is a membrane traffic protein that contains a tandem C2AB-domain located in its C-terminal that is responsible for the Ca^{2+}-dependent phospholipid binding and mediates interactions with regulatory proteins like SNAP25, CASK, Anexin A4 and Miosin V. We report here functional analyses to characterize the molecular determinants of the Rabphilin3A interaction with membranes. By using Isothermal Titration Calorimetry we have determined the affinities and thermodynamic properties of these interactions and the results indicate that the C2AB domain binds preferentially to membranes containing PIP2 and phosphatidylserine in the presence of Ca^{2+}. This is an exothermic reaction driven mainly by enthalpy changes. Dynamic Light Scattering assays have demonstrated that the main aggregation capacity resides in the C2B domain. Site-directed mutagenesis of key residues located at the different interacting surfaces of the C2AB domain shows that each one plays differential roles in the tandem. These is due to a collection of conserved key functional residues, but at the same time each one possess differential amino acids that confer them special abilities to interact with the membrane and with other proteins. These findings provide functional explanation about how these domains are regulated by a dual-target mechanism and reveal how this family of proteins can employ subtle structural changes to modulate their sensitivity and specificity to various celular signals.

P-03.3-03
Method of enrichment of human plasma extracellular vesicles for reliable quantification for biomarker discovery

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Introduction: Extracellular vesicles (EVs) are phospholipid bilayer-encased particles, released into body fluids by all cell types. EVs' molecular cargo, size and concentration reflect (pato)physiological state of their cell-of-origin. However, lack of standardised enrichment methods of EVs from complex sources hinders their full use as minimally invasive biomarkers. Here, we propose an EV-enrichment method for their reliable quantification from relatively small volumes of human blood plasma. Methods: EVs were enriched from plasma of 10 healthy volunteers using density-based (ultracentrifugation on 20% sucrose cushion; sUC) and size-based (size exclusion chromatography; SEC) methods. Concentration, size, and purity of EV-enriched samples were determined with nanoparticle tracking analysis (NTA), asymmetric flow field-flow fractionation (AF4-MALS) and TEM, while miRNA levels were measured with qPCR. Effects of proteinase K and DNase-I treatment on size/concentration of particles and repeatability of selected method (sUC) were also analyzed. Results: Average mode diameter and size profile of enriched particles in the samples were comparable between isolation methods, yet SEC led to a higher number but lower quality of isolated particles compared to sUC ($P < 0.001$). TEM indicated this could be result of lipoproteins and protein aggregates in SEC-samples. sUC was highly repeatable and resulted in purer EV samples with more miRNA cargo. After isolation with sUC from 1 mL of plasma, we measured $3.11*10^9$ particles (mean size 109 nm) using NTA and $0.66*10^9$ particles (2*Rgeom 195 nm) using AF4-MALS. Proteinase K and DNase-I did not significantly affect the size and concentration of sUC-enriched EVs in the sample, implying that potential impurities do not contribute significantly to the measurements. Conclusion: sUC method resulted in higher yield and purity of EVs in the enriched samples and could be used to explore plasma-EVs' size and concentration as potential biomarkers.

P-03.3-04
Mechanism of vitamin D3 effect on pacemaker and contractile activity of the isolated heart

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Deficiency of vitamin D_3 in the organism is one of the risk factors in the development of cardio-vascular disorders. However, the molecular mechanisms of its action on the cardiac activity remain unclear. The dynamics of vitamin D_3 influence on the functional indices (pacemaker rhythm, contractile activity) of the isolated heart was studied by the pharmacological analysis method. Application of 10^{-10} M (physiological concentration) vitamin D_3 on the isolated heart was conducted in combination with the blockers of the following structures: calcium channels (verapamil, 10^{-5} M), potassium channels (aminopiridin, 10^{-3} M), phosphodiesterase (theophillin, 10^{-4} M), proteinkinase C (staurosporin, 10^{-4} M). Vitamin D_3 caused biphasic shift of the inotropic-chronotropic activity of the heart. The contraction amplitude increased up to 150% during the first 20 min ("early" effect), then it decreased up to 50% of the initial level and again rose up to the initial one on the 90–100th min ("late" effect). The pacemaker activity decreased from 45 beat/min to 20 beat/min which later was restored up to the initial rate. The isolated heart was functioning up to 90–120 min (in the control – 20–30 min). The inotropic-potentiating effect of vitamin D_3 was not manifested when calcium channels were blocked by verapamil, but chronotropic effect underwent insignificant changes. The heart contracted for 40 min. In the presence of theophillin the dynamics of "early" shifts of inotropic-chronotropic activity were identical with the effect of vitamin D_3. However, the "late" effect did not occur. When vitamin D_3 was combined with staurosporin the heart stopped for 10 min. There were no significant changes when vitamin D_3 was used with aminopiridin. On the base of the data obtained it is supposed that "early" effect of vitamin D_3 on the functional activity of the heart is realized through calcium and cAMP-dependent mechanisms, the "late" effect – by activation of proteinkinase C.

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P-03.3-05
Effect of cholesterol on mixed hBest1/lipid monolayers

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Human bestrophin-1 (hBest1) is a calcium dependent chloride channel, expressed at the basolateral membrane domain of retinal pigment epithelium. Mutations in HBEST1 gene are linked to a group of pathologies referred as Bestrophinopathies. A new biological role for hBest1 has been proposed related to the neurodegenerative diseases such as Alzheimer's and Parkinson's. As a transmembrane protein, hBest1 functional activity is strongly dependent of its lipid environment. Here, we present the effect of cholesterol on mixed two-component protein-lipid monolayers: hBest1/1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) and hBest1/sphingomyelin (SM) (in the presence of Ca^{2+} ions). Using physicochemical experiments (Previously published in: Videv P et al. (2021) Membranes 11 art. no. 52, pp. 1–8) based on the surface pressure/mean molecular area (π/A) isotherms as a function of cholesterol concentration, we showed that under the given conditions (35°C, close to physiological), cholesterol exerts condensing effect on mixed hBest1/POPC and hBest1/SM monolayers. π/A isotherms are shifted to a higher surface pressure at a given molecular area, and to a lower molecular area at a given π (A_π). This surface behavior of cholesterol is associated with its structuring properties on membranes and association of hBest1 with enriched cholesterol microdomains. Acknowledgments: This work was financially supported by grants from Bulgarian Science Fund (№ KP-06-N23/7, 18.12.2018), the Ministry of Education and Science under Contract DO1-275 / 16.12.2019 / "Research Infrastructure for Cell Technologies in Biomedicine" of the Bulgarian NRRI 2017-2023 and under the National Program for Research "Young Scientists and Postdoctoral Students" (Bulgarian Ministry of Education and Science; the program was approved with PMC № 577 / 17.08.2018)

P-03.3-06
Small molecules as a regulators of the membrane activity of antimicrobial peptide cecropin A

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Antimicrobial peptides, cecropins, are characterized by a wide range of antibacterial activity. The molecular mechanisms of interaction of the peptides with cellular membranes have not fully understood. The small molecules that are capable to adsorb on membrane and change its physico-chemical properties can be effectively used to study the membranotropic action of cecropins. Using this approach we showed that the introduction of phloretin, RH421, RH237, bupivacaine, benzocaine or tetracaine led to decrease in the mean conductance of the single cecropin channels (g_{SC}). The addition of myricetin, lidocaine and procaine in the membrane-bathing solutions did not practically affect g_{SC}. The data are not in agreement with the results of the measurements of small molecule induced alteration in the dipole potential of negatively charged membranes. Taking into account the significant decrease of the temperature of the main phase transition of DPPC in the presence of phloretin, RH421, RH237, bupivacaine, benzocaine or tetracaine we concluded that the observed changes in g_{SC} are caused by uncoupling action of the small molecules on the lipid bilayer. Moreover, the addition of phloretin, piperine, dihydrocapsaicin, capsaicin, bupivacaine, mepivacaine, ropivacaine, benzocaine or tetracaine in the membrane-bathing solution induced the decrease in the steady-state number of open cecropin channels (Nop), while the introduction of the RH421 or RH237 led to an increase in the Nop. It was found that myricetin, pentoxifylline, synephrine, lidocaine and procaine did not practically influence the Nop. We concluded that the changes in Nop is due to the alteration in membrane dipole potential and g_{SC} changes. The study was supported by RFS (#19-14-00110).

P-03.3-07
Fragmentation of cells during extracellular vesicle isolation – a mechanism of vesicle formation

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Extracellular vesicles (EVs) are sub-micron sized membrane-enclosed fragments of cell origin. After the release from the mother cell they become free to move in the surrounding fluid. They can travel within the organism and also between organisms and reach adjacent and distant distant cells with which they can interact. In this way they contribute to intercellular communication with important physiological and patophysiological consequences. Different procedures for EV isolation from biological samples (including cell culture supernatant and biofluids) have been proposed and effort is underway to use them in diagnostics and therapeutics. Three main mechanisms for the natural formation of EVs have been acknowledged so far: apoptosis, plasma membrane exovesiculation and exosome formation within late endosomes and their subsequent release. Here, we outline another mechanism which however prevails in isolates, i.e. cellular fragmentation due to thermal and mechanical stress exerted upon cells and their fragments during sample processing. We isolated EVs by centrifugation/ultracentrifugation and washing of peripheral blood samples and analyzed them by flow cytometry, dynamic light scattering and scanning electron microscopy. We found that the number and the average size of patricles in the isolates depended on the centrifugal pull. Our results showed that in blood isolates obtained by centrifugation and ultracentrifugation, cell fragmentation was the main process of the formation of EVs in the size range from 50 to 1000 nm. Thereby we present evidence in favor of the hypothesis that EVs in isolates from blood are a self-assembled material with transient identity.

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P-03.3-08
AMPK suppresses phosphorylation of Tyr10 of the catalytic α1-subunit of the Na^+, K^+-ATPase in skeletal muscle and kidney cells

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Na^+,K^+-ATPase (NKA), a heterodimeric (α/β) ion pump, transports Na^+ and K^+ across plasmalemma, thus maintaining ion homeostasis, excitability and contractility of skeletal muscle. NKA is also a signal transducer that forms complexes with epidermal growth factor receptor (EGFR) and non-receptor tyrosine kinase Src. Phosphorylation of the NKA α-subunit is a major mechanism by which various signalling pathways regulate NKA function. Among the phosphosites of the α-subunit, the role and regulation of Tyr10, which is regulated by insulin in the kidney, has not been elucidated in the skeletal muscle. We explored whether AMP-activated protein kinase (AMPK), a cellular energy sensor, modulates Tyr10 phosphorylation. AICAR, the most widely used exogenous AMPK activator, induced a time-dependent decrease in Tyr10 phosphorylation in cultured human primary skeletal muscle cells. Other AMPK activators, such as A769662 (a direct AMPK activator), FCCP (a mitochondrial uncoupler), ionomycin (a Ca^{2+}-ionophore), and 2-deoxyglucose (an inhibitor of glycolysis), also suppressed Tyr10 phosphorylation, further indicating Tyr10 is regulated by AMPK. AICAR and A769662 also suppressed phosphorylation of Tyr10 in human proximal tubular cells HK2. In contrast, fetal bovine serum, epidermal growth factor, and ouabain, a cardiac glycoside that inhibits NKA, increased Tyr10 phosphorylation in skeletal muscle and HK2 cells. Using phosphosite prediction tools we identified Src as the potential Tyr10 kinase. Consistent with this notion, Src inhibitor PP2 blocked ouabain- and EGF-stimulated phosphorylation of Tyr10. Finally, we found that Tyr10 and the surrounding amino acid sequence are well-conserved in jawed vertebrates (Gnathostomata), suggesting functional importance of Tyr10 in the vertebrate lineage. Taken together, our results suggest that AMPK suppresses Tyr10 phosphorylation. Tyr10 may therefore provide a link between regulation of energy metabolism and NKA signalling in skeletal muscle.

P-03.3-09
Evidences on microalgal extracellular vesicles

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Vesicle-based products are being considered as drug-delivery systems in therapies of various diseases. Natural source vesicles are believed to be promising in relation to their stability and potential beneficial contents. However, protocols for a robust production with reliable purity and stability are not yet quite established, and methods for quality assessment of vesicles and their integrity are still being sought for. We have established a small size laboratory production of algal cultures in order to harvest the extracellular vesicles from the culture media. In this work, we report on isolates from different cultures of microalgae. The algal cultures were fractionized by differential centrifugation (dC) according to established extracellular vesicle isolation protocols. Flow cytometry was employed for monitoring of the processing. Selected fractions taken throughout the course of the procedure were assessed by flow cytometry; light scattering, and different microscopic techniques with an aim to understand the process of isolation. Scanning electron microscopy revealed some indications of ectovesiculation and apoptosis-like cell degradation. Formation of apoptotic body-like structures was observed under various stressors, including shear-induced cell damage caused by centrifugation. The dC isolates consisted of submicron-sized particles which, however, most often did not attain the expected characteristics of membrane enclosed entities without internal structure. We therefore suggest that the harvested material can be described as self-assemblies of cell constituents, most probably formed through destruction of cells in high shear stress centrifugation field. Our results indicate the importance of external parameters on isolation and provide new information on extracellular vesicles of microalgae. *The authors marked with an asterisk equally contributed to the work.

P-03.3-10
Cholinesterase as biomarker of blood cell vesiculation in clinical samples

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Extracellular vesicles (EVs) are submicron membrane enclosed particles which are continuously gaining attention due to their potential role in medicine and pharmacy. The clinical practices are still lacking practical biomarkers of EVs presence in samples. Activity of cholinesterase (ChE) has proved useful in following red blood cell vesiculation in extreme conditions with added amphiphilic molecules. However, in clinical samples these effects have not been fully explored. In this work we present results showing the applicability of ChE-based method for following vesiculation of blood cells in clinical samples. Peripheral blood was collected from eight volunteers with no record of disease. Blood was drawn from medial cubital vein after 12-hour fast. Blood samples were subjected to series of centrifugation. After removing majority of erythrocytes, platelet-poor (PPP) and platelet-rich plasma (PRP) were obtained with centrifugation of plasma samples. Small particles (SPs) with size and density corresponding to EVs were pelleted from plasma sample by centrifugation at 20 000 x g, 10 min, 22 °C. Two washing steps were performed at same conditions to obtain the final isolate. Samples of plasma, PRP, PPP, SPs isolates, and supernatants from SPs isolation (after sedimentation of SPs from plasma samples, and

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those from the washing steps) were assessed by flow cytometry. Also, in all samples, ChE activity was measured spectrophotometrically using Ellman's method adopted for microtiter plates. The correlations between number of SPs and ChE activity were found to be statistically significant for supernatant before washing ($P < 0.001$), supernatant after second washing ($P < 0.01$) and in isolates of SPs ($P < 0.01$). Our results indicate that ChE activity is promising as a potential biomarker for vesiculability of cells in clinical blood samples. *The authors marked with an asterisk equally contributed to the work.

P-03.3-11
Intracellular Na/K imbalance contributes to gene expression in endothelial cells exposed to elevated NaCl

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High-salt consumption contributes to the development of hypertension and is considered an independent risk factor for vascular remodelling, cardiac hypertrophy and stroke incidence. High-salt intake may contribute to the development of various disorders via signals triggered by augmented concentration of extracellular sodium ($[Na^+]_o$), chloride ($[Cl^-]_o$) and osmolarity of the extracellular fluids determined by combined elevation of $[Na^+]_o$ and $[Cl^-]_o$. Our results strongly suggest that Na^+_i/K^+_i alterations play a key role in affecting the transcription of genes in endothelial cells (HUVEC) exposed to hyperosmotic conditions. This conclusion is supported by the following evidences. First, an increase in extracellular NaCl from 125 to 140 mM influenced the Na^+_i/K^+_i gradient in HUVEC. Second, COX2, IL6, IL1LR1 were found to be downregulated upon cells exposure to elevated NaCl medium. Third, we did not detect an altered abundance of NFAT5 protein in response to the increase in extracellular NaCl by 15 mM within 24 hours. Forth, neither Na^+_i/K^+_i ratio, nor the content of COX2, IL6, IL1LR1 was affected by exposure of HUVEC to the medium containing 125 mM NaCl and 30 mM mannitol. Fifth, the change in osmolarity of the extracellular medium, either due to the addition of NaCl or mannitol, had no effect on the mechanical properties of endothelial cells. This work was supported by Russian Science Foundation, grant number 19-75-10009.

P-03.3-12
Looking for a needle in a haystack: How NLP proteins disrupt plant plasma membrane?

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Major diseases of crop plants that are caused by microbial plant pathogens are a substantial threat to global food security and lead to enormous economic loss. Microbial pathogen-plant interactions depend on complex molecular interactions. Nep1-like proteins (NLPs), secreted by taxonomically unrelated microorganisms, bacteria, fungi and oomyces, cause necrotic lesions of plant tissue and facilitate eudicot plant infection, but are not active against monocots. We have recently discovered that a mechanism of plant plasma membrane damage involves NLP's recognition of particular class of lipid molecules found in plant membranes (Lenarčič T et al. (2017) Science 358, 1431-1434). Although these lipids, glycosylinositol phosphorylceramides (GIPCs), represent a major class of plant sphingolipids, very little is known about their chemical properties and properties of lipid membranes containing them. We proposed a model of early steps of NLP–membrane interaction, however, the overall molecular mechanism by which NLP disrupts plant plasma membrane remains unknown. Identification of NLP's membrane receptor enabled exploitation of various GIPCs-containing lipid model systems. We prepared a series of model plant membranes and examined lipid–NLP interaction by liposome sedimentation assay, surface plasmon resonance (SPR), quartz crystal microbalance (QCM) and neutron reflectometry (NR). We showed that NLP protein binding is highly salt dependent which correlates with the low salt environment in the extracellular compartment where the toxin binds. The data provided important insights into stability and fluidity of GIPC-containing membranes, as well as an insight into molecular mechanism of membrane damage induced by NLP toxins, by either via pore-forming mechanism or other type of membrane integrity disruption.

P-03.3-13
The influence of mutations in gene alpB on the biogenesis of outer membrane vesicles of *Lysobacter* sp. XL1

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Outer membrane vesicles (OMV) are unique nanoparticles formed by Gram-negative bacteria. OMVs are regarded as promising nanocontainers for the targeted delivery of drugs in humans and animals to inhibit pathogenic bacteria and to suppress the development of cancer cells. A possible practical application of OMS requires understanding of their biogenesis. We studied the biogenesis of OMV in *Lysobacter* sp. XL1 able to

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produce lytic agents efficient against multidrug resistant pathogenic bacteria, specifically bacteriolytic enzyme L5, as factor of OMVs biogenesis by deletion of gene alpB (coding for protein L5). The plasmid containing a DNA fragment with a deletion variant of alpB and flanking gene alpB sequences of genomic DNA was constructed on the basis of pJQ200SK suicide vector. This plasmid was inserted in *Lysobacter* sp. XL1 by electroporation followed by the selection of merodiploids resistant to antibiotic Gm (a marker of the inserted plasmid) and to Tc (a marker of the inserted in the locus of alpB deletion) but sensitive to sucrose (a suicide gene sucB in pJQ200SK). When grown in the medium with sucrose, the isolated merodiploids underwent a double exchange between mutant and wild-type alpB alleles. This allowed us to isolate clones with eliminated plasmid, i.e. clones sensitive to Gm and resistant to sucrose and Tc. A comparative analysis of OMVs isolated from *Lysobacter* sp. XL1 wild type and mutant strain showed that the latter formed fewer vesicles which also differed in size. This finding indicates that bacteriolytic enzyme L5 is the factor of OMVs biogenesis in *Lysobacter* sp. XL1. The results obtained make it possible to propose a new mechanism of the OMVs biogenesis in Gram-negative bacteria. A successful knockout of alpB *Lysobacter* sp. XL1 opens wide perspectives for the creation of homologous expression systems for lytic enzymes important in biomedicine. This work was supported by the Russian Science Foundation (Project No. 19-74-00086).

P-03.3-14
The insight into the mechanisms responsible for the anticancer activity of natural biosurfactants

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Biosurfactants (BS) are surface-active compounds produced by various microorganisms that have gained scientist's great interest worldwide. The extensive research on the potential use of BS results from their biological activities such as antimicrobial, antiviral, and anticancer properties. Although there are several hypotheses regarding the mechanism of action of BS, the obtained knowledge remains incomplete. Because of the high toxicity and limited efficacy of commonly used chemotherapeutic agents and the multidrug resistance of cancer cells, the development of new therapeutic agents acting synergistically with the existing drugs is crucial for cancer treatment. Fundamental study on lipopeptides' influence on the cell membrane organization, especially in cancer cells, can provide a new perspective for cancer prevention and therapy. Here, we demonstrate the potential mechanism responsible for BS anticancer activity, which results from their interaction with the cell plasma membrane. The advanced biophysical techniques' application indicates significant alterations of membrane parameters induced by cyclic lipopeptide surfactin treatment. It should be noted that BS occurs as a group of similar compounds differing in single amino acids and the alkyl chain (length, branching). Therefore, the elucidation of the interplay between the molecular structure of lipopeptides and their influence on cancer cells seems crucial for understanding the mechanisms responsible for BS properties. Here, we present the structure-dependent anticancer activity of surfactin analogs. Explaining the mechanisms responsible for BS activity on living cells is a key factor limiting their future medical uses further development. Therefore, the present results can significantly influence the current view on BS application, especially in the potential anticancer therapies.

P-03.3-15
The pore-forming ability of mastoparan and pardaxin depends on the thickness of the membrane hydrocarbon core

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Antimicrobial peptides are the part of the innate defense system against the pathogenic microorganisms in many eukaryotic organisms, including humans. They might act as antibiotics or fungicides. It is believed that antimicrobial activity is related to increase in permeability of target cell membranes. Moreover, it is shown that the action of antimicrobial peptides on the membrane depends on its physicochemical properties, in particular, the thickness of the hydrocarbon core. In present work we have investigated the ability of mastoparan (MP) and pardaxin (PX) to form ion-permeable nanopores in lipid bilayers of different composition. Using Montall and Muller technique we have showed that the addition of MP up to concentration of 10 μM to bathing solution does not lead to a significant increase in the ion permeability of di(C18:1)PC, di(C18:1)PS, di(C18:1)PE, (C16:0/18:1)PC/(C16:0/18:1)PE/(C16:0/18:1)PG, and di(C18:1) PE/tetra(C18:1)CL bilayers. PX does not affect di(C18:1)PE/tetra (C18:1)CL membranes at the concentration less than 1 μM. The probability of the pore-forming activity of the peptides is about 100% for cholesterol-enriched di(C14:1)PC and di(C16:1)PC membranes as well as for di(C18:2)PC bilayers. Thus, the obtained data are in agreement to the assumption that the activity of MP and PX depends on the thickness of the membrane hydrocarbon core. Bobone et. al. [Biochemistry, 2012] also showed that MP ability to disengage fluorescent marker from liposomes made from di(C16:1)PC, di(C18:1)PC, di(C20:1)PC depended on the membrane thickness. The study was supported by the Russian Foundation of Science (19-14-00110).

P-03.3-16
Combination of NMR spectroscopy with MD simulation in detailed elucidation of the interaction of cytochrome P450 3A4 with microsomal cytochrome b5 in membrane mimetic environment

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Cytochrome P450 3A4 (CYP3A4) is the monooxygenase enzyme which bound to the membrane of the endoplasmic reticulum (ER). CYP3A4 is the most important human P450 as it has a significant role in the metabolism of xenobiotics, including drugs (metabolizes more than 50% of substances available on the pharmaceutical market). It was shown that microsomal cytochrome b5 (b5) has a stimulating effect on hydroxylation reactions

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catalyzed by CYP3A4. In turn, b5 is also anchored in the ER by C-terminal fragment. However, truncated variant of b5 (trb5) has an inhibitory effect on CYP3A4 hydroxylation as shown *in vitro*. Currently, there is no understanding of the precise mechanism underlying the stimulating role of b5 on P450 catalysis. To solve this problem, it is necessary to study in more details structural-dynamic characteristics of all components of the cytochrome P450-dependent system and the complexes between them at the atomic level under conditions close to natural. In this work, we have performed a comparative analysis of the interaction of CYP3A4 with b5 in membrane environment using heteronuclear NMR spectroscopy and molecular dynamics simulation. Experiments on the chemical shifts perturbation of resonances in 1H-15N HSQC spectra were used to determine the interaction interface between CYP3A4 embedded in peptide based nanodisc (CYP3A4-ND) and trb5. Molecular dynamics simulation was performed using AMBER lipid14 and ff14SB force fields in explicit POPC bilayer and in explicit water environment. NMR and MD are in a good agreement in the case of CYP3A4 and trb5 interaction interface determination. The interaction interface is located near the proximal region where the binding site to NADPH-cytochrome P450 reductase is also located. In the analysis of MD in the case of membrane-bound b5 the interactions with the proximal side are not detected, but occurs near the β-sheet. The reported study was funded by RFBR and BRFBR, project number 20-54-00041/X20P-159.

P-03.3-17
A pilot study of fresh wheat grass juice intestine absorption by Caco -2 model *in vitro*: Fe, Zn and Mg absorption for a vivid cell

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Wheatgrass – WG (*Triticum aestivum*) known as "living food" possess a variety of biological activities. It is rich source of proteins, vitamins, antioxidants, and minerals. Wheatgrass is a common health supplement, consumed in the form of fresh juice, powder or tablet to boost up the health and vitality. Since most of supplements are orally ingested, big question is active concentration of components absorbed cross the intestinal barrier. Caco-2 cell system is intestinal in vitro model which enables evaluation of compound transport mechanism and ability to pass through enterocyte membrane. Permeability values estimated with Caco-2 model correlate well with human *in vivo* absorption. The aim of this study is to establish cultivation conditions for Caco-2 model to determinate active concentrations of three minerals (Mg, Zn, Fe) indispensable for cells and enzyme activity. Five variety of WG were germinated over 12 days in controlled conditions (tem. 20°C, day and night light (10/14 h). Samples of WG were centrifuged (3000 rpm/10 min/RT) and applied to cells diluted (1:4) in HBSS/MES buffer. As a standard, commercial WG powder was applied. Samples were collected every 20 minutes (0–100 min) in duplicate and stored at -80°C before analysis. Concentration in time dependant manner were estimated by AAS. Caco-2 model was confirmed by measurement of TEER value for each well on the 19th day of cultivation. Concentration range for Zn, Fe and Mg were respectively as follows: 0 min (117.16; 826.54; 46.3 x103 μg/L), 20 min (93.85; 387.26; 23.13 x103 μg/L), 40 min (44.45; 66.92; 6.85 x103 μg/L), 60 min (29.45; 104.49; 6.54 x103 μg/L), 80 min (15.23; 54.24; 2.79 x103 μg/L), 100 min (6.67; 46.21; 1.75 x103 μg/L). Results indicate passive model of transport for analysed minerals with well notable tendency of reduced rate of absorption through the time. The highest rate of absorption, as anticipated, was in the first 20 minutes pointing to medium speed absorptive compounds.

P-03.3-18
Biophysical and biochemical studies on mitochondrial potassium channels using polymer nanodiscs

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Several potassium channel proteins are located in the inner mitochondrial membrane. They play role in the regulation of mitochondrial respiration, synthesis of reactive oxygen species, and cell death. One of them is the ATP-regulated potassium channel (mitoK$_{ATP}$), which seems to be comprised of the ROMK2 protein. Another channel is the large-conductance calcium-activated potassium channel (mitoBK$_{Ca}$). To investigate these channels, we used a novel approach, in which membrane proteins are extracted by copolymers of styrene-maleic acid (SMA). Commonly, detergents are used for this purpose. However, in detergents proteins often lose activity and their complexes are unstable. This can happen, because necessary for protein function lipids have been stripped away. SMA polymers produce membrane protein-containing particles (SMALPs) without the need for the use of detergents. Therefore, proteins isolated by this method maintain their native lipid and protein environment exhibiting higher stability and activity. Using SMA we carried out isolation of human ROMK2 protein expressed in *Escherichia coli* cells. The channel electric activity, similar to activities of mitoK$_{ATP}$ described previously, was observed after fusion of ROMK2-SMALPs with planar lipid bilayers. The impact of known mitoKATP channel modulators, such as ATP/Mg^{2+}, VU591 (ROMK channel blocker), and diazoxide (mitoK$_{ATP}$ opener) on the observed activity was investigated. In addition, we examined the impact of other potential modulators. Previously, it was shown that polymer nanodiscs can be used for the isolation and stabilization of protein complexes. I found that it is possible to solubilize by SMA potassium channels as large SMALPs of megadalton size with mitochondrial proteins. The isolation of these complexes allows for their more detailed biochemical characteristics. This work was supported by Polish National Science Center, grant no. 2015/19/B/NZ1/02794 and 2020/37/N/NZ1/01808.

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P-03.3-19
Membrane environment modulates ligand-binding properties of a bd-type terminal oxidase from *Escherichia coli*

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Cytochrome bd is a terminal oxidase of aerobic respiratory chains of many bacteria including pathogens. The enzyme couples reduction of O_2 by quinol to generation of membrane potential and carries three hemes, b_{558}, b_{595}, and d. Cytochrome bd plays important physiological roles and is a potential drug target. The study aimed at answering the question does the membrane environment affect ligand-binding properties of cytochrome bd from *Escherichia coli*. Recent studies of some membrane proteins suggest that lipid membranes can significantly affect their local structure, dynamics, and activity. There is still a lack of studies on any terminal oxidase in which the molecular mechanism of how the membrane affects its properties would be characterized. In this work, absorption spectroscopy and reconstitution of the enzyme into liposomes were used. Reactions of cytochrome bd as isolated, in intact and detergent-treated bacterial membranes, and azolectin liposomes, with cyanide and carbon monoxide (CO) were compared in detail. In the native membranes, cyanide and CO bind mainly to the high-spin heme d. Upon enzyme solubilization, the ligands begin to react also with part of the low-spin heme b_{558} population. Reconstitution of the isolated solubilized cytochrome bd into azolectin liposomes restores the ligand-binding pattern close to that detected in the intact bacterial membranes: both exogenous ligands no longer bind heme b_{558}. Substantial changes in the Soret absorption spectra caused by the addition of cyanide to the intact membranes are due to reduction of heme b_{558} and heme b_{595} with an endogenous electron donor rather than the ligand binding to a b-type heme. One can thus conclude that the membrane environment modulates ligand-binding properties of cytochrome bd from *E. coli* and possibly other bacteria. Previously published in: Borisov VB (2020) Biochemistry (Mosc) 85, 1603–1612. This work was supported by the Russian Science Foundation (grant 19-14-00063).

P-03.3-20
Regulation of the activity of mitoBK channel by hemin and hydrogen sulfide via new external heme-binding site

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Mitochondrial large-conductance calcium-activated potassium channel (mitoBK$_{Ca}$) plays an important role in cytoprotection during ischemia/reperfusion events. MitoBK$_{Ca}$ is formed by the DEC splice variant of the KCNMA1 gene, which encodes also plasmalemmal BK$_{Ca}$. The activity of mitoBK$_{Ca}$ is regulated by many modulators, including activators such as calcium ions and inhibitors such as heme and its oxidized form hemin. Heme/hemin binds to the heme-binding motif (HBM) containing just two cysteines and adjacent histidine (–CXXCH–) placed between two RCK (regulating conductance of K^+) domains located in the mitochondrial matrix. In this study, we performed patch-clamp experiments on mitoplasts from astrocytoma U-87MG cells and HEK293 cell line stably expressing DEC isoform of BK$_{Ca}$ channel (BK$_{Ca}$-DEC) in the outside-out configuration. In addition, in the inside-out configuration, we used a pipette perfusion system, which allowed for the application of channel modulators to the intermembrane space side of the mitoBK$_{Ca}$. We discovered that hemin applied from the intermembrane space also inhibits the activity of mitoBK$_{Ca}$ both in astrocytoma U-87MG cells and HEK293 cell line stably expressing DEC isoform of BK$_{Ca}$. In addition, we checked whether another metal-substituted protoporphyrin – Mn(III) protoporphyrin IX applied to the intermembrane space inhibits the activity of mitoBK$_{Ca}$ in a manner similar to hemin. We obtained HEK293 cell lines stably expressing BK$_{Ca}$-DEC mutants of cysteines and histidine, located in the intermembrane space side, which could constitute an external heme-binding site. Patch-clamp experiments will be carried out to find out whether introduced mutations affect channel inhibition by external heme. This work was supported by the Polish National Science Center, grant no. 2018/31/N/NZ1/00928.

P-03.3-21
Red blood cells as a source of negatively charged membranes

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Key reactions of the coagulation cascade are membrane-dependent. It's considered that the main sources of negatively charged membranes are activated platelets and microparticles. Recently it was discussed that RBCs can affect plasma coagulation in different ways. In this work, was investigated the ability of factor X activation by intrinsic fX-activating complex in presence of RBCs and the binding of the components of this complex. The study was approved by the institutional ethical committee, written informed consent was given by all participants. Cells were washed to isolate from whole blood. The binding of Alexa-647-labeled factors to the membrane was determined by flow cytometry. Chromogenic assay with fXa-specific substrate S-2765 was used to quantify generated fXa. To get phosphatidylserine (PS)-positive cells osmotic shock was used. To conclude the importance of effect the amount of generated fXa in presence of either RBCs, or platelets were compared. In these experiments, it was proportional to the concentration of added cells. The effect of RBCs in concentration 2×10^6 1/μl was similar to the effect of activated platelets in concentration 200×10^3 1/μl. Coagulation factors are predominantly bound to PS-positive RBCs. The quantity of generated fXa in presence of RBCs after the osmotic shock was proportional to the percent of PS-positive cells. We have shown that RBCs can be a good source of negatively charged membranes. This research is supported by grant of the Russian Science Foundation 20-74-00133.

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P-03.3-22
Membrane lipids and cellular water modulate G-protein-coupled receptor rhodopsin activation

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G-protein-coupled receptors (GPCRs) are membrane proteins that mediate cellular signal transduction. They exist as dynamic conformational ensembles with multiple inactive and active conformations. We used the archetypal GPCR rhodopsin to investigate how membrane lipids and cellular water modulate the conformational dynamics of these structural ensembles through GPCR activation, hypothesizing a flexible surface model. Rhodopsin in its native membrane and POPC only membrane vesicles were exposed to various osmotic pressures by incubating in buffered polyethylene glycol (PEG) solutions. The shifts in Metarhodopsin equilibrium due to the change in the lipid environment and osmotic pressure were probed using UV-Visible spectroscopy. Our results show a flood of ~80 water molecules into the rhodopsin interior during photoactivation, forming a solvent-swollen Meta-II active state [1, 2]. POPC recombinant membranes shift the equilibrium towards the inactive Meta-I state compared to the native membrane environment. The analysis of transducin peptide-binding isotherms reveals that the binding affinity of the peptide is significantly decreased when the lipid environment is changed from the native to complete POPC. We also performed a series of experiments to study how the binding affinity of the transducin C-terminal peptide to the Meta-II state is affected by the osmotic pressure in recombinant membranes. The effect of POPC lipids on the metarhodopsin equilibrium can be explained using the flexible surface model. The POPC lipid membrane has a zero-membrane curvature that shifts the equilibrium towards the more compact, Meta-I state compared to the native lipid membrane environment that has a negative curvature that favors the more expanded state of Meta-II. Thus, our results delineate the crucial role of soft matter in regulating the metarhodopsin equilibrium. [1] Chawla U et al. (2021) Angew Chem Int Ed 60, 2288–2295. [2] Weerasinghe N et al. (2018) Biophys J 114, 274a.

P-03.3-23
Role of ABCC6 in PXE and cancer therapy

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ABCC6 is a membrane transporter whose mutations cause the *Pseudoxanthoma elasticum* (PXE), a complex autosomal recessive disease characterized by ectopic mineralization of soft connective tissues. Although the mechanism of transport of ABCC6 is not completely clear, it is accepted that ABCC6 mediates the efflux of ATP, which is further hydrolyzed in AMP and PPi, a strong inhibitor of mineralization. Previous studies revealed that knockdown of ABCC6 in hepatoma-derived HepG2 caused an alteration in NT5E and TNAP expression[1]. Both enzymes are involved in the subsequential metabolism of extracellular ATP and with their activity modulate the balance between inorganic phosphate and pyrophosphate. In a recent study, we have found that silencing of ABCC6 or its pharmacological inhibition with probenecid in HepG2 cells caused a rearrangement of actin filaments in the cytoskeleton and a decrease in migration rate by reducing the efflux of ATP, thus suggesting the hypothesis that ABCC6 could be a target in cancer therapy[2]. A growing number of evidences are there that flavonoids can modulate the activity of many ABC transporters. We studied the effect of the prototypical flavonoid, quercetin on cell motility. The preliminary results suggest that ABCC6 activity was modulated by the oxidative stress. Previously published in: 1. Martinelli F et al. (2018) Front Mol Biosci 5, 75. 2. Ostuni A et al. (2020) Cells 9, 1410.

P-03.3-24
Response of blood cell lines to a Listeriolysin O mutant Y406A

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Listeriolysin O (LLO) is a toxin from the intracellular pathogen *Listeria monocytogenes*, which forms pores in cholesterol-rich lipid membranes of host cells. LLO has pH optimum at pH 5.5, a condition found in late endosomes, while also at neutral pH it can bind to the membrane and form pores, and damage cells. LLO mutant protein Y406A with specific activity at acidic pH that could be interesting for the applications in medicine and biotechnology was generated by our group. Mutant Y406A with substitution (Try to Ala) at the site 406 is able to bound to membranes and oligomerized similarly to the wtLLO, but the final membrane insertion step requires acidic pH. Mutant Y406A has pH optimum at pH 5–6. The cytotoxicity and the release of extracellular vesicles (EVs) was used to examine the response of the cells to LLO and its mutant Y406A. The effects of wtLLO and Y406A were tested on myelogenous leukemia cell line (K562), T lymphocyte cell line (Jurkat), and B lymphocyte cell line (Raji). The cytolethal concentration of wtLLO was between 10 nM for K562 and 0.1nM for Jurkat cells. Vesiculation level was increased at cytolethal concentration and at 10-fold lower concentration than cytolethal. At 100-fold lower concentrations than cytolethal, the effect was reversed and cells shedding less EVs than control cells. On the other hand, the viability of cells was not affected after treating with Y406A at neutral pH, even at high concentrations of protein, while at pH 6.0 Y406A showed almost the same citotoxicity as wtLLO at pH 7.4. However, a low pore-forming activity was detected at higher concentrations of Y406A (100 nM) at neutral pH. Besides, the level of EV secretion was slightly increased at higher concentrations of protein (100 nM) at neutral pH. Mutant Y406A is significantly less toxic than wtLLO under physiological conditions and becomes toxic under acidic conditions; this makes it a potential candidate for stimuli responsive applications and cancer treatment.

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New approaches in structure determination

P-04.1-01
Structural characterization of the Forkhead box O4 (FOXO4):p53 complex

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The forkhead box O (FOXO) transcription factor plays an important role in cell cycle regulation, metabolism, stress resistance and longevity of mammals. In human, FOXO protein family consists of four members: FOXO1, FOXO3, FOXO4 and FOXO6 which share a highly conserved DNA-binding domain called the forkhead box domain. They are expressed in all mammalian tissues and their signal is regulated by the interaction with other proteins and post-translation modification including phosphorylation, acetylation and ubiquitination. In many human cancers, FOXO4 has been considered as a tumour suppressor and involved in the interaction with several proteins including p53 to regulate apoptosis, cancer and age-related diseases. The tetrameric p53 protein is involved in the regulation of more than 500 target genes in the biological network. Recently, it has been reported that upon ionizing radiation (IR) damage, FOXO4 interacts with p53 which promotes cellular senescence over apoptosis and maintains the senescent cell viability by blocking p53 mediated apoptosis. However, the molecular details of FOXO4: p53 interaction remain unclear due to the absence of structural data. Therefore, the main aim of this project is to investigate the structural basis of the FOXO4:p53 complex. For that reason, sedimentation velocity analytical ultracentrifugation (SV-AUC), 2D 1H-15N HSQC NMR spectroscopy, chemical cross-linking coupled to mass spectroscopy and molecular docking experiments were conducted to obtain the molecular insight of the FOXO4: p53 complex with various constructs of FOXO4 and p53. Experimental data suggest that both proteins interact with the binding affinity in the micromolar (μM) range and that the N-terminal segments, as well as other regions of FOXO4-DNA binding domain are involved in the interaction with p53. This work was supported by the Charles University Grant Agency (project: 251203).

P-04.1-02
NMR-based oligomerization analysis of EBV latent membrane protein 1 reveals the mechanism of its inhibition by pentamidine

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The Epstein-Barr virus (EBV) is a hidden threat, present in 90–95% of the World's population. This virus may asymptomatically infect B-lymphocytes and some other cells to further induce different types of blood cancer. Latent membrane protein 1 (LMP1) is a gene product of EBV, which was shown to play a key role in EBV-associated malignancies, causing the immortalization and transformation of infected cells. Therefore, LMP1 is a prospective target to cure EBV-caused cancers. According to recent investigations, this protein is active only in oligomeric form. Several potential inhibitors able to disrupt the LMP1 oligomers were discovered, including the antimicrobial drug pentamidine. The 5th transmembrane domain (TMD-5) mediates the self-association of LMP1. TMD-5 contains an aspartate residue, D150, which was demonstrated to be critical for oligomerization. In this work, TMD-5 is used as a simplified model of LMP1 to examine its oligomers and the effect of pentamidine. First, we found that pKa of D150 is equal to 7.3 and studied the protonated (uncharged) and deprotonated (charged) forms of TMD-5 in DPC micelles. An NMR-based technique to treat the complex oligomerization equilibria was developed and applied. According to this method, the uncharged form of TMD-5 associates into dimers and trimers, while the deprotonation of D150 induces the high-order oligomerization of the protein and enhances dramatically its trimerization. Pentamidine interacts mainly with the charged TMD-5, destroying the high-order oligomers and stabilizing the monomer and trimer. The details of interaction between pentamidine and TMD-5 were also investigated by MD simulations. Our data suggest that D150 is charged in full-length LMP1 under native conditions. The obtained results and developed methods facilitate and encourage further examination of transmembrane drugs disrupting lateral protein interactions. The work is supported by the Russian Science Foundation, grant No.19-74-30014.

P-04.1-03
The first cryo-electron microscope facility in Slovenia to enable cutting edge research in the broader region

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Cryo-electron microscopy (cryo-EM) field has experienced fast development and significant technical improvements in the last decade. Perhaps the most important have been advancements in sample preparation, direct electron detectors and data processing, as well as data analysis, for which Nobel prize in Chemistry was awarded in 2017. Cryo-EM has become an important tool to gain structural insight into cells, organelles, protein complexes, proteins and recently also peptides and small crystalline molecules. At atomic or near-atomic resolution cryo-EM offers a unique combination of features. Through cooperation of multiple partners, the first cryo-EM microscope was acquired at the end of 2019 and is now installed at our Institute. 200 kV automated cryo-EM microscope "Glacios" from Thermo Fisher Scientific will enable researchers in Slovenia and also broader south-eastern EU region to accelerate their research and become more competitive in the international environment. This microscope stands out as it is one of the first cryo-EM microscopes in the region that offers the possibility of electron diffraction on microcrystals. This cryo-EM will enable researchers to test and screen many different samples but also perform the more and more important sample optimization for final data collection. It will enable atomic resolution data collection and also serve as a stepping-stone to apply for data collection at the high-end cryo-EM microscopes abroad. Cryo-EM facility, and the infrastructure that is growing around this instrument, will enable researchers to prepare their samples

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for cryo-EM, collect data and also analyze the data. The facility is open to all academic users as well as industry. Current state of this facility will be presented and the options it offers to researchers from various fields. Selected examples of data from our microscope will be presented to inform potential users how they can benefit from using the tools that our facility provides.

P-04.1-04
Crystallization of photosynthetic reaction centers and fixed-target serial crystallography data collection with the Roadrunner

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Photosynthetic reaction center (RC) of the bacterium *Rhodobacter sphaeroides* is a large (about 100 kDa) membrane pigment–protein complex. It participates in the initial stages of the photosynthesis process. Reaction centers are composed of three protein subunits and several pigment molecules. They convert solar energy into chemical bond energy through a series of electron and proton transportation. To date we have many crystal structures of *Rba. sphaeroides* RCs in a static state, but we do not have information about spatial configurations of intermediate states of these complexes. Obtaining structures of intermediates requires use of time-resolved serial crystallography. Serial crystallography demands a lot of small and high-quality crystals. It is often a challenging task for membrane proteins that usually do not crystallize well. We have found mesophase crystallization conditions that allowed us to get crystalline samples suitable for serial crystallography experiments. Successful experiments were conducted at the PETRAIII synchrotron. We obtained good-quality crystal structures both in cryogenic conditions (up to 1.8 Å) and at room temperature (up to 2.1 Å) using fixed-target sample delivery with micro-patterned silicon chips. Also, these crystals were successfully tested at the European X-ray free electron laser (EuXFEL). Smaller chips, suitable for standard goniometers, were used at the synchrotron, while larger chips were used with the Roadrunner goniometer at EuXFEL. This work was financially supported by the Russian Foundation for Basic Research (18-02-40008_mega).

P-04.1-05
Comparison of two methods for pre-crystallization buffer optimization – differential scanning fluorometry (DSF) and solubility test

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Introduction: Protein crystallization depends on several factors like protein concentration, precipitant type and concentration, temperature, pH, etc. Since crystallization drops are composed of mother liquid from the reservoir and protein solution, they both have an impact on the crystallization process and the quality of observed crystals. In the last 13 years there are many papers describing methods for optimization of pre-crystallization buffer, the buffer in which the protein is before setting up the crystallization drops. Mostly used are differential scanning fluorometry (DSF or so called Thermofluor) and solubility test. The result of each method is the list of best buffers to use but unfortunately both lists do not have that much in common we would expect. Methods: Differential scanning fluorometry is the method where fluorescent dye is added to the protein solution at room temperature or lower. The fluorescence is followed by RT-PCR. After rising temperature for each degree °C the fluorescence is measured. Fluorescent dye is not fluorescent in hydrophilic environment (folded proteins) and with temperature when proteins are unfolding, the hydrophobic areas are exposed and the fluorescence is rising up. Calculating the Tm for each experimental condition and comparing them gives the information about the protein thermal stability. Solubility test is the screening method for defining conditions where proteins are most soluble. First the protein is reversibly precipitated and then solubilized back in different conditions. Measuring the concentration of soluble protein is providing the information on good experimental conditions for protein crystallization. Results: Using both methods on the same protein we were trying to define the decision three. The results are not strait forward. The simple decision tree is not possible. Nevertheless, we were able by scoring combine the results of both methods which resulted in successful protein crystallization.

P-04.1-06
Inhibition of *Helicobacter pylori* purine nucleoside phosphorylase and bacteria cell cultures with 2-chloro-6-substituted purines

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Since its discovery in 1982, *Helicobacter pylori* was proven to be involved in development of several gastro-intestinal diseases, including chronic gastritis, peptic ulcer disease, gastric cancer and MALT lymphoma. It is estimated that over half of the world's population is infected with this pathogen. Although it colonizes extremely hostile acid environment of human stomach, this bacterium has developed ways to persevere - by adapting pH of its environment, burrowing into the mucus lining of the stomach, and employing high rate of mutations to develop antibiotic-resistant strains. Thus, there is a growing need for new drugs that could eradicate this pathogen. *H. pylori* lacks *de novo* purine synthesis genes and relies solely on the salvage pathway for acquisition of purines. This makes the enzymes of the salvage pathway promising targets for the development of new drugs. One of these enzymes is purine nucleoside phosphorylase (PNP), which catalyses the reversible phosphorolytic cleavage of the glycosidic bond of purine nucleosides in the presence of orthophosphate as a second substrate. We have previously produced *H. pylori* PNP in *E. coli* cells, characterized it kinetically and biochemically, and

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solved its 3D-structure. Here we present the results of inhibition studies of this enzyme. Several compounds were tested as potential inhibitors of *H. pylori* PNP. The highest inhibition levels were obtained with 2-chloro-6-substituted purines. Therefore, for these compounds detailed kinetic experiments were performed, which revealed that they inhibit *H. pylori* PNP through the mixed type mechanism with inhibition constants in low μM range. Complexes of enzyme with these inhibitors were crystallised by vapour diffusion method, X-ray diffraction data were collected at synchrotron (Elettra, Trieste, Italy) and 3D-structures solved by method of molecular replacement. The minimal inhibitory concentrations (MIC) of the best inhibitors vs. H. pylori 26695 strain were also determined.

P-04.1-07
The complexity of protein quaternary structure

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A large fraction of proteins possesses a quaternary structure, which often is an assembly to multiple copies of themselves. Functional, genetic and physicochemical needs are the driving force of the evolutionary selection of oligomeric complexes. The quaternary structure of a protein is determined by structural methods, most often, by X-ray crystallography, which requires high protein concentration and unique buffer composition to drive crystallization. As the equilibrium between different quaternary forms is concentration dependent, the registered quaternary subunit composition in the PDB or Uniprot is a reflection of the specific conditions of the structural method. Recent method developments provide us with the opportunity to re-evaluate the oligomeric state using numerous new methods on multiple proteins. We analyzed the oligomeric state of 17 different bacterial proteins by native mass-spectrometry (MS), mass photometry (MP), size exclusion chromatography, and small-angle x-ray scattering (SAXS). The results were compared to the entry in the PDB, SWISS-MODEL, Uniprot and PISA. Native MS and SAXS measurements were done in a range of protein concentrations, which enabled us to follow the changing equilibrium between the different oligomeric forms. Overall, we found that for all proteins at least some change in oligomeric composition was observed with varied concentrations. While these changes were small for some proteins, for others a major shift in the oligomeric form was recorded. For at least half the proteins, the registered oligomeric form was different from the one we identified in solution. Therefore, to rely on the structure to determine the oligomeric composition of the protein under-estimates the complexity of the protein in solution. This work demonstrates, that together with structure deposition, an additional small effort should be done to determine the quaternary structure in solution, and that good and approachable methods now exist to do this.

P-04.1-08
Single-molecule FRET studies and FRET-nanoscopy shed light on dynamic and structural properties of hGBP1

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Although the structure of proteins is the basis for their function, most modern methods used to obtain these structures are not using data from physiological conditions or even dissolved states, ignoring possible dynamics the protein might have in solution. One such protein, where the dynamic structure is important, is the human guanylate-binding protein 1 (hGBP1). It acts as a part of the cell's defence mechanism against viral and microbial attacks and can be posttranslationally modified with a farnesyl moiety.To further investigate the dynamic structure of hGBP1, we chose Förster resonance energy transfer (FRET) based methods. Acquisition of a more accurate dynamic structure of the non-farnesylated hGBP1 in solution required specifically labelled samples, and these were designed to form a "FRET-network". By using the distances and dynamics obtained from this "FRET-network" with single molecule FRET (smFRET), the possible movement for hGBP1 in solution could be simulated. Through the addition of more data and an extended FRET network to previous work, it was possible to refine the observed second conformation of hGBP1 monomer. Additionally, the polymers formed by farnesylated hGBP1 were investigated with a new method, which combines the benefits of stimulated emission depletion (STED) and FRET microscopy, to learn about the polymer and underlying protein structure under in vitro conditions. It could be shown that hGBP1 forms 3D macrostructures out of fibres with nucleotide analogue GDP-AlF_x. The chosen specifically labelled sample for these experiments showed a high FRET population as a monomer and even though no FRET was observed within the polymer, it was possible to extract information regarding the opening of the protein inside of the polymer through the hybrid FRET-nanoscopy approach, using the localization of the dyes to approximate the distance between donor and acceptor.

P-04.1-09
The blue protein from *Rhizostoma pulmo*: purification and some properties

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There is a wide range of coloration among marine creatures. One of them, jellyfish *Rhizostoma pulmo*, displays blue color due to the chromoprotein rpulFKz1 (Previously published in: M. E. Bulina et al. (2004) The Journal of Biological Chemistry 279, 43367–43370). The chromoprotein has an absorption spectrum with maxima at 420, 588 and 624 nm. It was found that rpulFKz1 contains a Frizzled cysteine-rich domain and a Kringle domain. These structures were never found in chromoproteins before. The aim of this work was to develop a purification

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method and to find out the conditions for rpulFKz1 crystallization. The purification scheme includes precipitation with ammonium sulfate, an ion-exchange and gel-filtration chromatography. LC-MS data and spectrophotometry showed that the purified protein is identical to rpulFKz1. The denaturing electrophoresis displays the band 32 kDa. Glycosylation was found after in-gel treatment with periodate and Schiff's reagent. Elution volume in gel-filtration chromatography corresponds to the dimeric forms. The optimal buffer conditions were found via thermal shift assay for pre-crystallization of rpulFKz1 protein. Additional spectroscopic experiments of proteolysis presumably led to the absorption spectrum of the released chromophore with the maximum of 326 nm. This work was supported by the President grant for leading scientific schools LS-2605.2020.4. *The authors marked with an asterisk equally contributed to the work.

P-04.1-10
Structural features of Cas9 protein complex with RNA-DNA duplex based on the HDX-MS technique

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Currently, the CRISPR-Cas9 system is widely used for genome editing. The establishment of detailed mechanisms of selectivity and specificity of target DNA recognition and cleavage by the CRISPR-Cas9 system is necessary both for fundamental science and for modern molecular medicine. We used the combination of computer modeling methods with hydrogen-deuterium exchange followed by mass-spectrometry. The first step of current work was computer modeling of Cas9 protein structure. We used modeling based on homology to obtain native protein structure (4CMQ, PDB as a reference) followed by structure editing in UCSF Chimera 1.14 to obtain Cas9 complex with RNA-DNA duplex. Complete system consists of Cas9 protein, RNA (103 nucleotides), DNA (33 nucleotides) and 3 Mg^{2+} ions. To test the system stability, we performed molecular dynamic simulations in implicit solvent model during 10 ns. The system stays relatively stable during all dynamics. It has to mentioned, that two Mg^{2+} ions migrated, which not influence on general system stability, as seen from RMSD plot. The next step is HDX-MS experiment. Firstly, we made a peptide map of Cas9 protein. After that the HDX-MS experiment was performed to determine native protein structure based on peptide map. The third experiment is Cas9-RNA complex hydrogen exchange reaction, that helps us to understand which peptides are involved in protein-substrate complex formation. The last step is Cas9-RNA-DNA complex HDX-MS analysis. This step gives us an information about the protein regions that takes part in interaction with DNA. Such step-by-step experiment procedure allows building the complete complex structure. As a result of theoretical and experimental data comparison, we obtained Cas9 protein structure and endonuclease-RNA-DNA complex structure in solution. This is an important step in studying the molecular mechanisms of the CRISPR-Cas9 system. This work was supported by grant from RSF (20-14-00214).

P-04.1-11
Characterizing conformational changes of proteins using switchSENSE®

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Conformational changes are at the heart of macromolecular interactions. A protein's three-dimensional structure often directs its function, activity and interaction behaviour. Vice-versa, external signals such as effector binding or variations of environmental conditions frequently trigger conformational changes. This mutual interplay of structure and function leads to complex signaling cascades which play a vital role in the biological context – defective conformations can result in severe diseases. Therefore, it is important to understand these structure-function relations thoroughly. Here, we introduce a novel method for the *in vitro* study of protein structure and interaction kinetics in physiological conditions, combining switchSENSE® with DNA Origami nanotechnology. This technique convinces with low sample consumption and a highly sensitive readout. Our approach not only allows to characterize interaction parameters (k_{on}, k_{off}, K_D) but also structural measures such as compaction or expansion of a protein by effector binding in a single assay. To illustrate the capabilities of this technique, we present conformational change measurements of the human Insulin receptor (IR). The interaction with the hormone Insulin plays a pivotal role in controlling the human carbohydrate metabolism and glucose homeostasis. The corresponding signaling pathway crucially relies on IR's three-dimensional structure. IR consists of an extracellular alpha and intracellular beta domain. Upon insulin binding both domains undergo a conformational change initiating autophosphorylation and further signal processing. This study revealed that our method is capable of determining conformational changes which agree well with previously reported crystal structure findings. Taken together, the data illustrate the potential of the switchSENSE® DNA origami technology for characterizing protein conformational changes for a wide range of biological applications.

P-04.1-12
The structure of SecA2 from *Clostridioides difficile* reveals differences in target recognition between general SecA1 and virulence factor-specific SecA2 export systems

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Bacterial SecA proteins are the driving force of extracellular protein export. By hydrolyzing ATP, they power the movement of the target protein through the SecY transmembrane channel. Two types of SecA systems exist in bacteria: The general SecA system (SecA(1)) that exports proteins involved in cell wall maintenance, nutrient acquisition and communication with the environment, and a more specialized SecA2 system. SecA2 system exports a different set of substrates, usually involved in bacterial virulence, and is found in some Gram-positive pathogens, like *Clostridioides difficile*. In order to contribute to understanding of the basis for the differential recognition of protein targets by SecA1 and SecA2 systems, we determined and analyzed the

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crystal structure of SecA2 from C. difficile (CDSecA2). Using X-ray crystallography and molecular replacement, structures of the whole length CDSecA2 in apo and ATP-bound form were determined. They revealed a closed monomer with multidomain organization similar to its homologs, and conserved regions for ATP binding. We confirmed the CDSecA2 enzymatic activity using ATPase assay. Modeling combined with structure-based sequence alignments and conservational analysis revealed, firstly, that the movement of CDSecA2 domains affects the conformation of the target protein binding site, and secondly, that an open conformation is necessary for CDSecA2 dimerization and SecY channel binding, ensuring target protein movement through the SecY channel. In addition, we identified differences between SecA(1) and SecA2 homologs that correspond to the adaptation of SecA2 proteins for a specific type of substrate. Our studies may provide the basis for development of SecA2-targeting therapies interrupting the virulence factors secretion pathway. Previously published in: Lindič et al. (2020) IJMS 21(17), 6153.

P-04.1-13
Human NEIL2 protein structure obtained with combination of computer modeling approaches and HDX-MS technique

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The repair system helps us to survive, despite tens of thousands of DNA damage that every cell in our body experiences daily, both under the influence of external causes and due to inevitable biochemical processes. One of the important ways of repair is excisional repair of the bases. BER begins with the fact that the damaged base is recognized and excised from DNA by one of the enzymes belonging to the class of DNA glycosylases. Then several more proteins act sequentially, and as a result, a normal DNA base replaces the damaged base. Human NEIL2 protein has DNA glycosylase activity towards 5-hydroxyuracil and other oxidized derivatives of cytosine with a preference for mismatched double-stranded DNA. We determined NEIL2 protein structure using combination of computer modeling methods and hydrogen-deuterium exchange followed by mass spectrometric analysis. Such combination allows to define high order structures of biological molecules and has several advantages over other biophysical methods. The most important advantage is that the structure of biomolecules are obtains in solvent. Determination of the protein structure started with homology modeling by Phyre2 webserver. The proposed structure was edited and specified using USCF Chimera software. We used refined structure in molecular dynamics (MD) simulations. During the 100ns MD in implicit solvent model the structure stayed stable. HDX-MS experiments are the next part of current work. Peptide map was determined firstly. After that we performed hydrogen-deuterium exchange reaction of the native protein. The exchange was stopped in certain time points and was frozen immediately. We analyzed samples by mass spectrometry methods. Final stage of the study is computer modeling and HDX-MS experiment data comparison. We defined that theoretical structure and experimental data are in good agreement. This work was supported by grants from the RFBR (19-34-90052) and the RSF (20-14-00214).

P-04.1-14
Preparation and characterization of novel membrane mimetic systems in cryo-EM GPCR studies

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Cryogenic electron microscopy (cryo-EM), a method used to determine structures of protein complexes, is steadily rising in popularity, judging by an increase in the number of resolved protein structures. However, this method remains hardly applicable for G-protein coupled receptors (GPCRs) which are hard to work with due to being unstable after extraction out of their native membrane environment and require special solutions called membrane mimetic systems (MMCs). On top of that, chosen MMCs have to meet requirements of cryo-EM such as being homogeneous and usable in low temperatures. Circularized nanodiscs (cNDs), assembled through the enzymatic circularization by transpeptidase Sortase A, possess homogeneity and thermostability, making them suitable for cryo-EM (Mahmoud L Nasr et al. (2017) Nat Methods 14(1):49–52). Recently there appeared a cryo-EM structure of a GPCR, using these cNDs (Meng Zhang et al. (2021) Nat Struct Mol Biol), proving the relevance of the latter. Moreover, there are cNDs acquired through post-translational processing of membrane scaffold protein (MSP) in *E. coli*, achieved by the introduction of intein fragments into the protein (Jonas Miehling et al. (2018) Chembiochem 2018 Sep 17;19(18):1927–1933). Intein based cNDs share the properties of those assembled through sortase mediated ligation but have an easier and faster purification process. Currently, however, there are no cryo-EM structures of GPCRs in these cNDs. To characterize GPCRs in novel MMCs, the properties of assembled intein-based nanodiscs with A_{2a} such as as thermostability of the model protein, oligomericity and homogeneity were analyzed by thermal shift assay, size exclusion chromatography and negative-stain EM respectively and compared to micelles, classic MSP1D1 nanodiscs and sortase-based cNDs. The work was supported by Russian Science Foundation (project no.19-74-00088). *The authors marked with an asterisk equally contributed to the work.

P-04.1-15
SAXS-based control of microcrystallization for 4D enzymology experiments

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Three-dimensional structure and its dynamic changes are the keys to understanding the protein function. The dose limit of the synchrotron X-ray experiments and the use of frozen samples to avoid radiation damage of protein crystals confine studies of protein motions in course of enzymatic reactions. Time-resolved serial crystallography at near-physiological temperature removes these restrictions. This technique needs samples containing a large number of highly ordered nano- to micron-sized crystals. However, the growth and the characterization of high-quality nanocrystals remain a bottleneck. Microcrystals are obtained by mixing a protein with precipitant solutions in a batch method, for which a microcrystallization phase diagram is needed to

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produce massive nucleation. Crystal growth must be monitored and can be stopped by rapid dilution in a high precipitant buffer at the desired crystal size [1]. Here, we have applied small-angle X-ray scattering (SAXS) to control the lysozyme microcrystallization process. The batch method was used for crystallization. SAXS allowed us to determine the time when the first crystals appear after the mixing of protein solution with the precipitant buffer. The experiment was performed at BioMUR beamline of Kurchatov synchrotron radiation source, Moscow, Russia. The appearance of Bragg peaks in the recorded SAXS scattering profiles indicated the starting point of first crystals formation. The technique can be useful for the preparation of microcrystal suspensions for time-resolved serial crystallography. This work was supported by RFBR grant 19-29-12054. Reference: 1.Kupitz C et al. (2014) Phil.Trans.Soc. B 369, 20130316.

Molecular machines

P-04.2-01
Role of C-terminal domains of yeast FACT complex in nucleosome unfolding

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FACT (facilitates chromatin transcription) is a histone chaperone that is involved in the processes of transcription initiation and elongation, DNA replication and repair [1]. Yeast FACT conducts large-scale ATP-independent nucleosome unfolding [2]; but the detailed mechanism of this process is unknown. Here we determined the role of Nhp6 protein and C-terminal domains of yeast FACT complex in nucleosome unfolding using single-particle Förster resonance energy transfer (spFRET) microscopy, EMSA and FRET-in-gel methods. Nhp6 protein interacts with the C-terminal domains of Spt16 and Pob3 subunits of yFACT, inducing unfolding of FACT in the absence of nucleosomes. Analysis of yeast FACT interaction with nucleosomes and hexasomes suggests that Spt16 subunit of FACT drives nucleosome unfolding, while Pob3 subunit is required for complete unfolding. Thus, FACT-dependent nucleosome unfolding is a tightly coordinated process that requires Nhp6 protein and C-terminal domains of Spt16 and Pob3 subunits of yFACT. Our data suggest a detailed model of FACT-dependent nucleosome unfolding. This work was supported by the Russian Science Foundation grant 19-74-30003. Previously published in: [1] K Gurova et al (2018) BBA – Gene regulatory Mechanism 1861, 892–904. [2] ME Valieva et al (2016) Nat Struct Mol Biol 23, 1111–1116.

P-04.2-02
Some kinetic features of Na,K-ATPase

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The following study assesses the comparative kinetic analysis of albino rat brain synaptosomal membrane and kidney microsomal fraction Na,K-ATPase, which helps us to understand different sensitivity of this fractions to neurotransmitter. It should be acknowledged that synaptosomal membrane Na,K-ATPase is sensitive to noradrenaline. This neurotransmitter inhibits enzyme system and changes catalytic cycle from Mg-ATP dependent ([Mg-ATP]>>[Mg^{2+}f]=[ATPf]) to Mg^{2+} dependent ([Mg^{2+}f]>>[ATPf]) cycle. These cycles have different stoichiometry for Na^+ and K^+. The stoichiometry of Mg-ATP dependent cycle is: 3Na^+:2K^+:1Mg-ATP, while the stoichiometry of Mg^{2+} dependent cycle is the following: 4Na^+:1K^+:1Mg-ATP. Kidney microsomal fraction Na,K-ATPase is not sensitive to noradrenaline. To investigate the mechanism, underlying this difference, we have studied the enzyme velocity dependence upon the concentration of Mg^{2+}. V=f(Mg^{2+}) function has shown different kinetic features for synaptosomal membrane and kidney microsomal fraction Na,K-ATPase. Adding EGTA to the reaction medium, effects the geometric form of V=f(Mg^{2+}) function differently. We have measured essential activator and full inhibitor numbers for Na^+ and K^+ with the surplus of Mg^{2+}, which resembles Mg-ATP cycle with the following stoichiometry: 3Na^+:2K^+:1MgATP. According to the results of this experiments it can be concluded, that kidney microsomal fraction Na,K-ATPase does not possess Mg^{2+} dependent cycle and thus cannot manifest sensitivity towards noradrenaline.

P-04.2-03
Structure comparison of two ketose 3-epimerases from *Shinella zoogloeoides* which are capable of D-allulose production

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D-Allulose, one of rare sugars is focused for human healthcare due to its functions such as moderating a blood glucose level and fat accumulation. For D-allulose production, various plausible enzymes have been reported, D-tagatose 3-epimerases from *Pseudomonas cichorii*, *Rhodobacter sphaeroides*, and *Sinorhizobium* sp., D-allulose 3-epimerases from *Agrobacterium tumefaciens*, *Agrobacterium* sp., *Clostridium bolteae*, *Clostridium cellulolyticum*, *Clostridium scindens*, *Clostridium* sp., *Desmospora* sp., *Dorea* sp., *Flavonifractor plautii*, *Ruminococcus* sp., and *Treponema primitia*, and ketose 3-epimerase from *Arthrobacter globiformis*. However only several crystal structures of them were reported. We previously reported the X-ray structure of a novel ketose 3-epiemrase from *Shinella zoogloeoides* which is a L-ribulose 3-epimerase (SzLREA) but shows the enzymatic activities not only toward L-ribulose but also D-allulose. Based on the genome analysis of *S. zoogloeoides*, we found there are some putative ketose 3-epimerases, and expressed one of them using the synthesized gene.

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The produced ketose 3-epimerase was another L-ribulose 3-epimerase (SzLREB) and showed the enzymatic activities not only toward L-ribulose but also D-allulose. The sequence identity between SzLREA and SzLREB is 41.6% and there is a significant difference in thermal stability between them. The optimum temperature of SzLREA and SzLREB is 80°C and 60°C, respectively. To understand the reason of the different thermal stability between two L-ribulose 3-epimerases in *S. zoogloeoides*, we determined the X-ray structure of SzLREB. In this study, we compared the X-ray structures of SzLREA and SzLREB. In SzLREA, there are some additional salt bridges and hydrogen bonds stabilizing the dimer interface, resulting in stabilizing tetramer structure.

P-04.2-04
Atomic-level investigations of the PSD-95 GK domain and its interaction with GKAP

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The postsynaptic density (PSD) is an elaborate network of proteins beneath the postsynaptic membrane. Understanding the 3D organization of the PSD is crucial in deciphering the mechanistic molecular events behind learning and memory, as well as a number of neurological disorders. Our work aims at detailed characterization of the structural changes accompanying the binding events between PSD-95 and GKAP, two of the most important scaffold proteins in the PSD. We have expressed a segment of the GK-binding region of GKAP, which is intrinsically disordered, as well as the globular GK domain of PSD-95. Their interaction was characterized in detail using biochemical and biophysical methods like pull-down assay and biolayer interferometry. The GK domain has been successfully expressed in 15N, 13C-labeled form and triple-resonance spectra have been obtained for NMR resonance assignment and for further structure and dynamics studies.

P-04.2-05
Sodium binding in cysteinyl leukotriene G protein-coupled receptors

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G protein-coupled receptors (GPCRs) recognize a large variety of extracellular signaling molecules, transmitting their signals across the cellular membrane to initiate specific intracellular responses. GPCRs play a key role in human physiology and represent targets for more than 30% of therapeutic drugs. Functional studies of GPCRs shed light on fundamental understanding of their mechanism of action and provide insights for the development of more efficient treatments. An important player in the class A GPCR signaling is the sodium ion, that binds in a conserved allosteric site and stabilizes the inactive receptor state. The focus of this work is on studying the differential effect of sodium ions on two Cysteinyl Leukotriene receptor subtypes, CysLT1 and CysLT2, structures of which have been solved recently (Luginina et al. (2019) Sci. Adv. 5, eaax2518, Gusach et al. (2019), Nat. Commun. 10, 5573). Interestingly, in the CysLT1R structure the sodium ion was found to be unusually tightly coordinated directly by four residues, two of which are acidic, while in CysLT2R, its nearest homologue, no sodium was observed in the pocket. To study sodium binding in Cysteinyl Leukotriene receptors we used protein thermal shift analysis (Alexandrov et al. (2008) Structure 16(3), 351–359). We obtained that sodium ions specifically stabilize CysLT1R, increasing the receptor melting temperature by 8°C. Meanwhile, the temperature shift is significantly diminished for the sodium binding site mutants. In CysLT2R sodium has no stabilizing effect on the protein. Based on the obtained results we suggest that the two receptors have distinct signaling patterns. The work was supported by Russian Science Foundation (project no.19-74-00088).

P-04.2-06
An integrative approach to study the structure and dynamics of the ribosome-inactivating protein Trichobakin

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Plant ribosome-inactivating proteins (RIP) are an extensive class of N-glycosidase enzymes that are necessary for protection against pathogens. They are also promising objects for the usage in biomedical developments since they exhibit antitumor, immunomodulatory and antiviral activities. Trichobakin (TBK) is a recombinant type-I RIP with very high inhibitory activity in the cell-free protein synthesis system (IC50: 3.5 pM). Thus, TBK can serve as a template for the creation of highly effective antitumor recombinant toxins. The goal of this work was the determination of the structure and dynamics of the TBK polypeptide chain with usage of the methods of integrative structural biology for the elucidation of the determinants responsible for the key steps towards achieving the resulting reaction, which include the process of penetration into the cell, release from endosomes into the cytosol, interaction with ribosomal proteins and specific recognition of the α-sarcin/ricin loop. We have determined the TBK solution structure by the NMR spectroscopy and the structure in crystalline state by the X-ray diffractions. In addition, small-angle X-ray scattering data were used for structures validation and comparison. To elucidate critical local region of the structure involved in potentially important conformational transition we used the NMR relaxation rates and heteronuclear NOE measurements of amide groups with model-free analysis approach. The data obtained indicate the possibility of conformational transition on the micro-millisecond time scale at the region of the C-terminal domain of TBK and are consistent with the results of the analysis of the trajectory of the accelerated molecular dynamics simulation on the microsecond time scale. The reported study was performed under the EAPR Call and funded by RFBR and BRFBR, project number 20-54-81015/X20EA-027.

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P-04.2-07
Nucleotide-bound conformations of the GroEL-GroES1 complex resolved by cryo-EM

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The GroEL-GroES complex is a bacterial protein folding machine working in an ATP-dependent manner. Despite being studied for decades, details of its functional cycle remain under debate. One of the discussed topics is whether the two rings of GroEL function simultaneously (implying the formation of a symmetric "football-shaped" complex) or alternately (through an asymmetric "bullet-shaped" form). Both football- and bullet-shaped complexes were resolved experimentally using X-ray crystallography or cryo-electron microscopy, and the corresponding atomic models are available in the Protein Data Bank. However, the deposited cryo-EM structures of the GroEL-GroES complex are of 7 Å or lower resolution and often with an imposed C7 symmetry, which limits their interpretation. In this work, we have obtained a cryo-EM structure of the bullet-shaped GroEL-GroES complex at 4.3 Å with no symmetry applied. A sample for cryo-EM was prepared as follows: GroEL (1 μM) was incubated with GroES (3 μM) in 50 mM Tris-HCl buffer, pH 7.5, containing 10 mM KCl, 10 mM $MgCl_2$, 3 mM ATP for 20 min. Then, the sample was concentrated 10 times using Vivaspin 500 with 100,000 MWCO PES membrane centrifugal filters. 3 μl of the sample was applied to a glow discharged grid (Quatifoil R1.2/1.3) and vitrified in Vitrobot Mark IV at 4.5°C. Cryo-EM movie stacks were recorded on a Titan Krios electron microscope (Thermo-Fisher) equipped with the direct electron detector Falcon II. 6100 image stacks were collected and further processed in Warp and CryoSPARC. 95208 particles were selected for the final symmetry-free 3D reconstruction. The local resolution of the equatorial domains reached 3.5 Å, which allowed us to distinguish extra densities in the ATP-binding pockets of both rings. The resolution variations also indicate that, compared to the GroES-bound ring, the second ring was more flexible at the apical domains. The authors acknowledge funding from the Russian Science Foundation (grant № 19-74-20055).

P-04.2-08
RNA polymerase collisions with dCas9

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CRISPR-Cas systems serve as powerful tools for genome and epigenome editing and gene-specific transcription regulation. Cas9 is a programmable nuclease that has found its use in numerous in vivo and in vitro applications. It was shown that the catalytically dead Cas9 (dCas9) from *Streptococcus pyogenes* can suppress transcription of the target genes *in vivo* both in bacteria and in eukaryotes, presumably by binding to the DNA template and blocking RNA polymerase progression. Interestingly, the effect of dCas9 strongly depends on its orientation relative to the direction of transcription, suggesting that it may be differently recognized by the moving RNA polymerase. We are investigating the molecular mechanisms underlying these observations. We obtained purified dCas9 protein and several sgRNAs that direct dCas9 to different positions and orientations on a DNA template designed for *in vitro* transcription experiments. We demonstrated that dCas9 specifically impedes transcription by RNA polymerase from *Escherichia coli* at the sites of binding. Surprisingly, this effect did not depend on the orientation of dCas9. We further showed that transcription factors GreB and Mfd may facilitate transcription through dCas9 bound to the template DNA strand. We use a reporter system to determine whether transcription factors may cause asymmetry in dCas9 action on transcription in *E. coli* cells. The results contribute to our understanding of how RNA polymerase interacts with roadblocks during mRNA synthesis. This work was supported by the Russian Science Foundation (20-74-10127). AA is supported by Skoltech Systems Biology Fellowship.

P-04.2-09
Analysis of a putative nuclease associated with the Argonaute protein from *Rhodobacter sphaeroides*

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The Argonaute protein from the alpha-proteobacterium *Rhodobacter sphaeroides* (RsAgo) binds small guide RNAs to recognize complementary DNA targets. In bacterial cells, RsAgo is bound to both small RNAs and small DNAs, with a preference for foreign genetic elements. RsAgo also promotes degradation of plasmid DNA *in vivo*. However, RsAgo itself lacks the slicer activity, suggesting the involvement of additional nucleases. To shed light on the molecular mechanism of target processing, we study a putative nuclease which is located in the same operon with RsAgo in the *R. sphaeroides* genome. This protein has motifs characteristic for the PD-(D/E)XK superfamily of nucleases. Numerous attempts to express the wild-type nuclease gene in *Escherichia coli* were not successful due to a low level of expression and low solubility of the recombinant protein. A codon-optimized sequence of the nuclease gene was cloned and successfully overexpressed in *E. coli*. Different expression vectors were used to produce histidine-tagged nuclease and a fusion protein with a chitin-binding domain, which allowed to obtain highly pure nuclease preparations. Initial assays did not reveal nuclease activity in these proteins. However, it was shown that co-expression of nuclease with the RsAgo protein in *E. coli* significantly increases the yield of RsAgo and changes the spectrum of genes preferentially targeted by RsAgo. We plan to further determine the role of nuclease in DNA processing in bacterial cells and test its interactions with RsAgo *in vitro* and *in vivo*. This work was supported in part by the Russian Science Foundation (grant 19-14-00359).

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P-04.2-10
How can we explain the activation of TREM2 associated with microglia diseases in cancer cells? CHIR 98014 inhibits the activation of a subset of p53-regulated genes

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In the last century, the discovery of the p53 protein set a new direction in understanding of the machinery of cancer cells. It appears that p53 may also play a key role in signaling pathways associated with immune system, not only in oncology. We discovered that p53 can be strongly activated in a cell due to the synergistic action of two substances: actinomycin D and nutlin-3a (A+N) and as a consequence, we observed the activation of many signaling pathways dependent on this stimulation. Unexpectedly, we found that the drug combination A+N induced the expression of several elements of TREM2 pathway in cancer cell lines of different origin. This signaling pathway functions mainly in cells involved especially in microglial cells, hence its appearance in lung cancer or osteosarcoma cells exposed to A+N was very surprising. Interestingly, the induction of TREM2 pathway genes was significantly impaired by CHIR98014, which is believed to be a specific inhibitor of the α and β isoforms of glycogen synthase kinase. The aim of this project is to understand how CHR98014 affects cells and how it inhibits p53, leading to the lack of activation of TREM2. We demonstrated that the activity of TREM2 signaling pathway is dependent on the TP53 gene, while the knockdown of GSK3A and GSK3B by the CRISP-CAS9 method did not significantly affect the activation of the TREM2. What is the relevant target of CHR98014? In order to find more broad effect of the inhibitor, we decided to sequence the transcriptome. Surprisingly, we found that CHIR98014 in combination with A+N induces the process of apoptosis. How does the p53 inhibition promote apoptosis? Our research could have a significant impact on the understanding of the poorly studied p53 function in immunology, which could shed light not only on the pathogenesis of cancer, but also on viral and neurodegenerative diseases. This work has been supported by grants no. 2017/27/N/NZ5/01079 to BŁ-S from the National Science Centre, Poland. *The authors marked with an asterisk equally contributed to the work.

P-04.2-11
ISCA1: a late actor of [4Fe-4S] cluster biosynthesis in the mitochondrial ISC assembly machinery

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Iron–sulfur clusters are essential cofactors found in all kingdoms of life; they had unique functional roles throughout evolution. These clusters, that are the second major form of complex iron cofactors in biology, are ubiquitous in all organisms, playing a key role in several biological pathway. In eukaryotic cells the cluster biosynthesis starting point is the iron-sulfur cluster assembly machinery (ISC) located in the mitochondria. This work is focused on the late-acting steps of the ISC assembly machinery that are still elusive, three proteins ISCA1, ISCA2 and NFU1 were shown to be implicated in the assembly of [4Fe-4S] clusters. Using NMR spectroscopy coupled with SEC chromatography we determine a detailed molecular model where ISCA1, ISCA2 and NFU1 assemble and make [4Fe-4S] clusters available to mitochondrial apo proteins. In this study, it was discovered that ISCA1 is the key player of the [4Fe-4S] protein maturation process due to its ability to interact with both NFU1 and ISCA2. ISCA1 is the promoter of the interaction between ISCA2 and NFU1 leading to the formation of a transient ISCA1-ISCA2-NFU1 ternary complex that could be necessary for the cluster transfer to the targeting protein. Exploiting paramagnetic NMR experiments it was also demonstrated that ISCA1, through its specific recognition with the C-domain of NFU1 (containing the cluster binding CXXC motif), mediates a [4Fe-4S] cluster transfer from the assembling site on the ISCA1-ISCA2 complex to a cluster binding site located on ISCA1 and NFU1 in the ternary complex. This mechanism allows the [4Fe-4S] cluster transfer from the ISCA1-ISCA2 complex to NFU1 making the [4Fe-4S] cluster available for mitochondrial apo proteins specifically requiring NFU1 for their maturation. *The authors marked with an asterisk equally contributed to the work.

P-04.2-12
Dependence of plasmid DNA replication on nucleoprotein complexes formation by plasmid replication initiator, TrfA protein

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DNA replication of iteron containing plasmids is initiated by the binding of plasmid-encoded initiator protein (Rep protein) to specific sequences (iterons) within an origin region. The formation of nucleoprotein complexes between the Rep and the double-stranded DNA (dsDNA) results in melting of helix in the region with the lowest thermodynamic stability, DUE (DNA unwinding element). The formed single-stranded DNA (ssDNA) becomes the site where the replisome assembly occurs. There are just a few structures of Rep proteins and their complexes with DNA available. For replication initiator of plasmid RK2, TrfA protein, there was neither structure of the whole protein alone nor in a nucleoprotein complex available. Therefore, firstly by using in silico methods accompanied with biochemical and mass spectrometry analysis, we predicted the structure of the TrfA protein in a complex with DNA. Next, we indicated the amino acid residues of TrfA protein, important for formation of the nucleoprotein complexes within plasmid origin region. Using in vitro analysis of point mutants in these residues, we showed the consequence of disturbances of TrfA–DNA interaction for plasmid DNA replication. Acknowledgements: This work was founded by the Polish National Science Centre grants [2012/04/A/NZ1/00048] to IK and [2017/26/D/NZ1/00239] to KW and

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P-04.2-13
5-Fluorouracil-enriched nanocellulose and pectin biomaterials determined inflammasome complex activation and ROS production in breast cancer cells

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Breast tumorigenesis is correlated with an inflammatory reaction, inflammasome activation and reactive oxygen species (ROS) generation. In this context, our study aimed to validate new biomaterials enriched with an anti-tumor agent, developed for breast tissue engineering post mastectomy. Threedimensional (3D) biomaterials based on nanocellulose and pectin (CNP) enriched with 5-fluorouracil (5FU) have been proposed for breast tissue reconstruction. To perform this study, normal and breast cancer cells were seeded in CNP 3D scaffolds. Cell viability was quantitatively and qualitatively determined, after two and seven days of culture. Caspase-1 levels were measured from culture media, in order to observe the inflammasome complex activity. p53 and caspase-1 expression from cells grown in CNP 3D systems were determined using Real-Time PCR and immunofluorescence techniques. Extracellular and intracellular ROS production was measured, in order to observe the 5FU's capacity to enhance oxidative stress in the tumor cells. The addition of 5FU affected normal cell behavior and decreased tumor cell viability. The highest levels of caspase-1 from inflammasome were observed on 3D systems with tumor cells and 5FU. Real-Time PCR and immunofluorescence assays revealed the overexpression of caspase-1 in tumor cells exposed to 5FU, as compared to control cells. An increase of 5FU concentrations determined an increase of ROS levels in a dose-dependent manner. CNP materials enriched with 5FU may serve as antitumor drug delivery instrument in breast cancer, while the nanocellulose and pectin synthesized in a 3D structure could support breast tissue engineering. 5FU had a high cytotoxic effect on breast cancer cells, activating the inflammasome complex, caspase-1 signaling cascade and induced ROS production. These CNP biomaterials with 5FU may represent a possible alternative for breast tissue engineering. This work was supported by PN-III-P1-1.2-PCCDI-2017-0782/REGMED.

P-04.2-14
Identification of genes presumably involved in cell division of *Ureaplasma parvum*

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In well-studied bacteria the cell division is based on formation of ring-like structure – the divisome. The divisome consists of several dozens of proteins, among which FtsZ is being the most conserved protein which present in nearly all bacteria, even in most genome-reduced species of Mollicutes class (or mycoplasmas). Mollicutes are considered to be one of the simplest living organisms, that are close to so-called "minimal" cell. It is believed that mycoplasmas utilize simplified machineries, including that which is involved in the cell division. However, some of mycoplasmas, for instance, *Ureaplasma* species, lack ftsZ as well as all other known cell division genes. So, it is not clear how *Ureaplasma* species divide and which proteins do they use in the cell division process. In this work we were able to identify several genes that could be possibly involved in the cell division process in *U. parvum*. Using bioinformatics analysis (BLAST), we found UPA3_RS00115 (hydrolase of unknown substrate, putative triacylglycerol hydrolase), UPA3_RS01710 (putative transmembrane protein) and UPA3_RS01870 (putative lipoprotein). These 3 genes were identified using homology to 3 of 7 genes, recently identified as those that affect cell morphology and probably participate in the cell division of synthetic mycoplasma JCVI-Syn3A. Also, we were able to identify UPA3_RS02030 gene which is located in the same cluster as ftsZ and ftsA genes in other bacteria and demonstrate weak homology to ftsZ gene. Moreover, using "triton ghosts" technique (extraction of cytoskeleton-like proteins using mild cell lysis in Triton-X100), we were able to identify UPA3_RS01765 gene product which presumably forms cytoskeleton structures and could possibly be involved in the cell division. Further work is necessary to find out what is the actual function of the genes identified in current study.

Imaging for life: from molecules to organisms

P-04.3-01
Fluorescein-labeled derivative for study of cytotoxicity mechanism of iron (II) clathrochelates

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We synthesized iron (II) clatrochelate complex labeled with fluorescent dye, characterized them *in vitro* and tested their subcellular localization in cancer cells. Clatrochelates are complex compounds with central metal ion incapsulated in tridimensional macrobicyclic organic ligand. Such complexes could be easily functionalized – their eight positions could be modified with different substituents, which opens a large potential variety of biological activity depending on the nature and geometry of those

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substituents. Recently, the high toxicity of functionalized iron (II) clatrochelate complexes (IC50 about 1 mkM) against cancer cells (line HL-60) was discovered. However, the mechanism of their activity is still unknown. To get hint about their mode of action we studied accumulation and redistribution of clatrochelates in cancer cells. For that purpose, we synthesized a labeled with fluorescein clatrochelate complex. Due to low stability of clatrochelates in alkaline environment, during the synthesis we used enzymatic catalysis for the deprotection of acetyl group of the acetylated fluorescein. CD spectroscopy showed that labelled clatrochelate binds with serum albumins, similarly to unlabeled model complex. Fluorescence polarization studies also confirmed binding of labeled clathrochelate to albumin. Despite of clatrochelate ability to quench fluorescence, due to high quantum yields of fluorescein such complexes possess sufficient emission intensity allowing its visualization in cells. Study on human ovarian cancer A2780 cell line has shown that the clathrochelate is able to penetrate through cell membrane, it doesn't enter into the nucleus and accumulates in cytoplasm. No accumulation in any intracellular organelle was apparent. Now we continue structure optimization of fluorescently labeled clathrochelate for its use in the thorough study of the mechanism of cytotoxicity of these compounds. This work was supported by the grant H2020-MSCA-RISE 778245.

P-04.3-02
Comparative study of PyA-cluster system and excimer forming oligonucleotide probes for the detection of miRNA

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Design of new fluorescent probes for nucleic acids detection and point mutations determination is the actual direction of modern bioorganic chemistry and molecular biology. Many types of fluorescent nucleic acid probes covalently linked with pyrene units were proposed. Excimer forming pyrene modified oligonucleotide probes are prospective instruments for RNA and DNA detection due to their high sensitivity to the microenviroment. Recently proposed PyA-cluster system that formed a three-way-junction structure, with two PyA units (8-(1-ethynylpyrenyl)-2'-deoxyadenosine) positioned close together, in the presence of the target miRNA demonstrated high sensitivity. The aim of our work is the design and comparative study of excimer forming oligonucleotide probes on the basis of pyrene bearing oligo(2'-O-methylribonucleotides) and probes containing PyA-cluster that formed a three-way-junction structure. MicroRNAs particularly microRNA let-7 are considered as diagnostics biomarkers and are the objects of investigation upon creation of new improved systems of RNA detection. The series of 5'-bis- and 5'-mono-pyrene tandem probes targeted to microRNA let-7a were synthesized. The method of synthesis is based on the activation of 5'-phosphate of deprotected oligo(2'-O-methylribonucleotide) in solution followed by interaction with pyrenemethylamine. The probes containing PyA-cluster on the basis of oligo(2'-O-methylribonucleotides) and oligodeoxyribonucleotides were synthesized by solid-phase phosphoramidite method. The most sensitive fluorescent probes were revealed using the comparative study of their fluorescent properties upon hybridization with synthetic miRNA let-7a target. Proposed excimer forming oligo(2'-O-methylribonucleotide) probes and probes containing PyA-cluster that formed a three-way-junction structure can by efficiently used for microRNAs detection. The work was partially supported by Russian State funded budget project of ICBFM SB RAS 0245-2021-0007.

P-04.3-03
Adhesins and motility of human pathogen *Borrelia burgdorferi*

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Lyme borreliosis (LB) is the most prevalent vector-borne disease in the Northern hemisphere and, as such, one of the major public human health concerns in Europe and North America. LB is caused by certain species of spirochetes of the *Borrelia burgdorferi* sensu lato complex. Borrelia is known to produce a number of adhesive surface molecules that are the key players in the pathogenic process of the bacterium. As opposed to pathogens permanently circulating in the blood of their host, *B. burgdorferi* is not known to be able of long-term survival in the blood. Yet, to be acquired by its tick vector, the spirochete has to leave its protective niche in the extracellular matrix (ECM) and reach the tick feeding pit. Using an *in vitro* system that mimics the natural tick feeding, we observed that a subset of borrelial surface proteins, particularly DbpA/B and BBK32, enhance the motility of the bacterium in ECM. Using immuno-scanning electron microscopy, we determined the surface distribution of the surface adhesins and, using atomic force microscopy, we measured the specific binding interactions between borrelial adhesins and various ECM compoments. We characterized the dynamic, nano-mechanical molecular properties of borrelial surface proteins with ECM proteins, aiming to understand the underlying physical processes that govern the motility/adhesion of this malicous agent. Additionally, using serial block-face scanning electron microscopy, we studied the interactions of Borrelia and human microvascular cells to visualize the key steps of Borrelia pathogenesis. Our results indicate that crucial, yet undiscovered role of some borrelial adhesins is to promote translational motion in a host by acting as transient binders of ECM. We provide evidence showing that adhesins directly influence and enhance motility of *B. burgdorferi* in fluid shear stress-less environment by coordinating host ECM adherence with pathogen propagation during the infection process.

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P-04.3-04
Heartbreaking beauty: updates in cardiac amyloid imaging

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Cardiac amyloidosis is one of the most rapidly progressing heart diseases with high mortality. For diagnosis of amyloidosis histological evidence of amyloid deposition is essential. The gold standard method for amyloid detection is the Congo red staining and birefringence under polarized microscopy. Here we describe new approaches to amyloid detection based on Congo red staining, which can be useful for diagnostic purposes. Myocardial autopsy samples from 10 random individuals (males and females, >70 years old) diagnosed with cardiac amyloidosis were stained with 0.1% Congo red or with 0.1% novel Congo red analogue – disodium salt of 2,7-(1-amino-4-sulfo-2-naphthylazo)-fluorene (DSNAF). Firstly, we found that pre-heating of tissue sections in citrate buffer (pH 6.0) for 15 minutes enhances the intensity of amyloid staining with Congo red. Congo red staining of pre-heated tissue gives the amyloid an intense dark salmon color. Secondly, we demonstrated that fluorescence microscopy is superior to polarized microscopy for detecting amyloid deposits in Congo red–stained myocardial specimens. With use of red fluorescence filter amyloid deposits appear bright red and so they are much easier to recognize than under polarization. Finally, we tested a new amyloid staining technique and demonstrated for the first time that new fluorescent dye DSNAF can be successfully used to identify amyloid deposits in histological sections of human myocardium. Compared to Congo red, fluorescence intensity of DSNAF upon binding to amyloid fibrils is significantly higher. Thus use of DSNAF enables detection of small amyloid deposits with high accuracy. Modifications in amyloid staining protocol described here could improve the amyloid detection accuracy in laboratory practice and clinical diagnostics. The work was supported by the grant of President of the Russian Federation (MK-560.2020.7).

P-04.3-05
Genetically encoded sensor for the detection of the extracellular pH by FLIM

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Maintaining the acid-base balance within certain narrow limits is necessary for the normal functioning of living organism. In our laboratory it was shown that a number of receptors, namely IRR, ErbB2 and some others can be activated by the alkaline extracellular medium. But these receptors are expressed in many organs and tissues that have a neutral or acidic pH. So, there may be local pH changes in the body that lead to the activation of these receptors, but currently there is no data on such changes. Detection of these changes must be performed *in vivo* with a resolution sufficient to visualize individual areas of organs and tissues. In case of small laboratory animals, the use of electrochemical pH-sensors is difficult because of the small size of the animal and the insufficient spatial resolution of such sensors. Modern methods of pH measurement, such as PET and MRS, require the introduction of special radioactive tags. The most suitable method in terms of detection time, resolution, required equipment and ease of use is fluorescent imaging. In this regard, for the first time genetically encoded fluorescent sensor of the extracellular mildly alkaline pH named SypHerExtra was created in our laboratory. This sensor is based on the pH-sensitive fluorescent protein SypHer3s with a leader peptide of mouse IgK, providing protein excretion into the extracellular media, and the membrane domain of the protein neurexin-1, that "anchors" the sensor on the outside of the cell membrane. We have shown that this sensor allows to measure pH using fluorescence lifetime microscopy (FLIM) that improves the accuracy of the data obtained during in vivo measurements in comparison with the ratiometric detection method. The ability to detect the pH value in vivo using the SypHerExtra sensor will shed light on the fundamental question of the role of local pH changes in various organs and tissues. This work was supported by the RFBR grants № 20-04-00880 A, 18-04-01369 and 19-04-01042 A.

P-04.3-06
Features of the safety assessment of novel food sources derived with microbial synthesis: design of experimental diets

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Recent years a worldwide interest in the use of alternative sources of food protein, in particular, the protein of microorganisms, has increased. Food raw materials derived from microbial synthesis belong to a novel group of food, that have not been previously consumed by humans, and ensuring the safety of such products in the Russia is regulated at the state level. The safety assessment procedure of the novel food includes comprehensive toxicological studies in *in vivo* tests, therefore, the formulation of experimental diets, not only providing all physiological needs of laboratory animals for a long time, but also allowing to include the studied product in a maximum possible amount, is an important scientific task. Semi-synthetic casein diet (SCD) for laboratory animals [Tyshko N., 2011], widely used in toxicological studies, has all the required characteristics. The major components of this diet (per 100 g) are casein, maize starch, sunflower oil and lard, the content of which are 23.9, 59.0, 5.0, and 2.0 g, responsible for the protein, carbohydrate, and fat components, respectively. The total caloric value of the diet is 358.4 kcal/100 g. The first step in the test diet designing is a limiting factor determination, which is used to calculate the maximum amount of the studied product that can be included in the diet without changing of chemical composition and caloric content. If the protein concentrate produced from microbial biomass contains 75.5 g of protein per 100 g, the limiting factor is protein, so the maximum possible amount of this concentrate per 100 g of diet will be 26.8 g (20.2 g converted to protein). The chemical composition of this product allows to include it in the diet, completely replacing casein and becoming the sole protein source, which

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provides the consuming of the product agravated amounts by test animals as a part of the balanced diet during the whole experiment. This work was supported by Russian Science Foundation grant 20-76-10014. *The authors marked with an asterisk equally contributed to the work.

P-04.3-07
Macro- and microelement composition of *Hermetia illucens* larvae biomass

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Food safety regulation in the Eurasian Economic Union is given a considerable attention. The current Technical Regulations (TR) establish requirements for food and raw material, as well as the processes of their production, storage, etc. Particular consideration is focused on novel food that derived from non-traditional food sources such as micro-organisms, microscopic algae, insects. According to TR 021/2011 the novel food is required to receive a state registration. The registration procedure includes a complex research, confirming a safety of this food. The chemical composition of larvae dry biomass of black soldier fly was investigated as a part of the safety assessment procedure of novel insect-based food. As insect larvae have the ability to accumulate macro- and micronutrients, one of the important steps of expertise is to study the elements content in raw material. The chemical composition of *Hermetia illucens* larvae biomass was studied for 23 parameters (Al, Ba, Be, V, Ga, Fe, K, Ca, Co, Li, Mg, Cu, Na, Ni, Rb, Ag, Sr, Tl, Cr, Cs, Zn, Se, P). The results' analysis and their comparison with the literature data indicates that in terms of Ca, K, P, Mg, Zn, Ba, Cu, Ga content the larvae did not differ from the data of other researchers. The content of Se was 297% lower than the literature, while the content of Na, Fe, Al, Co, was found to be significantly higher, ranging from 38 to 89%. It should be mentioned that in content of Na, K, Ca, Mg, P, Fe *Hermetia illucens* larvae were significantly superior to basic food of animal origin (beef, pork, etc.). Our data confirm the importance of a comprehensive examination of insect-based food chemical composition as a part of their state registration procedure. The result of this examination is the forming of a controlled indicators list for this food as well as the Technical Specification for the manufacturing of product with standardized properties. This work was supported by Russian Science Foundation grant 20-16-00083.

P-04.3-08
Label-free imaging with subcellular detail: Optical Coherence Microscopy in quality assessment of mammalian oocytes and embryos

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Confocal laser microscopy and other fluorescence imaging methods applied in biology allow for detailed structural and dynamic studies of a single cell but require fluorescent tags to visualize cellular architecture and may cause short- and long-term photodamage. Traditional light microscopy, although relatively non-invasive, does not provide detailed structural information. Optical coherence microscopy (OCM) is a promising alternative, as it does not require sample pre-processing or labelling and is capable of providing 3D images of intracellular structures. Here we examined whether intracellular features visualized by OCM imaging can be used as biomarkers of oocyte/embryo quality in the context of assisted reproduction techniques. We applied OCM to image: (i) immature mouse oocytes to examine their chromatin conformation corresponding with their transcriptional status and developmental competence; (ii) compact mouse morulas to calculate their cell number, which correlates with their ability to develop to the high-quality blastocysts. The abovementioned morphological parameters cannot be assessed non-invasively by traditional light microscopy. They were correlated with the oocyte/embryo's developmental success using techniques such as oocyte *in vitro* maturation, *in vitro* fertilization, embryo culture, immunofluorescence. We showed that OCM distinguished oocytes with so-called NSN (non-surrounded nucleoli) chromatin conformation that corresponds to unfinished transcription and lower developmental potential with high sensitivity and specificity (100% and 93% respectively). We also showed that OCM allowed for a precise nuclei count (a proxy for cell count) in compacted mouse embryos and that this data correlates with the embryo's ability to form high-quality blastocysts. The OCM scanning protocols were verified to be safe for oocytes and embryos. Our results suggest that OCM may be a valuable addition to the imaging toolkit used in assisted reproduction procedures.

P-04.3-09
HER2-specific nanocontainers with a genetically encoded BRET sensor for intravital bioimaging of tumors in model animals

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Optical bioimaging in contemporary experimental oncology is an indispensable approach which enables to evaluate tumor development in vivo, study its proliferative activity and metastatic potential, to estimate the therapeutic effect of antitumor agents in preclinical pharmacological studies. To date a large number of tumor models stably expressing fluorescent proteins have been created for this purpose. However, the use of an external light source to excite a fluorescent protein has a number of limitations associated with the loss of the intensity of the exciting light due to absorption and scattering of light by tissues. We propose to use a fluorescent protein excitation system based on bioluminescent resonance energy transfer (BRET) for a highly sensitive intravital detection of HER2-positive tumors and metastases. In our system, NanoLuc luciferase and a far-red fluorescent protein with a large Stokes shift LSSmKate1 (or LSSmKate2) exist in the single hybrid protein (BRET-sensor), in which the excitation of a fluorescent protein (acceptor) occurs as a result of non-radiative Förster energy transfer from the oxidized form of the luciferase substrate furimazine (donor). The targeted delivery of the BRET-sensor to HER2-positive tumors in vivo is performed using liposomal vector nanocontainers functionalized with designed ankyrin repeat protein (DARPin). Efficient encapsulation of the hybrid proteins into liposomes is achieved through electrostatic interaction between positively charged proteins and

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negatively charged liposome membrane. It was shown that the effectiveness of BRET increases with decreasing of buffer pH, reaching a maximum value at pH 6.0. Given the acidic values of pH in tumors, the proposed sensor can be an effective tool for intravital bioimaging of deep tumors and metastases in model organisms. The work is supported by the Russian Foundation for Basic Research (no.18-29-09023). *The authors marked with an asterisk equally contributed to the work.

P-04.3-10
Imaging of nanoparticles in optically cleared lung and bronchial specimens

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The spatial distribution of 100 nanometer-sized respiratory pathogens (such as virus particles) in the lungs at different phases of inflammation is an important but challenging research question. We developed a high-resolution CLSM-based technique for imaging of 100 nanometer-sized FluoSpheres in the optically cleared whole murine lungs and conducting airway specimens. Airways in the whole-lung specimens were fluorescently labeled with Streptavidin-Alexa488 (Previously published in: Bogorodskiy AO et al. (2020) Front Immunol 11:298.). Ce3D protocol (Previously published in: Li W et al. (2019) Nat Protoc 14, 1708–1733.) was used for optical clearing the lung and to prevent FluoSpheres degradation. For visualization of large blood vessels in the whole lung specimens we used second-harmonic generation (SHG) imaging. FluoSpheres were detected in the murine lungs up to 72 hours after application in both bronchial branches and the alveolar space. FluoSpheres were detected either as free particles or particle agglomerates 4 hours after application. After 24 hours, particles were mostly internalized by immune cells and were detected as agglomerates both in the luminal side of the airway epithelium and in the lung parenchyma. At all investigated time-points FluoSpheres were not detected in the large blood vessels. Using high-resolution imaging of conducting airways we identified CD169-positive macrophages and CD11c-positive dendritic cells contributing to FluoSpheres internalization. Our technique can be useful for investigations of the mechanisms of immune cell-virus particle interactions in the respiratory tract. The work was supported by RFBR, project 20-04-60311.

P-04.3-11
A single molecule atomic force microscopy study of PARP1 and PARP2 recognition of DNA strand breaks

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PARP1 and PARP2 catalyze the synthesis of poly(ADPribose) and use NAD^+ as a substrate for the polymer synthesis. Both proteins are able to directly recognize DNA breaks, and the binding to the ones results in activation of these enzymes. PARP1 and PARP2 are involved in DNA damage signaling and function as sensors of single-strand and double-strand DNA breaks (SSBs and DSBs). These PARPs catalyse poly(ADPribosyl)ation of itself and(or) broad spectrum of DNA damage response factors. Numerous studies have evaluated the activation of the PARPs by short DNA duplexes, however, the interaction of the proteins with single-strand damage and blunt ends on short DNA can overlap. Thus, long DNA duplexes with several DNA damages located at a distance from one another are better models to study the influence of DSBs (blunt DNA ends), undamaged DNA sequences and a single damaged site on the specificity of the PARP–DNA interaction. To characterize at the single molecule level the interaction of PARP1(2) with long DNA substrates we applied atomic force microscopy. We found that the distribution of PARP1 between DSBs and SSBs is relatively similar, although this protein has higher affinity and specificity for SSBs. In contrast to PARP1, PARP2 has a low affinity for DSBs, being more specialized in recognizing SSBs. PARP1 has the higher affinity for apurinic/apyrimidinic site and one nucleotide gap than PARP2, whereas both PARPs efficiently interact with the nick. The binding of DNA damage site by PARPs involved monomeric or dimeric form in depending on the type of the site. PARP dimerization influences the affinity of these proteins to DNA and affects the efficiency of the enzyme reaction. PARP2 suppresses PAR synthesis catalyzed by PARP1 after SSBs formation. The data from our study suggest that the functions of PARP1 and PARP2 overlap in SSB DNA signaling and provides evidence for a role of PARP2 in the regulation of PARP1 activity. The work was supported by RSF [19-14-00204].

Structures of nucleic acids

P-04.4-01
PARP1, PARP2 and PARP3 interaction with nucleosomes containing DNA-lesions

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The regulation of DNA repair is one of the most studied topics in molecular biology, not least because of the possibility of using these data for the development of anti-cancer agents. Poly(ADP-ribose)polymerase1 (PARP1) is an enzyme that involved in regulation DNA repair. It has been shown to be prospective target for anticancer monotherapy and anti-inflammatory therapy.

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However, cytotoxicity of current drugs does not allow to widespread using. This problem requires not only the developing of novel inhibitors, but also fundamental studies of PARP1. To this end we have developed a test-system, which allows analyzing of PARP1 activity in real time [1]. This approach is based on the detection of fluorescence anisotropy of nucleic acid-protein complexes. The fluorescein-labeled DNA duplex (31 b.p.) is used as a PARP1 cofactor. The PARP1-DNA complex formation leads to increase of anisotropy due to decreasing of FAM rotation. Under PARylation conditions the PARP-DNA complex dissociates leading to decrease of the anisotropy level. In current work we applied this method using nucleosome instead of short naked DNA, which is more similar to in vivo conditions. In this system the anisotropy is increased when PARP proteins bind to nucleosome in close proximity of FAM-labelled DNA-ends. We reconstituted nucleosomes with AP-site or gap in two positions with different proteins accessibility. We have shown the applicability of our method to detect PARP1, PARP2 and PARP3 interaction with nucleosome. Shielding of lesions in nucleosome particle leads to decrease of their recognition and binding capacity by PARPs. The reported study was funded by RFBR, project number 20-34-90095 and by RSF 17-74-20075. 1. Kurgina T.A., Anarbaev R.O., Sukhanova M.V., Lavrik O.I. A rapid fluorescent method for the real-time measurement of poly(ADP-ribose) polymerase 1 activity. Anal.Biochem. 2018.

P-04.4-02
AlkB dioxygenase repairs acrolein adducts to adenine, but not its isomer derived from Dimroth rearrangement

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1,N^6-α-hydroxypropanoadenine (α-HPA) is one of the exocyclic DNA adducts arising from alkylation of adenine by acrolein. Human DNA is endogenously exposed to acrolein as it is a byproduct of lipid peroxidation and polyamine metabolism, or exogenously by cigarette smoke, overheated food or the combustion of petroleum fuels and biodiesel. α-HPA is formed by the addition of an aza-Michael aldehyde to N1 of adenine, followed by cyclization, creating an asymmetric hydroxypropano ring outside of the nitrogen base. Such alkylated products have high mutagenic potential and may be implicated in the promotion of cancerogenesis. Interestingly, the combination of 1D and 2D 1H NMR (TOCSY, ROESY) showed that α-HPA can spontaneously convert into adenine and two other forms, γ-HPA and the acyclic adduct to the exocyclic N6, under moderate basic condition. The basis of this phenomenon can be Dimroth rearrangement, where the isomerization process takes place through an open-ring intermediate form. Mass Spectrometry data indicated that the acyclic adduct is N^6-(1,3-dihydroxypropano)-adenine. We have previously revealed that both α-HPA stereoisomers are repaired by Fe (II) and α-ketoglutarate-dependent dioxygenase AlkB, however, the putative R stereoisomer more efficiently than the S one. According to Molecular Modeling data, the S stereoisomer of γ-HPA may resemble the R one of α-HPA, so it is possible that the active center of AlkB can accommodate the γ form. In spite of this, HPLC based, in vitro experiments did not detect any γ-HPA repair product. Moreover, Differential Scanning Fluorimetry has excluded the putative inhibitory activity of γ-HPA against AlkB. Given that the alkaline environment of chromatin may promote Dimroth rearrangement of α-HPA, there is a strong need to look for the biological consequences of γ-HPA lesion and the potential mechanism(s) of its processing. This study was supported by the National Science Centre UMO-2018/29/B/NZ3/02285

P-04.4-03
The functional role of loops and flanking sequences of G-quadruplex aptamer to the hemagglutinin of influenza A virus

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The World Health Organization have mentioned influenza pandemic as one of ten threats to global health in 2019. The attachment of influenza virus to the host cell is mediated by hemagglutinin (HA), which is the most presented protein on the surface of influenza virus particle. These properties of HA make it an attractive target for the development of antiviral agents, such as aptamers. Nucleic acid aptamers are generally accepted as promising elements for the specific and high-affinity binding of various biomolecules. It has been shown for a number of aptamers that the complexes with several related proteins may possess a similar affinity. An outstanding example is the G-quadruplex DNA aptamer RHA0385, which binds to the HAs of various influenza A virus strains. These HAs have homologous tertiary structures but moderate-to-low amino acid sequence identities. Here, the experiment was inverted, targeting the same protein using a set of related, parallel G-quadruplexes. The RHA0385 derivatives were obtained by truncation of 5'- and 3'-flanking sequences and by alteration of loops, including the set of minimal, parallel G-quadruplexes with three single-nucleotide loops. These G-quadruplexes were characterized both structurally and functionally, and high affinities for both recombinant hemagglutinin and influenza virions were revealed. In summary, the parallel G-quadruplex represents a minimal core structure with functional activity that binds influenza A hemagglutinin. The flanking sequences and loops represent additional features that can be used to modulate the affinity. Thus, the RHA0385 serves as an excellent example of the hypothesis of a core structure that is decorated with additional recognizing elements capable of improving the binding properties of the aptamer. Acknowledgments: The reported study was supported by RFBR (№19-315-90100), and Russian Science Foundation (№18-74-10019).

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P-04.4-04
Existence of two RNA G-quadruplex structures in the human NRAS mRNA

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RNA guanine (G)-rich sequences can self-assemble through hydrogen bond interactions into a G-quartet structure, which is additionally stabilized by monovalent cations such as potassium and sodium ion. G-quartets stack with each other and form a non-canonical RNA G-quadruplex structure. RNA G-quadruplexes have a large diversity of loop lengths and arrangements. Nevertheless, they commonly adopt the parallel conformation in which all four strands are oriented in the same direction. RNA G-quadruplexes are dynamic and transient in cells. They are involved in many biological processes, including translation, regulation of alternative splicing and the subcellular transport of mRNAs, and hold great potential in therapy, both as therapeutic targets as well as therapeutic agents themselves. RNA G-quadruplex forming sequence in the 5' untranslated region (UTR) of neuroblastoma RAS viral oncogene homologue (NRAS) proto-oncogene has been determined to have a suppressive role in translation. NRAS proto-oncogene encodes for protein called N-Ras, which is involved in regulating cell division through signal transduction. We will present our results on RNA G-quadruplex structures in the 5'-UTR of the NRAS mRNA utilizing NMR, CD and UV spectroscopies. We further prepared modified RNA G-quadruplexes with different G-tract and loop lengths in order to stabilize the two most probable structures.

P-04.4-05
Roles of active-site amino acid residues in RNA cleavage by human AP endonuclease APE1

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Human apurinic/apyrimidinic endonuclease 1 (APE1) is multifunctional DNA repair and stress-response protein. APE1 exhibits a range of nuclease activities, including AP endonuclease, 3′–5′ exonuclease, 3′-repair diesterase, nucleotide incision repair, damaged or site-specific RNA cleavage, and multiple transcription regulatory roles. It should be noted that APE1 has single distinct active site. Activities of APE1 require different optimal conditions; in particular, lower pH and lower Mg^{2+} concentration are preferred for nucleotide incision repair comparing with AP endonuclease activity. Moreover, it was found that APE1 catalyzes RNA incision in the absence of divalent metal. The question of how APE1 is able to perform such different functions is still open. Herein, we aimed to gain a better understanding of the role of active-site amino acid residues Tyr-171, Arg-177, Arg-181, Asp-210, Asn-212, Thr-268, Met-270, Asp-308 and His-309 in the process of RNA binding and cleavage by APE1. These amino acid residues form the active site pocket of enzyme. It was previously shown that substitution of these residues lead to decrease in AP endonuclease activity. We used model RNA hairpins as substrates for investigating of endoribonuclease activity. In contrary to AP endonuclease cleavage the most severe reduction in RNA incision activity was observed with N212A and D308A mutations, whereas T268D mutation led to increase in RNA incision activity. Mutant forms APE1 T268D and M270A led to lose the specificity of enzyme to pyrimidine-purine sequence as compare with WT enzyme. D210N, T171F and M270A had slight decrease effect on RNA incision activity comparing with wild type APE1. Obtained data support that tested active-site amino acid residues have different roles in the catalytic mechanism of RNA and abasic DNA cleavage by APE1. This work was supported by Russian Science Foundation grant No. 19-74-10034.

P-04.4-06
Mechanism of target nucleotide recognition and cleavage in the model non-canonical DNA and RNA structures by human AP-endonuclease APE1

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Human apurinic/apyrimidinic endonuclease APE1 is responsible for detecting and initiating the elimination of AP-sites from DNA. The major physiological function of this enzyme is hydrolysis of the phosphodiester bond in DNA from the 5′-side of the AP-site. Moreover, APE1 makes an incision of the phosphodiester bond on the 5′-side of some damaged nucleotides. This enzyme also possesses 3′-5′ exonuclease, 3′-phosphodiesterase, 3′-phosphatase, and RNase H activities. However it remains unclear how such structurally different substrates can be recognized by the only active site of the enzyme. The ability of a damaged nucleotide to flip out of DNA and to get into the enzyme pocket during the formation of contacts between DNA and APE1 is proposed to be the key factor responsible for this wide substrate specificity of the enzyme. This model allows to suggest that the substrate specificity of APE1 is controlled by the ability of a target nucleotide to flip out from the DNA duplex in response to an enzyme-induced DNA distortion. Therefore the objective of the present study was to elucidate APE1 catalyzed mechanism of target nucleotide cleavage in various non-canonical structures such as tandem repeats, forming DNA or RNA quadruplexes, and various loop- and hairpin-containing structures, which can facilitate nucleotide eversion stage. Analysis of product accumulation using gel-electrophoresis has shown that APE1 excises all of the DNA substrates used, i.e. DNA quadruplexes, containing the single F-site in core or loop regions, bulged DNA duplexes, containing the F-site, as well as undamaged RNA hairpin-structures. Taking together, obtained data demonstrate the ability of APE1 to excise AP-site containing DNA as well as undamaged RNA in different non-canonical structures on the basis of substrate topology. This work was supported by the Russian Science Foundation № 19-74-10034.

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P-04.4-07
Gene polymorphisms associated with primary and secondary recurrent pregnancy loss in Russian women

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Unexplained recurrent early pregnancy loss (REPL) is one of the more complex human reproductive problems around the world. REPL is classified into primary REPL when women had repeated successive losses and never had full-term pregnancy and secondary REPL when women had a full-term pregnancy before the repeated losses. Several studies reported that aberrant embryonic DNA methylation, placental thrombophilia, deficiency of chorionic villous vascularization and fetal chromosome abnormalities may lead to a failure of fetal early growth and development. Thus, we aimed to investigate some gene polymorphisms that might be implicated in the aforementioned biological disorders to screen their association with REPL in Russian women. Whole blood samples were drawn from 50 patients with REPL (22 primary REPL and 28 secondary REPL) and 56 healthy women living in Central Russia. Genotyping was done for DNMT1 (rs8101626, rs2228611), DNMT3A rs7590760, DNMT3B (rs1569686, rs2424913), DNMT3L (rs2070565, rs2276248), SYCP3 rs769825641, MTR rs1805087, MTRR rs1801394, MTHFR (rs1801131, rs1801133), VEGF (rs3025039, rs699947), FII rs1799963, and FVL rs6025 polymorphisms using the polymerase chain reaction. Our study results have reported a significant difference in genotype and allele frequencies for DNMT1 rs2228611 polymorphism in primary REPL and secondary REPL comparing with healthy women. Women carrying the minor genotype GG are at higher risk of primary and secondary REPL by 2.2-fold (OR: 6.2, 95% CI: 2.7–13.8; OR: 5.4, 95% CI: 2.2–11.9 respectively). The minor genotype TT for DNMT3B rs2424913 polymorphism also increased the risk of primary and secondary REPL in women by 1.9 and 2.0- fold (OR: 4.4, 95% CI: 1.5- 12.9, OR: 3.8, 95% CI: 1.2–11.9 respectively). Consequently, we propose that the DNMT1 rs2228611 and DNMT3B rs2424913 polymorphisms might be associated with the REPL and used as genetic predictors for primary and secondary REPL.

P-04.4-08
Significance of rs2368564 and rs41317140 renin gene polymorphisms for coronary artery in-stent restenosis development

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The lumen diameter reduction or in-stent restenosis (ISR) of the target vessel is the most common complication after percutaneous coronary intervention (PCI). ISR often leads to the recurrence of angina pectoris what requires re-PCI. Neointimal proliferation is the main cause of ISR. The renin-angiotensin-aldosterone system (RAAS) may affect neointimal hyperplasia due to production of growth factors for smooth muscle cells. The objective of our research was to study the rs2368564 (I9–83G/A) and rs41317140 (C4063T) polymorphisms in the renin gene as predisposing risk factors in the development of ISR in patients with stable coronary artery disease (SCAD) after PCI with drug-eluting stent implantation. We analyzed the angiographic and clinical data of 113 patients after stent implantation and follow-up angiography. 62 healthy individuals participated in the study as a control group. All of them were Russians. The patients were divided into two groups - those with significant ISR (n = 53) and those without ISR (n = 60). All groups were comparable; there were no significant differences in age, sex, or lipid profiles. Genotyping for the REN I9 –83G/A polymorphism was performed using the Real-time PCR, for the REN C4063T we used the PCR-RFLP method. The Chi-square test and Fisher's exact test were used to estimate differences between groups. The REN rs2368564 AA genotype showed association with ISR. Its frequency was significantly higher in patients with ISR comparing patients without restenosis (24.1% vs 10.2%, respectively; $P < 0.05$). The frequency of REN rs41317140 TT genotype was significantly higher in patients with SCAD and ISR comparing with the control group (6%, 8% vs 0%, respectively; $P < 0.05$). Our results show that the AA homozygosity for the REN I9–83G/A polymorphism may predispose to restenosis development, whereas the TT homozygosity for the REN C4063T polymorphism is associated with an increased risk of SCAD in general.

P-04.4-09
COL1A1_1 rs1107946, COL1A1_1 rs1800012, END1 rs180054, TNF rs1800629 polymorphisms as a risk factor of liver fibrosis development in patients abusing alcohol

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Hepatitis B and C, alcohol abuse, and genetic factors are the main causes of liver fibrosis. The current research attempts to identify the genetic risk factors of liver fibrosis development in patients abusing alcohol. 35 patients with liver fibrosis stages 3 and 4 (26 men and 9 women; the period of alcohol consumption was 15.6 ± 9.5 years) and 30 healthy individuals participated in the study. The average age of the patients was 50.2 ± 11.52 years. Patients with hereditary liver diseases or infected by hepatitis B and C viruses were excluded from the study. COL1A1_1 rs1107946, COL1A1_1 rs1800012, END1 rs180054, TNF rs1800629 single nucleotide polymorphisms were determined using the Real-Time PCR. The stage of liver fibrosis was identified with the elastography method (FibroScan, Echosens, France). Our data showed the association of the COL1 A1_1 rs1107946 polymorphism with the risk of liver fibrosis development in patients abusing alcohol. The frequency of allele A of the COL1 A1_1 gene (rs1107946) among patients was 38.5% which is significantly higher than in the control group (12.5%; $\chi^2 = 8.388$; $P = 0.004$). The frequency of heterozygotes CA was not significantly different between the group of patients and the control group (42.8% and 30% respectively, $P = 0.186$). Homozygous AA genotypes were detected only in patients with liver fibrosis, the frequency was 17.1%. The association between COL1A1_1 rs1800012, END1 rs1800541, TNF rs1800629 polymorphisms and liver fibrosis in patients abusing alcohol was not revealed. We suggest that the presence of the minor allele A of the COL1A1_1 gene (rs1107946) may be one of the genetic

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factors involved in the development of liver fibrosis in patients abusing alcohol.

P-04.4-10
The effect of BER enzymes on the activity of APE1 polymorphic variants

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Base excision repair (BER) is a multi-step process and one of the major pathways to remove DNA lesions. Coordination of the enzymatic activities of BER participants is essential to ensure a complete repair of the damaged bases. A "passing-the-baton" model proposed for BER is consistent with the findings that DNA glycosylases coordinate with other proteins to process the damaged DNA. In this study, we focused on the issue of damaged DNA transfer from the complex with various human DNA glycosylases (OGG1, UNG, AAG), DNA polymerase β, XRCC1 or PCNA to AP endonuclease APE1. We tested several single nucleotide polymorphic (SNP) variants of APE1 (R221C, N222H, R237A, G241R, M270T, R274Q, P311S) on its ability to influence on protein-protein interactions with different DNA repair enzymes. The activity of SNP forms in the presence of BER enzymes was detected by the stopped-flow method with FRET detection and by direct PAGE analysis of the product accumulation. The AP-site analog (F-site) cleavage activity of WT APE1 and SNP forms was estimated in terms of observed catalytic rate constant k_{obs}. It was shown that OGG1, AAG, Pol β, and XRCC1 stimulate WT, R237A and P311S APE1, but revealed a negligible effect on other SNP variants, indicating that these amino acids can be involved in the protein-protein interactions during damage repair. Interestingly to note that UNG and PCNA slightly stimulate WT APE1, decrease the activity of R221C, M270T, and R274Q, but have no effect on N222H and G241R. Obtained data support that enzymes involved in the BER have a mutual effect on the activity of each other and some amino acid substitution caused by SNPs leads to a change of this effect. This work was supported by a grant MD-3775.2019.4.

P-04.4-11
Functional roles of active-site amino acid residues Asp210, Asn212, Thr268, Met270, Asp308 in the course of damage recognition and catalysis by human AP endonuclease APE1

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Human apurinic/apyrimidinic (AP) endonuclease APE1 is one of the participants of DNA base excision repair. APE1 is responsible for processing of AP-sites and many other types of DNA damage by hydrolysis of the phosphodiester bond on the 5′-side of the lesion. To date, the role of the metal ion and functions of some amino acid residues of the DNA-binding site and active site in the cleavage of DNA and RNA substrates are still discussed. A few alternative catalytic mechanisms of the phosphodiester bond hydrolysis in DNA by APE1 have been suggested. Therefore, in this study, we aimed to gain a better insight into the process of the target nucleotide recognition and cleavage of AP-site containing DNA-substrates by wild type human AP endonuclease APE1 and set of mutant forms. The catalytic amino acid residues Asp210, Asn212 and Asp308 and residues Thr268 and Met270 from the substrate binding site were substituted to obtain the following APE1 variants: D210N, N212A, D308A, T268D, and M270A. Direct detection of the product formation by PAGE allowed for estimate the efficiency of enzymatic hydrolysis of damaged DNA. Because of recognition of specific sites in real time accompanied by conformation adjustment of APE1 and DNA to optimize specific contacts, we performed a pre-steady-state kinetic analysis of conformational changes of APE1 and specific DNA substrates during their interactions. Real-time conformational rearrangements of DNA during interactions with APE1 were visualized by stopped-flow kinetic assays with detection changes of intrinsic Trp fluorescence intensity of enzymes, 2-aminopurine residue located opposite the abasic site or Förster resonance energy transfer. Taken together, our data elucidate functional roles of these amino acid residues in the mechanism of substrate recognition, catalytic complex formation and hydrolysis of phosphodiester bond. This work was supported by grant MD-3775.2019.4.

P-04.4-12
Recognition and removal of DNA clustered damages via NER

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Clustered DNA damages are defined as two or more damages situated within one to two helical turns of DNA. Clusters of damages may be generated following massive exposure of DNA injuring factors in combination with repair deficiency and are often difficult to repair correctly. In this study, we examined how the presence of an apurinic/apyrimidinic site analog, a non-nucleoside residue consisting of diethylene glycol phosphodiester, affects the recognition and removal from DNA of bulky damage N-[6-(dipivaloyl-5(6)-fluoresceinyl-carbamoyl)hexanoyl]-O1-(4,4'-dimethoxytrityl)-O2-[(diisopropylamino)(2-cyanoethoxy) phosphino]-3-amino-1,2-propanediol (nFlu) by mammalian nucleotide excision repair. In global genome NER, complexes of protein factor XPC perform the initial recognition of damage. Results of fluorescence anisotropy measurements have shown that protein factor XPC has a strong affinity to cluster-containing DNAs. The XPD helicase is an essential subunit of TFIIH involved in the next step of NER process, verification of DNA damage. Using photoaffinity modification we have analyzed the interactions between XPD protein and DNA probes bearing clustered damages. Furthermore, we have shown that the NER-catalyzed excision of DNA fragments that contain bulky lesion is fully abrogated in the presence of AP site analog in certain positions of the complementary strand. The excision efficiency from the model DNAs, containing a bulky lesion and an AP site analog in one DNA strand was not impeded. Alterations of double-stranded DNA geometry caused by the presence of clustered damage were assessed too. These experimental data, together with the results of molecular dynamics simulations, contribute to an understanding the mechanisms of repair of clusters containing bulky lesions of various topologies in mammalian cells. This study was supported by the RFBR grant № 19-04-00018 and the RSF grant № 19-74-10056.

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P-04.4-13
DNA binding and catalysis by single-nucleotide polymorphic variants of human uracil-DNA glycosylases SMUG1 and MBD4

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Uracil in DNA can be introduced via two mechanisms, deamination of cytosine and misincorporation of dUMP during replication. Deamination of cytosine has been calculated from measured deamination rates to occur at a rate of 100–500 per human cell/-day to yield mutagenic U/G mispairs. Uracil may also appear as a consequence of misincorporation of dUMP instead of dTMP during replication, resulting in a U/A base pair. Human uracil-DNA glycosylases SMUG1 and MBD4 are crucial enzymes of the base excision repair pathway, responsible for uracil repair. It is known that amino acid substitutions in BER enzymes associated with single-nucleotide polymorphisms (SNPs) are widespread in the human population. It should be noted that a decrease in the functional activity of individual BER enzymes and disruption of coordination between them can have negative consequences. Therefore, in the present study our aim was to determine the influence of known SNPs on the conformation and activity of human SMUG1 and MBD4. Using the NCBI dbSNP database (http://www.ncbi.nlm.nih.gov/SNP/), polymorphic variants of the human SMUG1 and MBD4 were selected. Four SMUG1 (G90C, P240H, N244S and N248Y) and four MBD4 (S470L, G507S, R512W and H557D) SNP variants were chosen for experimental analyses of enzyme activity. The activity of SNP variants was studied by direct PAGE analysis of the kinetics of accumulation of products. The conformational transitions in the molecules of WT enzymes and its SNP variants in the course of interaction with DNA-substrate were monitored in the real time using stopped-flow method with fluorescence detection. Obtained results allowed to suggest the kinetic mechanism of enzymes interaction with DNA substrates and to calculate the rate constants of the elementary stages, as well as to identify the stages of the process affected by mutation associated with SNP. This work was supported by the Russian Science Foundation № 16-14-10038.

P-04.4-14
Developing a novel web application for thermodynamic characterization of oligonucleotide unfolding

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The laws of thermodynamics provide tools for the use of elegant mathematical expressions to describe stabilities and interactions of biological macromolecules. Thermodynamic studies have fought their way to become the essential and necessary complement to structural studies, to fully define the driving forces of folding and binding interactions. Complete thermodynamic profiles of simple transitions (e.g., two state) can now be obtained in a relatively straightforward manner using software solutions provided by instrument manufactures. However, if thermodynamic analysis requires implementation of complex/ multistep transitions or global analysis of experimental data obtained from several experimental techniques researchers have no other option but to develop their own solutions. We have started to develop a web application that will help users to perform thermodynamic characterizations of oligonucleotide unfolding. The application can perform global fitting of calorimetric and spectroscopic data, and uses either two- or three-state equilibrium model to obtain thermodynamic parameters for each transition step - namely, the Gibbs energy, the enthalpy, and the heat capacity. In addition, the application can define the number of K^+ ions and the number of water molecules being released or taken up during unfolding. To test our application, we used UV spectroscopy, circular dichroism, and differential scanning calorimetry to monitor folding and unfolding of a model 22-nucleotide-long sequence of a human 3'-telomeric overhang, known as Tel22. The obtained data were uploaded to the web application and the global fit revealed that unfolding of Tel22 involves at least one intermediate state, and that K^+ ions are released during the unfolding, whereas water molecules are taken up. The web application is accessible through the following link: http://ltpo2.fri1.uni-lj.si/dna/. *The authors marked with an asterisk equally contributed to the work.

P-04.4-15
Click-chemistry for molecular barcoded transcript analysis

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Oligonucleotides (ON) fixed on the substrate are widely used in the technologies of genetic single-cell analysis. Synthesis of long ONs (>60 nucleotides), carrying a molecular barcode, is complicated by a decrease in the nucleophilicity of the 3'-OH. Besides, the technically unsolved condition of such ONs application is the fixing of several sequences on a substrate. We have developed a method for the formation of long ONs joining any target-specific sequence by the Click-chemistry method (CuAAC). For this purpose, we synthesized modified nucleotides with alkyne- and azido-moieties. The 5'-hydroxyl of the obtained ON was functioned *in situ* up to 5'-iodo by $(PhO)_3PCH_3I$ with the following maturing in solution of NaN_3 in DMF and formation of 5'-N_3-ON. Synthesis of the 3'-alkyne-ON was performed using 3'-Propargyl-5-Me-dC CPG. Cleavage was carried out in ammonia solution at 55°C for 5 hours. ONs were mixed in equimolar quantities for the [3+2]-cycloaddition reaction using Cu(I) as a catalyst and THPTA as a ligand. The reaction was carried out under oxygen-free environment at r.t. in 2 hours. Synthesis quality was checked up by MALDI-TOF. The performance of the obtained ON was tested by the synthesis of cDNA of a gene of the human T-cell receptor alpha-chain. The efficiency of cDNA production was evaluated by agarose gel electrophoresis. The developed technology will be used for ON immobilization on a solid support according to the method described earlier [Kozlov et al, 2019]. Our approach will make it possible to remove restrictions on the length of site-specific ON and, thus, to insert molecular barcodes for a great number of analyzed cells without loss of analysis accuracy. Click-attachment of different ONs to a

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single substrate will create new opportunities to identify genomic markers that undergo changes in the development of pathology (oncopathology, immunodeficiencies, allergies). Acknowledgments: The reported study was funded by RFBR, project number 19-33-90076.

P-04.4-16
Guanine substitutions modulate guanine quadruplex conformation in dehydrating conditions

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The behaviour of nucleic acids in cells might significantly differ from that followed in vitro in water solutions, due to molecular crowding, that is a combination of an excluded volume effect and dehydration. Guanine quadruplexes represent a broad spectrum of non-canonical conformations of nucleic acids. It is well known that crowding or dehydrating conditions support guanine quadruplex formation and influence quadruplex conformation. By using circular dichroism and UV absorption spectroscopy, we systematically studied the effect of crowding conditions simulated by ethanol on the structure of three model sequences with guanine substitutions. We have observed, that most guanine substitutions prevent the conformational switch from antiparallel or hybrid forms to parallel ones when induced by dehydrating agents. The inhibitory effect does not depend on the position of the substitution, but on the type of substitution and on its destabilising potential. A parallel form might be induced in some cases by ligands and even this ligand-induced switch is inhibited by guanine substitution. The ability or inability to have a conformation switch, based on actual conditions, might significantly influence potential conformation-dependent quadruplex interactions. Our last studies indicate, that the switching off effect of guanine substitutions is reduced in case of four-tetrad guanine quadruplexes, due to their potential to adopt three-tetrad quadruplex with excluded substituted guanine from the quadruplex core. Previously published in: Bednarova K et al. (2019) Chem. Eur. J. 25, 13422-13428.

P-04.4-17
Development of a dye-labeled DNA system for detection of TERT promoter region by surface enhanced Raman spectroscopy

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The search for novel biomarkers of cancer has been conducted for many years. Extracellular nucleic acids are prospective biomarkers for monitoring the state of an organism. There are two well-studied driver somatic mutations (228T and 250T) in the promoter of the telomerase reverse transcriptase (TERT) gene; these mutations are responsible for TERT overexpression that may cause certain types of cancer: bladder cancer, glioma and some others. The detection of extracellular nucleic acids containing regions of TERT-promoter could potentially be used for the predictive diagnosis of certain types of cancer. However, commonly used methods such as qPCR generally fail to detect these at low concentrations. This study proposes an application of a disparate method based on surface-enhanced Raman spectroscopy (SERS) to determine the nucleic acids of interest at low concentrations. In this process, we used metal nanoparticles to create a SERS-active surface and a DNA-probe consisting of an aptamer fragment interacting with silver nanoparticles and DNA-sequence complementary to the target with linked SERS-active labels. This study was carried out within detection of TERT wild type. We were able to obtain constructions containing regions with the two above-mentioned mutations. Currently, we are adapting this system to detect underrepresented DNA fragments containing mutations. This work is supported by RFBR project 18-29-08040.

P-04.4-18
G-quadruplexes in plants: Hic sunt Dracones

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G-quadruplexes are noncanonical four-stranded structures of nucleic acids, which play important molecular roles in all living organisms and viruses. Surprisingly, there are very few studies about G-quadruplexes in plants, thus we have carried out the pioneer bioinformatics study of G-quadruplex forming sequences occurrence in fully sequenced genomes of selected algal species, higher model plant (*Arabidopsis thaliana*), and non-model (but economically important) opium poppy (*Papaver somniferum*). We have statistically compared the frequencies of G-quadruplex forming sequences among nuclear, mitochondrial, and plastid genomes. This analysis revealed that frequencies of G-quadruplex forming sequences in plastid and mitochondrial genomes are generally much lower than in nuclear genomes. Subsequent analysis of selected genes involved in drought response, alkaloid synthesis, photoreception, and housekeeping processes indicated variable patterns and regulatory roles of G-quadruplexes in particular molecular pathways. Besides, we for the first time present an unprecedented example of a G-quadruplex forming sequence in the gene rpb1 coding for RNA polymerase II large subunit that is highly evolutionarily conserved across the whole Plant kingdom with a common ancestor more than one billion years old, and our bioinformatics results are supported with the complex circular dichroism measurements. Finally, we have inspected the presence of arginine-glycine rich G-quadruplex binding motif in all known nucleic acid-binding proteins from *Arabidopsis thaliana*. We have found that the G-quadruplex binding motif is relatively ubiquitous and there are tens or even hundreds of hot G-quadruplex-binding protein candidates waiting for their *in vitro* and *in vivo* validation. Our work provides novel and original synopsis of G-quadruplex moiety in plants with a couple of promising perspectives in applied research and agriculture. *The authors marked with an asterisk equally contributed to the work.

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P-04.4-19
Preliminary X-ray crystallographic studies of the *Candida albicans* 80S ribosome

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Fungal infections still affect many patients, and the risk is especially high for immunocompromised individuals. *Candida albicans* is a harmful pathogen responsible for disseminated, often deadly, infections. European surveys indicate that this pathogen is responsible for more than the half cases of invasive candidemia, with mortality rates ranging from 28 to 59% for Candida infections. At present there are several antifungal drugs effective against *C. albicans* available. However, most of the synthetic treatments have side effects and there is an urgent need to search for new effective and non-toxic drugs against this pathogen. For this specific purpose one of the most promising solutions is compounds which can specifically block the eukaryotic ribosome. The main challenge is to find specific inhibitors, which only act against the chosen target, such as the *C. albicans* ribosome. To date X-ray crystallography is applied for investigation of the binding mode for distinct compounds known to inhibit or modulate the protein-translation function of the ribosome. Initially, a very homogenous sample for crystallization was isolated, which allowed further work on crystallization. Subsequently we demonstrated that the selection of a detergent molecule is crucial for the *C. albicans* ribosome crystallization. For the first time crystallization conditions for a sufficient crystal size have been found. In this study we obtained a full dataset at 4.2 Å resolution of the vacant *C. albicans* ribosome by X-ray crystallography. In addition, the initial screening of inhibitors provided information that anisomycin binds to the *C. albicans* ribosome at the A-site. Improving these results and performing further screens of potential compounds we aim to find highly specific inhibitors of protein translation. This work was supported by Russian Science Foundation grant 20-65-47031.

Metabolic engineering

P-05.1-01
Recombination-based artificial evolution of *Saccharomyces cerevisiae* for acidotolerance

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Yeast *Saccharomyces cerevisiae* has been associated with humans for thousands of years because of its ability to make fermented foods and beverages, such as wine and beer. Moreover, in the last few decades we have been exploiting yeast in other areas of biotechnology, such as for production of variety of products, from bioethanol to vaccines and medicines. However, to use yeast in industry, strains that are phenotypically suitable for industrial conditions have to be designed. One of such desirable features is ability to grow at low pH. Acidotolerance is a polygenic trait, meaning that there are many genes, whose exact number is unknown, involved in determining this trait. We decided to exploit natural diversity of natural *Saccharomyces cerevisiae* strains, and for that purpose we developed a method that uses iterative crossing and harsh selection to obtain extreme phenotype from a very large pool of segregants. We used 6 different natural *S. cerevisiae* strains and by systemic crossing and selection we significantly improved the acidotolerance of the selected strains. The final, winning segregant was able to grow at pH as low as 1.8, while none of the parental strains comes even close to growing at such harsh conditions. Furthermore, we crossed the winning segregant with a reference strain and prepared a large pool of segregants for the purpose of X-QTL analysis. After pool DNA sequencing and bioinformatic analysis, we detected 45 potential QTLs and within them proposed 91 potential causative genes, of which several have been previously associated with acidotolarence in yeast. We thus successfully developed a method for obtaining biotechnologically important extreme traits just by using natural diversity of yeast S. cerevisiae.

P-05.1-02
New prospective tools for biomedical research from polychaete *Odontosyllis undecimdonta*

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Nowadays various *in vitro* and *in vivo* bioluminescence-based molecular instruments are widely used in biomedical research for detection of different analytes, drug discovery and real-time bioimaging of live systems. *Odontosyllis undecimdonta* is a species of marine syllid polychaete that produces bright bioluminescence. The bioluminescence of Odontosyllis is a well-known phenomenon, but the structure of the key components, luciferin and the luciferase, remained unknown for a long time. In our previous work we opted to extract and purify the Odontosyllis luciferase and successfully identified the luciferase gene. Also we performed isolation and structural characterization of the Odontosyllis luciferin, oxyluciferin and the product of luciferin nonspecific oxidation. The elucidation of Odontosyllis luciferin and oxyluciferin structures, allowed us to propose the possible mechanisms of the luminescent and nonenzymatic oxidative decarboxylation pathways of this bioluminescent system, as well as shed light on the luciferin biosynthesis pathway. Investigation of this metabolic pathway is of fundamental and practical interest, since it might stimulate the development of new bioluminescence-based applications in the future. Therefore Odontosyllis bioluminescent system together with luciferin biosynthesis pathway and its components have a good potential for biotechnology and biomedical research. The study was supported by the President of Russian Federation grant for Leading Scientific Schools LS-2605.2020.4.

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P-05.1-03
Determination of metabolites produced by fungal bioluminescence enzymes in heterologous hosts

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Luciferin of luminescent fungi (3-hydroxyhispidin) is produced from caffeic acid (CA) via hispidin (Hs) by two enzymes: hispidin synthase HispS and hispidin-3-hydroxylase H3H. As fungal bioluminescence system is only a few enzymatic steps from ubiquitous metabolic pathways it is attractive for creating autonomously bioluminescent organisms. We have introduced HispS, H3H and luciferase Luz into the genomes of yeast and plant heterologous hosts, *P. pastoris* and *N. tabacum*, making these organisms able to enzymatically produce light. In this study we aimed at direct determination of the metabolites relevant to genetically encoded bioluminescence and at finding a metabolic bottleneck limiting the production of light. To achieve these goals, we developed an LCMS approach to CA and Hs determination in the yeast and plant biomass. The procedure for the analysis of plant tissues included homogenization with subsequent lyophilization, methanol extraction and centrifugation followed by HPLC of supernatant on C18 column with triple quadrupole MS detection in negative mode. Yeast biomass analysis included methanol extraction with homogenization and centrifugation, supernatant lyophilization followed by reconstitution with concentrating and subsequent UHPLC of supernatant on C18 column with Orbitrap MS detection in negative mode. Results: all *N. tabacum* lines examined contained CA at μg/g level with circadian changes of concentration. Wild and transgenic lines contained Hs at tens ng/g level, higher in transgenic than in wild plants and higher in flowers than in leaves, corresponding to luminosity. All *P. pastoris* strains contained CA at μg/g level. Hs was found only in transgenic strains with the level depending on HispS type (to improve the efficiency of Hs biosynthesis we assayed several hispidin synthases from plants). Hs concentration increased manyfold after yeast treatment by CA. This work was supported by the Russian Science Foundation, grant 17-14-01169p.

P-05.1-04
Exploring novel plasma membrane carboxylate transporters from the yeast *Cyberlindnera jadinii*

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Organic acids have displayed great applicability since they are used as a platform-chemicals in several industries. The expression of carboxylate transporters in microbial cell factories is crucial for organic acid export and consequently its efficient and cost-effective bioproduction. Herein, we identified and characterized novel carboxylate transporters from the yeast *Cyberlindnera jadinii*. The *C. jadinii* transportome was analysed using two approaches: i) direct genetics to search for *C. jadinii* genes homologous to the carboxylate transporters Jen1 and Ady2; ii) using a bioinformatic approach where the *C. jadinii* NRRL-1542 proteome was explored to uncover the complete transportome. In this last approach, we designed a software tool to detect membrane proteins, identify conserved domains and determine the existing homologs from an established local database that contains a single representative genome on the species level. Thus, multiple matches within species directly reflect the presence of homologs within the same genome [1]. Selected genes were functionally characterized by expression in the *Saccharomyces cerevisiae* IMX1000 strain [2]. Protein expression and localization were determined by microscopy evaluation of GFP-fused transporter proteins. Transporter activity was evaluated by growth on different carbon sources and determination of radiolabelled carboxylic acid uptake. Full characterization of newly identified *C. jadinii* transporters is currently underway. [1]Ribas D et al. (2018) FUNGAL GENET BIOL. 122:1-0 [2]Mans R et al. (2017) FEMS Yeast Res, 17:8 Acknowledgments: This work was supported by the strategic programme UID/BIA/04050/2019 funded by portuguese funds through the FCT I.P. and EcoAgriFood (NORTE-01-0145-FEDER-000009), from NORTE-2020, under the PORTUGAL 2020 Partnership Agreement. MSS acknowledges Norte2020 for UMINHO/BD/25/2016 grant (NORTE-08-5369-FSE-000060).

P-05.1-05
Regulation of proteasomal genes in the non-conventional yeast *Debaryomyces hansenii*

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CRISPR/SpyCas9-based genome editing systems are developed for various yeasts that have importance for biotechnology, food industry, and medicine. A non-conventional yeast, *Debaryomyces hansenii*, has an application in the food industry and biotechnology. In academic research, this yeast serves as a model for the investigation of halo- and osmotolerance. Earlier, we have created an effective CRISPR/Cas9-based genome editing tool to elucidate the functions of *D. hansenii* genes. We focused on the system for transcriptional regulation of proteasomal genes in *D. hansenii*. Bioinformatic analysis of its genome revealed the presence of three genes encoding Rpn4-like transcription factors. These factors share some similarities only in their DNA binding domains. Using the CRISPR/Cas9 system, we introduced mutations in these genes to decrease or abolish the corresponding transcription factors' activity. Mutant strains were assessed for the expression levels of proteasomal genes, proteasome activity, and the resistance to various genotoxic, proteotoxic, and oxidative agents. We find that DhRPN4-I is a transcriptional activator of proteasomal genes, while DhRPN4-II and DhRPN4-III are their repressors acting under normal and stressed conditions. Mutant strains have different profiles of sensitivity to toxic agents. Stress resistance tests suggest that DhRPN4-I is responsible for resistance to DNA- and protein-damaging agents, oxidative stress-causing agents, and high osmolarity, but not high salt concentrations. On the contrary, DhRPN4-II and DhRPN4-III are required for the resistance to high salt concentrations. Our data indicate that Rpn4-like transcription factors have distinct

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functions upon stressed conditions, and their interplay may adjust proteasome activity to cope with particular stress efficiently. This work was financially supported by the Russian Science Foundation project # 17–74-30030.

P-05.1-06
Assay for caffeoyl pyruvate hydrolase activity in yeast

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The genetically encodable bioluminescent system from fungi is promising as a tool for luminescence imaging *in vivo*. The bioluminescence gene cluster of various glowing fungi includes a gene that was suggested to encode a caffeoyl pyruvate hydrolase (CPH). Here, we report the development of an assay for caffeoyl pyruvate hydrolase activity in heterologous host — yeast cells — and demonstrate that expression of CPH from fungus *Neonothopanus nambi* results in recycling of fungal oxyluciferin to luciferin precursor caffeic acid. We demonstrate the utility of the assay studying and comparing pyruvate hydrolase homologs from different species of fungi. These results provide further evidence that confirms the suggested function of CPH, thus increasing understanding of the mechanism of fungal bioluminescence. This work is supported by the Russian Science Foundation, grant 17-14-01169 P.

P-05.1-07
Comparison of tyrosine ammonia-lyases for engineering of autonomously luminescent mammalian cells

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Tyrosine ammonia-lyases (TALs) catalyze conversion of amino acid L-tyrosine to p-coumaric acid, the precursor of phenylpropanoid compounds with a wide range of biochemical functions. This class of the enzymes (EC 4.3.1.23) is found in bacteria and plants, but is absent in mammalian cells. In this work we developed a protocol and compared the efficiency of TALs from different natural sources in a model mammalian heterological host – HEK293NT cell line. The chosen TAL genes were optimized for expression in mammalian cells and co-expressed with several additional enzymes: coumaroyl-3-hydroxylase, which converts p-coumaric acid to caffeic acid, and *Neonothopanus nambi* caffeic acid cycle genes, which sequentially convert caffeic acid to fungal luciferin and then to oxyluciferin with simultaneous light emission. Although the luminescent signal was dependent on the experimental conditions such as temperature and composition of the medium, the direct comparison of different TALs in a standardized setup allowed to choose TAL enzyme with the highest activity in mammalian cell culture. We expect these findings to help engineer biosynthesis of phenylpropanoids, including fungal luciferin, in mammalian cells. This work was supported by Russian Science Foundation, grant 17-14-01169 P.

P-05.1-08
Assay for functional characterization of luciferases from higher fungi

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There are approximately a hundred glowing fungal species in the world and all of them share the same bioluminescent mechanism. The main enzyme is fungal luciferase (Luz) and though - belonging to the same enzyme family luciferases from various fungi - perform differently in different heterologous hosts. In this work, we have identified multiple luciferase candidate genes (luz) in genomes of bioluminescent fungi. We report an assay functional characterisation of luciferases and comparison of their performance in different heterologous hosts, such as yeast and mammalian cell cultures. Genes coding for luciferases were identified in fungal genomes, codon-optimized for expression in these hosts and cloned into expression vectors. Luciferase genes with high activity were identified for further engineering of bright bioluminescence based on fungal pathway. We can recommend the assay itself for further evaluation of new luciferases or their mutant forms performance in these hosts. This work was supported by the Russian Science Foundation grant 17-14-01169P.

P-05.1-09
Expanding the palette of antibiotics for *Pichia pastoris* selection

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Pichia pastoris yeast is a frequently used eukaryotic model organism with many advantages over other yeast species. Some scientific problems require the insertion of several genes into the yeast genome using vectors with different selectable markers. Among all markers, antibiotic-resistance genes prevail. Several widely used for *P. pastoris* selection antibiotics include zeocin, blasticidin, nourseothricin, hygromycin, G418. However, this range could be expanded to increase the number of genetic constructs that could be transformed at the same time. Studies on *Saccharomyces cerevisiae* have shown that this yeast could be selected

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using antibiotics puromycin and methotrexate and corresponding antibiotic-resistance genes as selectable markers. We have shown that the growth of the wild type *P. pastoris* GS115 strain is suppressed with the puromycin concentration of 7 mM in growth media. So, it is possible to conduct a selection of the yeast using this concentration of puromycin. We confirmed that *P. pastoris* could be successfully selected on puromycin using the puromycin N-acetyltransferase gene from *Streptomyces alboniger* as a selectable marker. To prove it, we engineered a genetic construct with the puromycin-resistance gene and a fungal luciferase gene, transformed yeast and cultivated them on growth media with 7 mM puromycin. All transformants glowed after fungal luciferin addition. Also, we have shown that a concentration of methotrexate lower than 2.75 mM is ineffective for *P. pastoris* selection. This result is unexpected because of *S. cerevisiae* sensibility to methotrexate in low concentration. So, we have shown the efficient concentration of puromycin to conduct the selection of *P. pastoris* GS115 strain and the fact that methotrexate is ineffective for these purposes in all tested concentrations. The study was supported by the President of Russian Federation grant for Leading Scientific Schools LS-2605.2020.4.

P-05.1-10
Development of β-lactamase based surface display systems in *Saccharomyces cerevisiae* for testing the effects of mutation in protein glycosylation on surface display efficiency

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Surface display in yeast represents a valuable alternative to enzyme immobilization on solid surfaces as it is usually less complicated, less time-consuming, and eliminates the need for expensive enzyme purification. Instead, it provides constant production of recombinant protein composed of enzyme of interest fused with fragments of native yeast cell wall proteins. This way modified cells can be used in production processes for longer time compared to the purified enzymes. However, major disadvantages of this technique are low surface display efficiency and possible hyperglycosylation of recombinant proteins. By lowering the amount of glycosylation in cells, several effects are expected. Decreased glycosylation level of enzyme of interest could result in less disturbance of its conformation. It also causes a lesser density of the outer mannan layer of the cell wall, possibly increasing the availability of the substrate. To test the effects of inactivation of genes involved in O-glycosylation (genes from PMT group) and N-glycosylation (OCH1 and genes from MMN group) on the enzyme activity and surface display efficiency, two different systems using β-lactamase as a reporter were developed. In one, bla gene coding for β-lactamase was fused with HSP150 gene coding for cell wall protein resulting in recombinant protein covalently immobilized on the wall through linkage on its N-terminal end. Other system consists of bla gene fused with a fragment of CCW12 gene coding for its signalling sequence for binding of C-terminal part of protein onto GPI anchor. Immobilization can cause differences in protein folding, possibly leading to changes in enzyme activity. To reduce interference of this effect with observed changes in enzyme activity in mutant strains, we used different immobilization approach described earlier. Protein amount in the cell wall is assessed by measuring β-lactamase activity using nitrocefin as substrate and in a semi-quantitative manner by western blot.

P-05.1-11
Application of bioluminescent reporter system for the detection of auxin *in vivo*

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Auxin is a key regulator in plant growth and development. Measuring concentrations of auxin in plant tissues and its localization in vivo allows to study plant physiology in detail. We propose a method to visualize the presence of auxin based on autonomous bioluminescence in plants. Bioluminescence results from a chain of reactions, catalyzed by different enzymes from the fungus *Neonothopanus nambi*. To link bioluminescence to the presence of auxin, we placed key genes responsible for bioluminescence under the control of an auxin-dependent promoter DR5. In transient expression assays, we showed that this system is functional. We plan to test the system further to learn whether it reports auxin localization in a concentration-dependent way, producing light quanta proportionally to the amount of auxin in the analyzed tissue. The reported study was funded by RFBR and GACR, project number 20-54-26009.

P-05.1-12
An *in situ* study of bioenergetic propertis of BHK21/C13 cells treated with Karnozin EXTRA® and NOW L-Carnosine®

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BHK21/C13 cell line are healthy fibroblasts derived from baby hamster kidneys of five unsexed, 1-day-old hamsters, which produce ATP predominantly by oxidative phosphorylation. Little is known about the effect of carnosine on their metabolic profile. Herein, in the presence of aqueous solution of the capsules Karnozin EXTRA® and NOW L-Carnosine®, corresponding to the concentractions of l-carnosine from the capsules of 2, 5 and 10 mM, cells were incubated for 24 h. Afterwards, we analysed basal respiration of intact cells, the maximal capacity of the mitochondrial electron transport system and the activity of respiratory chain complexes I, II and IV of treated cells. Mitochondrial respiration is measured using polarography with a Clark-type electrode (Oxygraph system, UK) at 37°C. The results demonstrate that the presence of 2 and 5 mM aforementioned capsules leads to an increase in the value of all analyzed parameters compared to control ($P < 0.001$). The mean value of basal respiration, maximal capacity of the mitochondrial electron transport system and the activities of complexes I, II and IV in Karnozin EXTRA® treated cells are higher in comparison with control and the groups treated with NOW L-Carnosine®

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($P < 0.001$). In contrast, in the presence of 10 mM aqueous solution of tested capsules there was a decrease in the value of all parameters, comparing to control ($P < 0.001$). We conclude that Karnozin EXTRA® led to a significant improvement in BHK21/C13 cells metabolic profile, compared to NOW L-Carnosine®. Carnosine in this formulation may be an endogenous regulator of fibroblast energy metabolism and a clinically safe therapeutic agent.

P-05.1-13
Regulation of the activity of pyridoxal-5′-phosphate-dependent transaminases by water-miscible organic solvents

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Pyridoxal-5'-phosphate (PLP) -dependent transaminases catalyze stereoselective amination of organic compounds and are highly important for industrial applications. Catalysis by transaminases often requires organic solvents to increase the solubility of reactants. Three transaminases were examined for stability in water – organic solvent mixtures: thermostable transaminase from *Thermobaculum terrenum*, cold-active transaminase from *Psychrobacter cryohalolentis*, and transaminase from mesophilic bacterium *Haliscomenobacter hydrossis*. We observed a 1.5-1.8-fold increase in the activity of transaminases from *T. terrenum* and *P. cryohalolentis* when adding 10–15% DMSO or methanol in the transamination reaction [Bezsudnova et al (2020) Catalysts 10, 1024; Bezsudnova et al (2020) Extremophiles 24, 537—549]. A two-fold increase in the activity of transaminase from *H. hydrossis* in half-reaction with D-leucine was also detected. To interpret the effects of water-miscible organic solvents on the activity of the studied transaminases we studied the stability of the transaminases by tryptophan fluorescence and analyzed the organization of their functional dimers. We focused on the analysis of the hydrogen-bonding network as a crucial structural factor of the stability of enzymes in harsh conditions. We suggested that the observed activation of the transaminases can be explained by the increase in flexibility of particular surface regions and the release of extra tension in regions of the enzyme that are important for catalysis. Despite the challenge of the prediction of co-solvent effects, their great influence on the counterbalance of stability and activity is a useful tool for fine-tuning the efficiency of biocatalytic processes. The work was supported by the Russian Science Foundation project 19-14-00164.

Posters - Research

Bionanotechnology

P-05.2-01
Engineering fluorescent probiotic bacteria against vaginal infections

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Vaginal infections are among the most frequent health problems in women and can be caused by several microorganisms, including bacteria, yeasts and parasites. One of the efficient ways to treat vaginal infections is with probiotic bacteria. Probiotics are live microorganisms that can confer health benefits to the host when administered in adequate amounts. Their antimicrobial properties are related to the production of hydrogen peroxide, lactic acid and bacteriocins. In order to better understand the mechanisms of action of probiotics it is important to track them inside the host, and one of the ways to achieve this is through imaging the bacteria with fluorescent proteins. We have successfully transformed the most important vaginal species *Lactobacillus gasseri* [1], *Lactobacillus plantarum* [2] and *Lactobacillus crispatus* [3] with three different plasmids that enable expression of fluorescent proteins with different spectral properties (IRFP, GFP and mCherry). The transformation into the bacteria was performed by electroporation using specific cell wall weakening agents. The genes for the fluorescent proteins were cloned under the control of the strong LDH promoter by direct fusion of the promoter with fluorescent genes using overlap extension PCR. The purpose of the engineered lactobacilli expressing different fluorescent proteins is to allow observation of their cell distribution. This will allow tracking of the lactobacilli in vivo and learning more about their effects and characteristics. Furthermore, to enhance the viability of lactobacilli and to protect them from the environment, we plan to incorporate them into electrospun synthetic polymer nanofibers. Thereby, the nanofibers would serve as an efficient intravaginal delivery system for probiotic lactobacilli. References: [1] Allain T et al. (2016) FEMS Microbiol. Lett 363, fnw117. [2] Berthier F et al. (1996) Microbiology 142, 1273–1279. [3]. Beasley SS et al. (2004) Poult Sci. 83, 45–48.

P-05.2-02
De novo design of self-assembling protein lattices and chains based on coiled-coil building units

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Designed protein assemblies and lattices are based on natural oligomerizing domains that self-assemble. They have much potential in biotechnology, for example as composite materials, but natural protein domains limit the achievable geometries and spatial resolution of the lattice, which restricts the range of possible applications. Self-assembling bionanostructures prepared by the *de novo* modular approach using coiled-coils (CC) as the building units can be tuned extensively, as we understand quite

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well the rules underlying the specificity of the CC dimer forming peptides. The idea is to use these concatenated orthogonal CC building modules linked by flexible peptide linkers to guide the assembly of the lattice of a selected geometry via orthogonal CC pairs. Our goal is to achieve large ordered structures such as long chains or lattices with unique 2D assembly with minimal packing defects formation. We further extended the modularity and diversity of our structures by incorporating other types of building modules, such as multimerization domains (collagen, foldon) and splicing proteins (split inteins). An important stage of my research is establishing the quality of different protein lattice designs. We analyze secondary structure, stability and other characteristics of the assembled lattices using a variety of biochemical, and biophysical methods, such as CD, DLS, SEC-MALS, ITC, SAXS and cryo-EM. One of the recent designs involves trimerization protein domain – foldon – which is fused to CC-forming peptide segment that orthogonally interacts with the corresponding pair fused to another foldon. Characterization of protein structure in solution by SAXS showed that our construct trimerizes through foldons and that interaction between CC segments caused formation of closed 6-meric structure. We are convinced that this design is a step in the right direction and that modifying it will provide us desired results.

P-05.2-03
Serum albumin penetration in the fluid lipid bilayer of liposomes loaded with a melphalan lipophilic prodrug can be prevented by inclusion of phosphatidylinositol or ganglioside GM1

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Liposomes are intended for systemic injections thus interactions with blood components are the first barrier on the way to cells and tissues. We studied binding of the most abundant blood protein – serum albumin (BSA) – to 100-nm antitumor liposomes by FTIR-spectroscopy. The liposomes were made on the basis of egg phosphatidylcholine (ePC) with inclusion of 10 mol % dioleoylglycerol ester of melphalan (MlphDG) as a prodrug into bilayer, and 10 mol % of phosphatidylinositol (PI) or ganglioside GM_1 that could alter BSA binding. We registered spectra for liposomes in PBS, then added BSA and incubated mixtures for 20 min at 37°C in a cell of spectrometer with recording spectra every 5 min. We analyzed changes in peak positions and forms. Liposome-protein interactions were estimated by (i) changes of membrane fluidity (shifts in CH2 stretching mode bands at 2930-2850 cm^{-1}), (ii) changes in the bonds of lipid ester carbonyls with water (contribution of highly hydrated lipids to the band interval 1719–1732 cm^{-1}), and (iii) changes in BSA structure (redistribution of α-helix/β-shift/β-turn/random in Amide I). For neutral ePC liposomes, BSA did not cause changes in the fluidity of membrane which means that it binds to bilayer without embedding. Membrane binding itself did not alter protein structure. For negatively charged GM_1 containing liposomes (ePC-GM_1, ePC-MlphDG-GM_1) and for the ePC-MlphDG-PI liposomes, we observed similar effects. ePC-PI liposomes were the only negatively charged sample that caused significant changes in BSA structure. Protein helicity decreased from 60 to 50%. Positively charged ePC-MlphDG liposomes undergone most prominent changes accompanied by increased membrane fluidity probably due to BSA embedding, and protein itself lost up to 20 % of helicity. We conclude that PI and GM_1 serve as shielding molecules for our prodrug in the liposomal formulation. This work was supported by the Russian Science Foundation (project 21-74-20177).

P-05.2-04
Nanoparticle-mediated delivery of Cpf1 for the generation of improved gene editing tools

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The CRISPR/Cas technology allows for the efficient manipulation of DNA sequences. It consists of a Cas nuclease in complex with a guide RNA complementary to a target DNA. Following guide recognition, the nuclease generates a double strand break on the DNA that can be repaired through non-homologous end joining and homology directed repair, less efficient but required for precise editing. Although to date, research has mainly focused on Cas9, its analogous, Cpf1 (or Cas12a), is receiving increasing attention due to its higher specificity [1]. The correction of disease-causing mutations through gene editing has a great therapeutic potential, however the efficient delivery of CRISPR molecules remains a major challenge [2]. This project aims to design CRISPR nanostructures able to overcome current delivery issues that can be used for the efficient in vivo editing of oncogenic mutations. These nanostructures consist of Cpf1 nuclease conjugated with biocompatible nanoparticles (NPs) including magnetic and albumin NPs and albumin-coated gold nanoclusters. Cpf1-NP binding was carried out through two main strategies: electrostatic interaction and covalent conjugation. For the latter, the NPs were previously modified with a smart linker for the controlled intracellular release of Cpf1. All the NPs tested showed Cpf1-binding capacity. Additionally, HeLa cells were shown to internalize the conjugates. Moreover, Cpf1 was engineered for improved delivery. One of the main obstacles to nanoparticle-mediated cell delivery is endosomal entrapment. Thus, we produced Cpf1 variants fused to peptides known to enhance endosomal escape. Cpf1 activity remained intact upon modification as shown by in vitro tests. [1] A.C. Komor et al. CRISPR-based technologies for the manipulation of eukaryotic genomes, Cell (2017) [2] C. Liu et al. Delivery strategies of the CRISPR-Cas9 gene-editing system for therapeutic applications, J. Control. Release (2017)

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P-05.2-05
The conjugation of antitumor unsymmetrical bisacridines with quantum dots enhance cytotoxicity and change the biological response in lung and colon cancer cells

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Quantum dots (QDs) are a novel class of semiconducting nanomaterials with unique properties, including drug delivery systems and biomedical applications. Unsymmetrical bisacridines (UAs), synthesized in our laboratory, patented in Europe [EP 3070078B1] and USA [US 10202349 B2], are a new class of antitumor agents highly active against many experimental cellular and tumor models, including lung, colon, pancreatic and prostate. Here, we investigated whether the conjugation of UAs with QDs has an impact on cytotoxicity and biological response against human lung H460 and colon HCT116 cancer cells, compared to UAs alone. UAs (C-2028 and C-2045) were non-covalently attached to the $QD_{red/green}$ (Ag-In-Zn-S/MUA). The externalization of phosphatidylserine, the decrease of mitochondrial transmembrane potential and the induction of senescence following QDs-UAs treatment at IC_{80} were examined. Our results indicate that QDs-UAs conjugates exhibited higher cytotoxic activity towards H460 cells than UAs alone, without altering HCT116 cells cytotoxicity. In both cell lines, studied compounds and their conjugates induced apoptotic cell death which reached a higher level in H460 than in HCT116 cells. Interestingly, the number of apoptotic cells (A+/PI+) significantly decreased since 72 h of QDs-C-2028 conjugates treatment compared to C-2028 alone in H460 and HCT116 cells. These results are consistent with the data from mitochondrial transmembrane potential. Furthermore, UAs induced cellular senescence in H460 cells (flattened cells, expression of β-galactosidase) and this process was intensified following QDs-UAs conjugates treatment. Surprisingly, the senescence process was not observed in HCT116 cells. Summing up, QDs-UAs nanoconjugates enhanced the cytotoxicity and cellular senescence in H460 cells, but weaken the cellular response (QDs-C-2028) compared to C-2028 alone in both cell lines. These studies were supported by the National Science Center, Poland, UMO-2016/23/B/NZ7/03324.

P-05.2-06
Lysosome dysfunction induced by magnetic nanoparticles: is it an answer how to regulate the cell death remotely?

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Magnetic nanoparticles (NP) became a cornerstone in modern biomedicine and cell biology as a promising tool for diagnostics, targeted drug delivery and bioimaging. Magnetic nanoparticles have demonstrated an ability to modulate lysosomal dysregulation and specific cell death signaling. However, precise underlying mechanisms of such modulation as well as impact of cellular genetic background remain enigmatic. We investigated cell lysosomal-mediated signaling in three hepatic cell lines (Huh7, HepG2 and Alexander) over magnetic nanoparticles treatment in different concentrations. Nanoparticle treatment resulted in reorganization of F-actin and tubulin cytoskeletal filaments. Lysosomal accumulation of nanoparticles induced significant lysosomal dysfunction and affected mTOR activity. Further, we found that nanoparticle treatment triggered autophagic flux in Alexander and Huh7 cells, whereas HepG2 remained unresponsive to the treatment. Indeed, Bcl-2 protein expression level was a key to the resistance of HepG2 cells to nanoparticle treatment. Additionally, we compared p53 protein subcellular localization within all three cell lines. Alexander cells increased cytosolic fraction of p53 upon nanoparticle treatment, contrary in Huh7 nanoparticles triggered nuclear localization of p53. Overall, this study provides knowledge that gains insight of molecular mechanisms of lysosome-mediated cell response upon nanoparticle treatment. Our findings make an inspiring background for the further study of remotely controlled cell death and nanoparticle based treatment. *The authors marked with an asterisk equally contributed to the work.

P-05.2-07
Delivery and targeting of fibrinolytic agents to blood clots

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Cardiovascular disease (CVD) accounts for nearly one-third of deaths worldwide. There are increasing evidences for a consistent association between denser fibrin clot structure, more resistant to degradation (fibrinolysis), with CVD and (athero)thrombotic disorders. Fibrin polymerization starts by thrombin-mediated cleavage of fibrinopeptides A and B from the N-terminal of Aα- and Bβ-chains of fibrinogen, respectively. This exposes small residue sequences, knobs A and B, that interact with their respective binding pockets on the C-terminal region of the Aα- and γ-chains of another fibrinogen molecule, leading to the formation of fibrin fibres. Liposome nanoparticles have drawn interest as pharmaceutical nanocarriers, due to their stability and content release in a controlled manner. The aim of the work is to develop an encapsulated fibrinolytic nanoparticle strategy with lower bleeding risk, to be incorporated in the clot structure. We studied the impact of the empty liposome nanoparticle on blood clot formation and lysis and demonstrated that it does not affect haemostasis properties, by recording clot polymerization and lysis kinetics. Using dynamic light scattering and zeta potential assays, we concluded that the nanoparticle is stable over time, without any measurable aggregation or change in its surface charge for 28 days. Turbidimetry studies showed that the presence of the nanoparticles was associated to a non-significant small increase in fibrin fiber radius, protofibril packing and protein content with increasing lipid concentrations. The fibrinolytic agent tissue plasminogen activator (tPA) was added as liposome cargo, achieving 60–80% encapsulation efficiency. Preliminary results demonstrated a controlled release of tPA in a solid emulation of a clot, without activity loss. The work is now focused on optimizing the nanocarrier by surface decoration with a targeting element toward fibrin clots.

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P-05.2-08
The efficacy of designed anti-measles virus peptides depends on the stability of self-assembled clusters

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The resurgence of several infectious diseases, like measles, has driven the search for new chemotherapeutics to prevent and treat viral infections. Self-assembling antiviral peptides are a promising class of entry inhibitors capable of meeting this need. Fusion inhibitory peptides derived from the heptad repeat of the C-terminal (HRC) of the measles fusion protein, dimerized and conjugated with lipophilic groups, were found to be efficacious against measles virus. The structures of the self-assembled nanoparticles formed by these peptides modulated their activity. Based on the analysis of a L454W mutation in the fusion protein of a naturally occurring measles viral isolate, HRC peptides bearing the tryptophan residue at position 454 (HRC-L454W) were synthesized with the goal of improving membrane anchoring and manipulating self-assembly. Monomeric and dimeric peptides, whether conjugated or not to a single lipophilic group, reduced infection in vivo. Bis-conjugation with lipophilic groups, in contrast, abrogated activity. Based on the physicochemical properties of self-assembly and membrane insertion kinetics of the HRC-L454W peptides we show that bis-conjugation increases the stability and order of the inner core of the spontaneously self-assembled nanoparticles, resulting in their compaction. The presence of the tryptophan residue also increases steric hindrance effects in the nanoparticle of the dimeric peptides, contributing to inter-peptide cluster meshing, but the same level of compaction is not achieved. We propose that the highly ordered packing and stability of molecular clusters forming the inner core of self-assembled nanoparticles prevent efficient dissociation of the peptides in vivo, hindering their release and therefore eliminating their antiviral efficacy.

P-05.2-09
Application of peroxidase-mimicking DNAzyme-nanobody conjugate for the detection of harmful microalgae

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We site-specifically conjugated a nanobody specific for Alexandrium minutum with a single-stranded G-quadruplex DNA complexed with hemin and possessing peroxidase mimicking (DNAzyme) using the self-labeling tag mVirD2 derived from a truncated VirD2 protein of Agrobacterium tumefaciens. The conjugate was capable of specific binding to the surface antigen on A. minutum and showed catalytic activity when the chromogenic 2,2′-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) was used in combination with hydrogen peroxide as a substrate, resulting in the accumulation of metastable blue-green radical cation as it is for peroxidase enzymes. We successfully developed a stable and sensitive detection system without the need of additional enzymes/antibodies as well as no significant loss in catalytic/binding ability. This approach enables the flexible design of DNAzyme conjugates with potential applications in electrochemical sensor technologies for the detection of biological elements.

P-05.2-10
Vinca species: from plant extracts to green-synthesized nanoparticles

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Natural compounds in plants are studied for the chemical structure of the molecules and their potential as chemotherapeutic drugs. Thus, medicinal plants are more often used as reducing agents to prepare metal nanoparticles through a technique defined as 'green-synthesis'. Based on previous studies in the Vinca extracts, *Vinca minor* was used for green-synthesis of Ag-MnO_2 nanocomposites, along with the well-known *Chelidonium majus*. Three types of nanoparticles (NPs) were obtained: VmNPs (using *V. minor*), CmNPs (using *C. majus*), and MNPs (using a mix of the two extracts) which were characterized through S/TEM, EDX, XRD, and FTIR methods. Their antibacterial properties were tested on *S. aureus* and *E. coli*, and their cytotoxicity was assessed on HaCaT keratinocytes and A375 melanoma cells. The NPs were uniformly shaped and sized, depending on the extract used. Thus, MNPs were the smallest, followed by VmNPs, and CmNPs, with manganese oxide in the middle, silver oxide and an additional layer of organic compound, on the exterior. Both bacterial strains were inhibited, with better results against *S. aureus* by VmNPs, followed by MNPs, and CmNPs which had no effect on the bacterial strains. When cytotoxicity was examined, melanoma cells were significantly more affected than keratinocytes ($P < 0.0001$) when VmNPs were used at concentrations above 500 μg/mL, MNPs negatively affected both cell lines and CmNPs did not inhibit cell growth but had a rather proliferative effect, in same experimental conditions. The results indicated that the combination of plant extracts in a 1:1 ratio is highly efficient for NP synthesis, but the small size and high content of secondary metabolites harmed both normal keratinocytes and skin melanoma cells. Further studies are required to find the optimal extract concentration and to investigate the potential damages induced even when cell viability is not affected. *The authors marked with an asterisk equally contributed to the work.

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P-05.2-11
Typical 2-cys peroxiredoxin: applications of a morpheein in bionanotechnology

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Morpheeins are proteins that reversibly self-assemble into different quaternary oligomers, whose architectures are governed by conformational changes of the subunits [previously published in: Jaffe EK (2005) Trends Biochem Sci 30, 490–497]. This property is useful in bionanotechnology where "bottom-up" building of new, high-ordered nanostructures is required: by capitalizing on morpheeins' self-assembly into patterned structures and exploiting their inborn affinity towards inorganic and living matter, responsive nanostructures and nanodevices can be obtained and exploited as such or as molecular scaffolds for more complex materials in practical purposes. In this context, peroxiredoxins (Prxs) represent the paradigm of a morpheein that can be applied to bionanotechnology. Here, we report on the structural and functional transitions that Prxs undergo to form patterned oligomers, e.g., rings, tubes, particles, and catenanes, and collect chemical and genetic engineering approaches to employ them in the generation of responsive nanometric items [previously published in: Ardini M et al (2021) Bioconjugate Chem 32, 43–62]. A relevant case of study is emphasized where we describe bio-tailored assembly of optically active plasmonic silver nanorings (AgNRs) over graphene-coated nanopore arrays exploiting the Prx ring scaffold [previously published in: Giovannini G & Ardini M et al (2020) Adv Opt Mater 8, 1901583]. Ag^+ ions are adsorbed over Prx and chemically reduced to yield small colloidal AgNRs, which are then placed on solid-state arrays of nanopores coated with graphene monolayers by taking into account of the affinity of Prx towards graphene. Moreover, drilling of 2 nm holes is easily achieved by focusing electron beam at the AgNRs center to get a final hybrid nanopore endowed with enhanced fluorescence. We foresee the application of such hybrid device for flow-through analysis of biomolecules, e.g., next-generation sequencing and single-molecule detection. *The authors marked with an asterisk equally contributed to the work.

P-05.2-12
Stefin A-functionalized liposomes as a system for cathepsins S and L-targeted drug delivery

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Cysteine cathepsins are papain-like lysosomal proteases, synthesized as proenzymes and predominantly localized in the endolysosomal vesicles. They have been shown to play a role in different stages of cancer progression. Among the cathepsins, upregulation of cathepsins S and L has been reported for different types of cancer. Cathepsins S and L can also be secreted into the tumor microenvironment, either by tumor or immune cells. Their extracellular localization and overexpression in different cancer types make them promising targets for targeted drug delivery and diagnostics. Stefin A is an endogenous inhibitor of cysteine cathepsins, including cathepsins S and L. Its small size, high stability and absence of disulfide bonds make it a suitable ligand for active drug targeting. In our work, we used stefin A [D61C] mutant, in which the mutation of Asp61 to Cys enables the conjugation of the protein through the maleimide-cysteine reaction. To target cathepsins S and L, we have prepared stefin A[D61C]-conjugated liposomes. Liposomes are a widely used drug delivery system for small molecules as well as proteins. An advantage of the liposomal delivery system is also the possibility of functionalizing the surface. Stefin A[D61C]-conjugated liposomes were shown to bind efficiently to recombinant cathepsins S and L, and also block their activity. Administered Stefin A targeted liposomes have been demonstrated to accumulate in tumors of a mouse breast cancer model, thereby demonstrating a potential for the cancer targeting diagnostic and/or therapeutic approaches.

P-05.2-13
De novo designed nanostructures functionalized as recognition sensors

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Conceptual and computational advances triggered an explosion of designed proteins in the last decade. De novo proteins that imitate or even supersede natural proteins, promises revolution in synthetic and structural biology, as well as in biotechnology and medicine. We developed a strategy to create *de novo* protein nanostructures, called coiled-coil protein origami (CCPO). [1,2] CCPOs are single-chain polyhedral structures constructed from concatenated coiled coil-forming building modules of different lengths and properties. Using orthogonal coiled coil-forming pairs whose dimerization chemistry is specific and well understood, we can construct protein self-assembling nanostructures of various sizes, shapes and properties. One of opportunities is to exploit CCPOs with architecture as the scaffolds for functionalization with bio-specific recognition molecules since CCPOs have potential for biomedical applications for diagnostics, prevention and therapy. [3] CCPO technology enabled us to assemble triangular constructs, based on a single polypeptide chain, composed of six concatenated CC-forming modules. They were assembled in bacterial cells, according to their predicted design. We analyzed secondary structure, stability and other properties with a set of biochemical and biophysical methods (CD, DLS and SEC-MALS). SAXS analysis and high resolution cryo-EM imaging were performed to confirm the agreement of the structure with computationally designed models. At selected positions we incorporated domains in order to introduce functions, such as recognition of target molecules. Lectins added into CCPO can act as bio-specific recognition molecules for glycans that play a role in infections, cell adhesion and progression of various diseases and are thus of great interest for diagnostic and therapeutic applications. References: [1] Gradišar H et al. (2013) Nat Chem Biol 9, 362–366. [2] Ljubetič A et al. (2017) Nat Biotechnol 35, 1094–1101. [3]. Lapenta F et al. (2018) Chem Soc Rev 47, 3530–3542.

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P-05.2-14
Lactococcus lactis simultaneously displaying protein binders of tumor-associated antigens and proinflammatory cytokines for targeted therapy of colorectal cancer

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Proinflammatory cytokines interleukin (IL)-6, tumor necrosis factor (TNF), and IL-8 promote the development of colorectal cancer (CRC). The delivery of cytokine-binding proteins into the gut using tumor-targeted safe probiotic lactic acid bacteria as a vector might represent an alternative therapeutic approach in inflammation-associated CRC. We engineered six *Lactococcus lactis* strains to simultaneously display a combination of small protein binder of tumor-associated antigen (HER2-binding affibody or EpCam-binding affitin) and a binder of proinflammatory cytokine (IL-6-binding affibody, TNF-α-binding affibody or IL-8-binding evasin) on their surface. Surface display of two proteins was achieved by cloning the genes into a lactococcal plasmid for dual protein expression (constructed by doubling nisin promoter). Protein binders were fused to Usp secretion signal and AcmA anchor to enable their attachment onto the surface. The expression of HER/EpCam-binding ligands was confirmed by western blot or flow cytometry. The functionality of cytokine-binding moieties on the bacterial surface was demonstrated by testing their ability to bind cytokines using ELISA. Engineered bacteria sequestered 80–100% of the corresponding cytokine spiked into the solution, whereby L. lactis displaying IL-6-binding affibody proved the most efficient. Furthermore, engineered bacteria were co-cultured with immunostimulated cancer cells and showed that it is able to bind cytokines released in the culture medium. Strains displaying IL-8-binding evasin removed 60–65% of IL-8 secreted by Caco-2 and 58–62% of IL-8 secreted by HT29 colorectal cancer cells. Strain displaying IL-6-binding affibody removed 92–94% of IL-6 secreted by human monocytic THP-1 cells (undifferentiated and differentiated) and up to 96% of IL-6 secreted by myeloid leukaemia U-937 cells. Adhesion of engineered bacteria to Caco-2 cells is currently being tested to assess their tumor-targeting capacity.

P-05.2-15
Immobilization of cysteine proteases on chitosan with possible portion desorption

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Ficin (EC 3.4.22.3), papain (EC 3.4.22.2) and bromelain (EC 3.4.22.32) are thiol proteases plant origin. These enzymes can be used in antimicrobial therapy, as they destroy bacterial films. Due to the fact that native cysteine proteases are subject to autolysis and rapid inactivation under the influence of various factors, the immobilization of biocatalysts on an insoluble matrix is of high applied value. Chitosan can be used in the development of methods for the targeted delivery of drugs through the controlled release of medical substances. The aim of the work was to study the portion desorption of proteolytic enzymes from the surface of chitosan. Medium molecular weight (200 kDa) and high molecular weight (350 kDa) acid soluble chitosans were used as a matrix to immobilize cysteine proteolytic enzymes by adsorption. Enzymes were desorbed from the chitosan matrix in 50 mM Tris-HCl buffer (pH 7.5) and 0.9 % sodium chloride solution for 48 hours. The desorption of ficin, papain and bromelain from the chitosan matrix after 48 hours of incubation in 50 mM Tris-HCl buffer (pH 7.5) at 37 °C did not exceed 27 %. When immobilized ficin, papain and bromelain were placed in physiological saline, more than 80% desorption was observed for high molecular weight chitosan and 70–80% for medium molecular weight chitosan after 48 hours of incubation. Immobilized enzyme preparations with controlled desorption can be used in the development of plasters, dressings and other materials for the treatment of purulent wounds. This work was financially supported in the form of a grant from the President of the Russian Federation for state support to young Russian scientists - doctors of sciences MD-1982.2020.4.

P-05.2-16
Inhibition of immobilized acetylcholinesterase

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Neurons are among the most metabolically active cells in the body. The expressed metabolic activity of neurons is indicated by the presence of numerous enzymatic systems by which neurotransmitters are synthesized from starting materials. Following synthesis, they are transported along the axons and stored in synaptic vesicles, which are facilitated by transport proteins. Acetylcholine (Ach) is synthesized in the cytoplasm of presynaptic nerve endings from the amino alcohols of choline and acetyl coenzyme A. The released acetylcholine is very short-lived because it is rapidly degraded by the enzyme acetylcholinesterase. First, ACh is released from the presynaptic nerve into the synaptic cleft due to the action potential traveling by the nerve fiber. At the membrane of the postsynaptic nerve fiber, Ach binds to the receptor, resulting in a change in resting potential, which generates action potential in the postsynaptic neuron. β-caryophyllen is a natural bicyclic sesquiterpene that is an ingredient in many essential oils. In addition to its proven anti-inflammatory effects, recent research indicates that it has a positive effect on the treatment of arthritis, osteoporosis, and cancer. The inhibition of terpene by acetylcholinesterase activity was examined by an electrochemical method. We have come to the conclusion that terpene acts as a competitive inhibitor because it binds to the enzyme / substrate complex and prevents dissociation of the enzyme / product of the complex.

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P-05.2-17
Peptide ligands of IgG Fc region for purification and immobilization of antibodies

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We developed a tailored screening protocol to isolate peptide binders of the IgG Fc region from a large combinatorial phage display library (previously published in: Kruljec et al. (2018) Bioconjugate Chem 29(8), 2763–2775). The top candidate peptide was characterized in detail by convenient on-phage trimming and alanine scanning analysis to identify residues crucial for binding. We further constructed a focused phagemid-based peptide library by randomizing non-essential residues of the lead peptide and subjected it to a single selection round against human IgG pool, followed by next-generation sequencing for unbiased monitoring of clone enrichment. An affinity matrix was prepared by coupling the synthetic affinity-matured peptide to cross-linked agarose beads via a short bifurcated linker tris(2-aminoethyl)amine. The peptide-based affinity chromatographic column facilitated isolation of human IgG of all subclasses at purities comparable to those achieved with commercial protein A column. The dynamic binding capacity for both affinity columns was ~40 mg IgG/mL resin. Importantly, the peptide-based affinity chromatographic column withstood harsh cleaning-in-place conditions (e.g. 0.5 M NaOH and 30% isopropanol) without functionality loss. The peptides reported here represent a promising alternative to staphylococcal immunoglobulin-binding protein A in antibody downstream processing due to affordable synthesis, chemical stability, expected low immunogenicity, and moderate affinity for the Fc region. In addition, we envision that our peptides might find use as ligands for reversible and homogenous immobilization of antibodies to biosensor surfaces and/or immunoprecipitation beads.

P-05.2-18
Biological evaluation of novel amphiphilic nucleic acid – polymer nanoparticles in eukaryotic cells

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Polymer-based gene delivery systems are globally studied, as safer and less immunogenic substitutes to viral systems. However, as nucleic acids (NAs) are stable and hydrophilic molecules, and their intracellular delivery and targeting rely on secondary carriers. In this work we are presenting the biological effects of novel nucleic acid-polymer conjugates (NAPCs) on two cell lines – A549 and HepG2. NAPCs are composed by oligonucleotides functionalized with different polyethers such as poly(ethoxyethyl glycidyl ether) and polyesters such as poly(ε-caprolactone). The NAPC assemblies were found to carry thousands of oligonucleotide strands per particle. Using MTT and crystal violet assays we investigate cytotoxicity of NAPCs applied on cells at different concentrations. The effect on cell viability was determined by incubating the cells for 48 h with nanoparticles in a concentration-dependent manner. NAPCs behavior into cells was investigated by microscopy observations after Neutral red staining and SYBR Green I staining (Previously published in: Bakardzhiev P et. al. (2020) European Polymer Journal, 131). Our results suggest that the biological properties of the NAPCs include low toxicity and rapid cellular uptake without the need of other transfection agents. We conclude that these novel NAPCs are promising carriers for the delivery of DNA with potential for biomedical applications. Acknowledgments: This work was supported by grant DN 19/8-2017 from Bulgarian National Science Fund.

P-05.2-19
Anti-Stokes fluorescence of phycobilisomes quenched by orange carotenoid protein

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Phycobilisomes (PBs) are water soluble megadalton light-harvesting complexes of cyanobacteria and red algae. They consist of hundreds of phycobiliproteins containing thousands of bilin pigments, which are organized in an "energy funnel" to effectively deliver excitation to photosystems. Quenching of excess excitation energy is necessary for the photoprotection of light-harvesting complexes. In cyanobacteria quenching of PBs excitation is induced by the orange carotenoid protein (OCP) which photoactivation occurs under high light conditions. However, recent studies have shown that PBs can switch among various states depending on lighting conditions without OCP, presumably due to intramolecular charge-transfer within a phycobilins, which might play an essential physiological role. In this work using time-resolved fluorescence spectroscopy with picosecond time resolution in single photon counting mode we investigate spectroscopic signatures of Synechocystis sp. PBs and their components in absence and in presence of activated OCP. Since PBs is a multilevel system with energy transfer, we decided to use low energy quanta to excite the lowest energy levels of pigments in PBs core. Analysis of anti-Stokes fluorescence showed that the maximum fluorescence intensity of PBs is shifted to longer wavelengths by about 30 nm compared to excitation to the absorption maximum of phycocyanin. These long-wavelength excited states are characterized by approximately 2 ns lifetime, which does not change when the OCP complex with the PBs is formed. We assume that investigation of the nature of excited states, selectively excited in the infrared region, can provide a better understanding of the processes of energy conversion in light-harvesting complexes and the mechanisms of photoprotective reactions. This study was supported by Russian Science Foundation (grant no. 21-44-00005).

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P-05.2-20
Hydrogel with antimicrobial peptide Pentadefenin eliminates infection in a murine model of bacterial vaginitis

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Vaginitis (colpitis) is an inflammation of vaginal mucosa. Most often it's caused by specific pathogens (*Gonococcus* spp., *Chlamydia* spp., etc.) and followed by opportunistic infection (*Staphylococcus* spp., *Streptococcus* spp., *Candida* spp., etc.). Vaginitis is diagnosed at least once in up to 80% of women with relapse in a year in 50% cases. Its treatment becomes less effective due to the spread of bacteria resistant to common antibiotics. To overcome such resistance antimicrobial peptides may be used. This work aimed to develop and preclinically test a candidate drug for bacterial vaginitis treatment based on novel synthetic antimicrobial peptide Pentadefenin. For use in vaginitis treatment, it was incorporated in a buffered hydroxyethylcellulose hydrogel. The gel was tested in a murine model of bacterial vaginitis caused by *Staphylococcus aureus* and *Streptococcus agalactiae*. After the treatment course, these bacteria were bacteriologically detected only in 10% of mice. Opportunistic bacteria (*Gardnerella vaginalis*, *Prevotella bivia*, *Porphyromonas* spp.) DNA level detected with qPCR was lowered 100-fold. Gel application also increased the level of bacteriospecific secreted IgA and suppressed inflammation of murine uterus caused by infection. Pentadefenin caused no chronic toxicity, no local irritation, and no pyrogenic effect. In the acute toxicity experiment it was not toxic at dose 2 g/kg, which is 10,000 times higher than the intended human therapeutic dose. Its preclinical trials as a vaginal microbicide are now completed. We can conclude that the gel with Pentadefenin suppresses an infectious process in the vagina and could be potentially used for the treatment of bacterial vaginitis.

P-05.2-21
Immobilized gold nanoparticle based plasmonic assay platform for biomolecule and microorganism detection

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Plasmonic sensors are suitable tools for study of molecular interactions. Localized Surface Plasmon Resonance (LSPR) based sensors detect spectral changes associated with intramolecular interactions between analyte molecules and recognition elements. Due to its label-free and highly sensitive features, LSPR based methods have high potential for biosensing applications. In this study, we aim to develop a sensitive, label-free, rapid and simple biosensing platform. For this purpose, a novel refractive index (RI) sensitivity enhancement methodology is proposed by immobilizing gold nanoparticles (GNPs) for platform-based LSPR. Fabrication of platform was carried out by GNP synthesis, immobilization of GNPs on polystyrene solid support, and growth of GNPs. Validation of response to RI changes of developed sensor platform was carried out by tests with varying concentrations of sucrose and ethanol. Then as a proof-of-concept, detection ability and detection limit determination of *E. coli* BL21 (DE3) and protein Bovine Serum Albumin (BSA) was carried out. Adsorption of *E. coli* BL21 (DE3) via bulk interactions showed that the developed LSPR platform exhibit high enough binding affinity for bacteria detection, and was able to detect down to concentrations as low as 10^2 CFU/mL. Immune capturing of BSA via anti-BSA antibody showed that the developed LSPR platform was able to detect BSA protein–antibody interaction down to 10 μM concentration range. Herein we propose an LSPR-based sensing platform, promising possibility of application in immunoassays and also in microorganism monitoring. The platform simplifies current procedures and enhances sensitivity; both in terms of molecular size of analyte and analyte concentration.

P-05.2-22
Nanofibers enable high loading and long-term viability of *Lactobacillus* spp.

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The interest in *Lactobacillus* spp. has grown in recent years due to increased awareness of the importance of probiotics and microbiota for human health. Electrospinning is an established technique to produce fibers with small diameter in the range of several nanometers to micrometers, often called nanofibers. Incorporation of *Lactobacillus* spp. in nanofibers allows for the concomitant drying of the bacteria and the preparation of solid dosage form, thereby offering considerable advantage over, e.g., lyophilization. We have incorporated ten different species of lactobacilli in poly(ethylene oxide) nanofibers and characterized them using scanning electron microscopy. All bacteria retained considerable viability (1–2 logs decrease) that correlated with cell hydrophobicity. We have focused our efforts on the *Lactobacillus plantarum* ATCC 8014 by developing poly(ethylene oxide) and composite poly(ethylene oxide)/ lyoprotectant nanofibers. High loading was achieved for *L. plantarum* cells (up to 7.6×10^8 colony-forming unit /mg) that were either unmodified or expressing mCherry fluorescent protein. The initial concentration of *L. plantarum* electrospinning solution was reported as the most critical parameter for its high viability after electrospinning. The presence of amorphous lyoprotectant (especially trehalose) in the nanofibers promoted *L. plantarum* survival due to lyoprotectant interactions with *L. plantarum* cells. *L. plantarum* cells in nanofibers were stable over 24 weeks at low temperature, thereby achieving stability comparable with that in lyophilizates. The poly(ethylene oxide) nanofibers released almost all of the *L. plantarum* cells over 30 min, which will be adequate for their local administration. Our integrated approach enabled development of a promising local nanodelivery system for lactobacilli for topical or vaginal application.

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P-05.2-23
Targeted mutations near type 1 cooper center of two-domain laccase from *Streptomyces griseoflavus*

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Laccases (EC 1.10.3.2) are multicopper enzymes that catalyze oxidation of phenolic and non-phenolic substrates coupled to reduction of molecular oxygen to water. Known laccase substrates include phenols, arylamines, anilines, benzenethiols, and metal ions. Laccases are widespread class of enzymes involved in pathogenesis, immunogenesis and morphogenesis of organisms, metabolic conversion of different organic substances. The active centre of all laccases consists of 4 copper sites. Type 1 (T1) responsible for oxidation of substrates, type 2 (T2), and a coupled binuclear type 3 pair (T3) which are assembled in a T2/T3 trinuclear cluster (TNC) where molecular oxygen is reduced to water molecule. Along with the typical three-domain (3D) laccases bacteria from *Streptomyces* species produce small two-domain (2D) enzymes, which are active at acidic and basic pH, thermostable, and resistant to inhibitors but their activity is lower than that of 3D laccases. Based on analysis of three-dimension structures of two-domain laccases, we have chosen methionine at position 199 of 2D laccase from *Streptomyces griseoflavus* (SgfSL) near T1 copper site for mutational analysis. Met199 can play a key role in organization of substrate binding pocket of 2D laccases. We have constructed two mutant forms with the substitution of Met199 to amino acids with a smaller side chain – alanine and glycine and studied their enzymatic activity. We demonstrated that the activity of both mutants increased 2.5-5-fold for ABTS and for Met199Gly variant activity increased 5-fold using 2,6-DMP as substrate. Decolorization activity of both mutant forms against Malachite Green and Indigo Carmine dyes is also increased. This work was financially supported by the Russian Foundation for Basic Research № 19-34-90121

P-05.2-24
Aqueous fullerene dispersions as superoxide dismutase mimics

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Nanomedicine applications, in addition to physicochemical properties, require the knowledge of the biochemical activity of studied nanomaterials toward reactive oxygen species, especially superoxide anion radical (SAR), as a critical participant of free-radical homeostasis. Aqueous dispersions of unfunctionalized fullerenes (AFD) are of interest as they do not have groups that can be involved in metabolic processes. Aqueous dispersions of Gd endofullerenes (Gd-AFD) are considering as conceivable contrast agents for MRI. This work aimed at studying SOD-like properties of aqueous dispersions of fullerenes and endofullerenes towards superoxide-producing cell systems. The SOD-like activity was assessed by enhanced chemiluminescence (CL) in the lucigenin+NADH with rat brain homogenates. Using this model allows us to study the effect of nanoparticles (NPs) on the intracellular production of a SAR by a microsomal respiratory chain. The CL registered to the plateau up to 30 min. The total emission under the curve and the emission suppression vs. a blank sample have calculated as ($K = S/S_0$). Objects are AFDs of C_{60}, C_{70}, and C_{82}@Gd; conc. was up to 20 μM. All the samples have SOD-like activity to fibroblasts; $C_{60}(K = 0.15)$ turned out to be 2–3-fold more active than $C_{70}(K = 0.35)$, probably due to a higher permeability of C_{60} through membranes due to smaller cluster size. The activity of AFD of C_{82}@Gd($K = 0.62$) is half that of AFD C_{70} and five-fold lower than AFD C_{60}, also due to large particle size. Previously, we have shown pronounced SOD-like properties of AFDs and Gd-AFDs for the xanthine/xanthine oxidase model. Thus, this ability retained for cell cultures, with the activity inversely proportional to the NP size. The inertness of Gd-AFDs towards the SAR in tissues makes them promising contrasting nanopharmaceuticals due to a minimal effect on free-radical homeostasis. Acknowledgments. The Russian Science Foundation contr.No.19-73-00143 supports this work. *The authors marked with an asterisk equally contributed to the work.

P-05.2-25
In silico analysis of amino acid sequences of glioblastoma homing peptides

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To increase the specificity of existing and newly developed drugs for cancer treatment, short tumor-targeting peptides could be used. Earlier in our laboratory, using phage display, tumor-targeting phage particles to glioma cell line U-87 MG were obtained, which have been analyzed for binding efficiency of selected phages to glioma cell line U-87 MG cells in vitro, and for biodistribution and specificity of accumulation of bacteriophages in the tumor tissue of U87 MG (M.D. Dmitrieva et al. 2020). The objective of this study was to carry out a comparative in silico analysis of amino acid sequences of selected tumor-targeting peptides (№№ 19, 26, 49, 83, 92, 19C, 36C, 18C, 23C, 2C) with sequences of peptides and proteins that already have in the available databases using PepBank (S. F. Altschul et al. 1997). We found that sequence №19 was producing significant alignments with CAMPATH-1 antigen precursor; №19C with apolipoprotein A-I; №18C contained CR2-binding sites; №92 contained avidin and streptavidin binding motifs; the remain peptides did not have homology with any known peptides. We compared them with those already known from the literature. Thus, for comparative analysis, we took 10 peptides (this study) and 13 peptides from literature data, all selected to human glioma cell line U-87 MG. The most of the analyzed peptides belonged to two large branched clusters on Maximum- Parsimony tree (MEGA-X). Using WebLogo we generated a sequence logo. Despite significant polymorphism in the sequence the peptide motif, it can be possible to highlight the motif: R(A,P,S)P(Q,W)TR(L,S)GG(P)P(R,S,W), which is most often found in this peptide analysis. Both peptides in our research №92 (HPSSGSA) and №26 (SWTFGVQFALQH) partly contained a motif. In the future, we are going to investigate the targets - unique set of proteins or receptors on the glioblastoma cell surface. The present study is supported by the Russian Science Foundation project № 19-44-02006.

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P-05.2-26
Plant virus particles as potential adjuvants

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There is growing evidence that plant viruses and their structurally modified particles may have immunopotentiation effect on target antigens and can be applied in vaccine candidates without any additional immunostimulating substances. Herein we present data on the adjuvant properties of plant viruses with various shapes (rod-like virions of Tobacco mosaic virus (TMV); filamentous virions of Potato virus X (PVX); icosahedral virions of Cauliflower mosaic virus (CaMV)), and structurally modified TMV spherical particles (SPs). As a model antigen we selected ovalbumin (OVA) (42.7 kDa, Sigma, A5503-1G). We have analyzed the immune response to individual OVA and to OVA in compositions with plant viruses/SPs. The antibody titers to potential adjuvants were also evaluated after immunisations by OVA-plant viruses/SPs compositions. We have shown that CaMV, TMV and SPs can effectively induce total IgG titers to OVA. Intriguing data were obtained when analyzing immune responses to the virions/SPs themselves. Strong immunity was induced in response to CaMV and PVX, whereas TMV and SPs stimulated considerably lower self-IgG titers. Given the adjuvant effect on the immune response to OVA and self-immunity (IgG titers to virions/SPs), in our opinion, the most promising objects are SPs and TMV. We believe that our data provide new insights into plant viruses' immune potentiating properties and may be useful in further rational development of adjuvants and vaccine candidates. Previously published in: Evtushenko EA et al. (2020) Sci Rep 10, 10365. The work was funded in part by the Russian Science Foundation grant №18-14-00044 (obtaining and studying SPs) and by the grant of the RF President №075-15-2019-188.

P-05.2-27
Antimicrobial vesicles of *Lysobacter capsici* VKM B-2533T

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A rapid spread of antibiotic-resistant pathogenic microorganisms is one of the burning problems, the mankind encounters nowadays, which solution demands the urgent search, isolation and study of new antimicrobial agents. It was found out that some species of the genus Lysobacter are unique sources of such compounds. *L. capsici* VKM B-2533^T demonstrates an efficient antibacterial and antifungal activity. The aim of this work was to confirm that *L. capsici* is able to form unique nanoparticles - outer membrane vesicles, and to study their lytic potential. Vesicles were active against Gram-positive bacteria (*Micrococcus luteus*, *Bacillus cereus*, *Staphylococcus aureus*), filamentous fungi (*Fusarium solani*, *Sclerotinia sclerotiorum*, *Aspergilus niger*) and yeast (*Candida boidinii*, *C. utilis*). The antifungal activity of the culture liquid of *L. capsici* was associated with vesicles that could serve as vehicles promoting the release of the antifungal agents into the ambient medium. We supposed that antifungal and antibacterial activities may be associated with functionally different groups of vesicles. To confirm this supposition, the total preparation of vesicles was fractionated in a sucrose density gradient of 30–55%. As a result, 22 fractions were obtained. Using electron microscopy, it was found out that the vesicles were distributed in the middle fractions of the gradient fractions. All vesicular fractions demonstrated a high antibacterial activity, while one of them (the 9th) was active also against fungi and was thus associated with a specific group of vesicles. To isolate the antifungal agent from the total fraction of vesicles, a peculiar scheme of purification was developed consisting of multiple extractions and reverse phase HPLC. The isolated antifungal agent turned out to be active also against phytopathogenic fungi. Such its ability makes it a promising remedy for plant protection. Establishing of its structure will be the subject of the further research.

P-05.2-28
The new water-soluble C70 fullerenes substituents effect on the key antioxidant response human genes expression

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Modern medicine advances are promoted by nanotechnology progress. Fullerene C70 derivatives more effectively bind free radicals than fullerene C60 derivatives [Sergeeva, 2019]. However, the various substituents effects on the fullerene C70 derivatives antioxidant properties were not studied. The effect of two fullerene derivatives F1 C70[C6H4CH2CH2CH(COOK)2]8 and F2 C70[C4H2SCH2COOK]8 on human embryonic lung fibroblasts (HELF) was studied. The compounds toxicity was analyzed by the MTT test. H2DCFH-DA was applied for ROS synthesis assessment. The gene expression was studied by real-time PCR on the StepOnePlus system (Applied Byosystems, USA). The protein level was analyzed by flow cytometry (Cytoflex, Germany) and the protein localization was evaluated by fluorescence microscopy. The concentrations of F2 below 28 μg/mL, F1 below 6.4 μg/mL are non-cytotoxic for HELF. The most significant decrease in the cellular ROS level (1.6–2 times, $P < 0.05$) was observed for F1 at the 18.3 ng/mL concentration for 1–24 hours, but F1 did not reduce the ROS level in the cells 36–72 hours later. F2(18.3 ng/mL) had a prolonged antioxidant effect on HELF reducing the ROS level by 20–35% ($P < 0.05$) for 72 hours. The F1 addition (18.3 ng/mL) to HELF causes an increase in the NOX4 gene and protein expression by 1.5–2.8 times in 3–72 hours, which may induce an increase in the ROS level; the transcription factor NRF2 expression increases by 6–8 times ($P < 0.05$) in 3 hours, decreasing in 24 hours to control values. F2(18.3 ng/mL) does not induce NOX4 expression induction in HELF at the gene and protein levels in 72 hours; at the same time, the NRF2 expression of the increases 6–7 times in 24 hours after F2 was added to the cells. Flow cytofluorometry and fluorescence microscopy confirmed the data for NRF2. The work was performed as part of the state assignment of the Ministry of Education and Science of the Russian Federation with the support of the Russian Science Foundation grant No. 18-15-00437.

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P-05.2-29
The effect of minor modifications of fullerenes on SOD-like activity

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Fullerenes are particular interest among nanopharmaceuticals as intracellular regulators of the balance of reactive oxygen species, especially their activity with respect to the superoxide anion radical. The aim of the work was to study the role of the chemical structure of modifying substituents of C60 fullerenes in their manifestation of SOD-like properties. SOD-like activity was defined by activated luminescence in a fibroblast culture model (1.0 million in 1 mL) + lucigenin + NADH. This model permit to study the effect of nanoparticles on the intracellular production of a superoxide radical by a microsomal respiratory chain, which is the main source of a superoxide radical along with mitochondria. The luminescence was recorded until reaching the stationary level, the light sum under the curve for 30 min (S) and the degree of repression of luminescence with respect to control sample were calculated from chemiluminograms: K = S/S0. The fullerenes were studied: F-548 (M = 2275 g/mol), F-694 (M = 2387 g/mol), F-697 (M = 2499 g/mol) - C60 modified with potassium thiophenylcarboxylates (acetate, propionate, butyrate, respectively); F-966 (M = 2835 g/mol), modified with potassium diphenylacetate, and F-702 (M = 2161 g/mol) of a same structure, but containing fluorine in aromatic rings. The fullerenes F-548, F-694, F-697, having a same chemical structure of the substituents, showed almost the same and significant SOD-like activity - repression ratio 0.08 ± 0.01, 0.10 ± 0.02, 0.12 ± 0.01, respectively. It can be seen that the side chain length affects the SOD-like activity. F-966 and F-702 significantly differed in antioxidant properties - the repression ratio of F-966 was 0.61 ± 0.05; for F-702 0.02 ± 0.01, which is explained by the presence of fluorine. Thus, minor modifications such as the presence of heteroatoms and chain length have a major effect on SOD-like properties. Varying structure of the substituents makes it possible to obtain fullerenes with desired antioxidant properties.

P-05.2-30
Soluble cyanobacterial carotenoprotein as a robust antioxidant nanocarrier and delivery module

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Formation of reactive oxygen species (ROS) accompanies electron transfer reactions during aerobic respiration or photosynthesis. Since high ROS levels may be harmful to cells, antioxidants are crucial for maintaining their normal functioning. Carotenoids are excellent natural antioxidants playing important roles in photoprotection and regulation of photosynthetic activity of higher plants, algae, and cyanobacteria, but their exploitation and directed delivery to cells is extremely limited due to their hydrophobic nature and fast photodestruction. Mammalian cells cannot produce carotenoids, but they have multiple effects on the human and animal organism, not only as antioxidants. Interest in systems securing antioxidant stability and facilitating targeted delivery is largely explained by prospects of their using for the design of medical agents. We demonstrated that the recently discovered water-soluble carotenoprotein from Anabaena sp. PCC 7120 (AnaCTDH) can interact with liposomes to efficiently extract carotenoids, yielding violet–purple protein samples. We found that carotenoid transfer between the protein and lipid membranes is reversible and that the efficiency of this process depends on protein–membrane and protein–carotenoid interactions. We demonstrated that AnaCTDH can deliver of carotenoids into membranes of mammalian cells with almost 70% efficiency and decrease the hazard caused by ROS. We have designed the TagRFP-AnaCTDH chimera, where the addition of the TagRFP module (~26 kDa) to the N-terminus of AnaCTDH (~15 kDa) did not break the carotenoid binding and transfer capacity. The ability of AnaCTDH to extract CAN from membranes could potentially be utilized for curing pathological conditions. This study was supported by Russian Scientific Foundation project №.17-74-30019. The study was partially supported by the Russian Foundation for Basic Research and the German Research Foundation joint grant (no. 20-54-12018 and no. FR1276/6-1).

P-05.2-31
Listeria innocua biofilm development on abiotic surfaces

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Biofilms are an emerging form of bacterial growth and play an important role in human and environmental health as they can serve as a reservoir for pathogens. *Listeria monocytogenes* is an important foodborne pathogen associated with high hospitalization and mortality rates. A closely related species *Listeria innocua* is often used as a model organism for *L. monocytogenes*. Here, the dynamics *of L. innocua* ŽM39 biofilm growth on plastic material (polystyrene) was investigated using differential fluorescent staining and microscopy. The effect of inoculum size, adhesion and incubation time, and temperature on the growth, architecture and composition of biofilm was determined. The inoculum size and the adhesion time had no effect on the biofilm properties within 24 hours. However, there was a notable difference in biofilm development at the temperatures tested, with biofilm growth being supressed at 4 °C even after three days. Also, no loss of viability was observed and the amount of exopolysaccharides remained the same. Our results demonstrate that *L. innocua* adheres easily to plastic surfaces and that the biofilm can persist for several days implying an important ecological role of the biofilm in Listeria environmental persistence. *The authors marked with an asterisk equally contributed to the work.

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P-05.2-32
Heterologous production of AmiN kinase provides the resistance of mammalian cells toward ribosome-targeting antibiotic amicoumacin

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Amicoumacin A (Ami) is a broad-spectrum antibiotic displaying an inhibitory effect on protein translation by the disintegration of initiation complex array and the shifting of mRNA-tRNA complex motion during relocation. Ami exhibits not only strong antimicrobial properties but also potent anti-inflammatory, antiulcer and antitumor activity. The study of the biosynthetic gene cluster of the Ami producing strain *Bacillus pumilus* 124 led to the discovery of the self-resistance kinase AmiN inactivating Ami by phosphorylation that is reversed *in vivo* by the respective phosphatase AmiO. Here we evaluated the effect of heterologous expression of amiN on the resistance of prokaryotic and eukaryotic cells toward Ami. Preliminary evaluation of Ami antibacterial potential was performed on the model *E. coli* ΔtolC cells highly sensitive to different antimicrobials. The heterologous expression of amiN increased the minimum inhibitory concentration (MIC) of Ami by 100 times in this model bacterium. Similarly, the transfection of HEK293-T cells with a genetic construct containing the amiN gene elevated the half-maximal inhibitory concentration (IC50) of Ami by more than 300 folds, assessed by the MTT-test and flow cytometry. Thus, we suggest that a remarkable difference in inhibitory concentrations between wild type and recombinant cells ensures high selection efficiency. We believe that the described anti-Ami activity will provide essential insight into the antibiotic resistance problem and find numerous applications in the field of synthetic biology and biotechnology. This work is supported by RSF grant 19-14-00331.

P-05.2-33
Amphotericin-loaded flavonosomes as a new prototype for lipid-based formulations of polyene antibiotic

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Lipid-based formulations of amphotericin B (AmB) provide well proven opportunities to reduce the nephrotoxicity of the antibiotic. The toxicity of AmB is due to its ability to bind not only to ergosterol in the membranes of fungal cells but also to cholesterol in the membranes of human cells. Therefore, in order to reduce toxicity, we tried to find a sterol, the affinity to which of AmB is less than ergosterol, but more than to cholesterol. Using the assay with calcein leakage from large unilamellar liposomes mimicking the membranes of mammalian and fungal cells, we found that the most suitable candidates with "intermediate" affinity are the 7-dehydrocholesterol, cholesterol precursor in its biosynthetic pathway, and the desmosterol, one of the plant sterols. Moreover, we demonstrated that the inclusion of flavonoid phloretin into the AmB-modified liposomes led to increase in the ability to disengage a fluorescent marker from large unilamellar lipid vesicles that indicated the increased membrane permeability and should have a clinical consequence for enhanced efficiency of such polyene-loaded flavosomes. The replacement of phloretin to the other flavonoids, biochanin A, genistein, quercetin, showed that the phloretin is the most active agent. The current – voltage characteristics of the planar lipid bilayers modified by AmB-loaded flavonosomes revealed the functioning of double-length polyene channels that are usually formed at one order of magnitude less antibiotic concentrations than single-length channels. Thus, the developed AmB-loaded flavonosomes might be a prototype of a new lipid-based formulation of polyene macrolides with improved pharmacological characteristics for treatment invasive fungal infections and leishmaniasis. The study was supported by grants of the President of RF # MD-2711.2019.4 and RFS # 19-14-00110.

P-05.2-34
Cardoon (*Cynara cardunculus*) seed oilcake as natural source for bioplastics production

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The production of innovative environment-friendly materials as substitutes of the high pollutant petroleum-derived plastics is in the spotlight. Oilseed crops produce a huge amount of protein containing by-products known as oilseed cakes, which can be considered as novel bioplastics sources. Protein concentrates (PCs) from cardoon defatted seeds were prepared and used to obtain different film forming solutions (FFSs) containing different amounts of glycerol (GLY) as plasticizer. The FFSs were characterized by dynamic light scattering and zeta-potential and the derived films analysed for mechanical properties. The results showed that the presence of 30% GLY (w/w protein) was strictly needed to provide handleable materials. Both tensile strength (TS) and Young's modulus (YM) of the films containing different amounts of PCs (200–400 mg) decreased as a function of plasticizer concentrations, whereas their elongation at break (EB) simultaneously increased. However, film TS, EB and thickness values were higher, whereas those of YM were lower, when PC concentrations were increased. Thermogravimetric and differential scanning calorimetry analyses showed that plasticized cardoon-based films were more stable than the films obtained in the absence of GLY, shifting the maximum of degradation peaks towards higher temperatures. In the attempt to reinforce film matrix, cardoon PCs were also analyzed as possible substrates of microbial transglutaminase (mTG), an enzyme catalysing the formation of protein intermolecular ε-(γ-glutamyl)-lysine crosslinks. Preliminary results showed that cardoon PC contained effective mTG protein substrates able to act as both acyl donors and acceptors giving rise to high mol.wt. polymers. Acknowledgements: Authors acknowledge italian Ministero dell'Istruzione, dell'Università e della Ricerca for the financial support (PRIN, Progetti di Ricerca di Interesse Nazionale, 2017, COD. 2017KBTK93).

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P-05.2-35
Nanoscopic structure analyses of dorsal skin samples in wound healing rat model by using spider silk materials

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Spider silk is a natural protein having good biocompability that provides ease of use in tissue engineering applications such as skin wound covers, nerve damage repair, artificial joints. This study investigates the application of spider dragline silk as a surgical suture material. Each spider silk filaments were prepared by using 360 ± 15 draglines with length of 20 ± 1 cm and diameter of 0.8 ± 0.2 mm. Sterilization was done by gamma rays with a convenient energy and dose for biomedical applications. Dorsal skin flap model was used to evaluate the effects of different suture materials in wound healing. Twenty seven Wistar albino rats were randomly divided into five groups. Four types of different sutures *Argiope bruennichi* silk, *Aloe vera* coated *A. bruennichi* silk, commercial suture, *A. vera* coated commercial suture were used on the 2 cm x 4 cm dorsal flaps, as well as a control group (without any operation) was included. Each group was evaluated on the 7th and 14th days for wound-healing. Small-angle X-ray scattering (SAXS) method was used to investigate nanoscale structural changes according to the control groups. HECUS System including Kratky Optic was used with X-Ray source. 1024 Scattering data was used in determination and refinement of the nano scaled 3D ab-initio models. The best models were determined and electron density differences with their size and distributions are also obtained for the studied biological samples. Additionally, the obtained inner-phase structural details were indicated as evidence of the successful surgical applications and well tissue repairs with newly designed spider silk filaments. Acknowledgements: This research was supported by Hacettepe University Scientific Research Projects Coordination Unit (Grant No: FBA-2018-16615).

P-05.2-36
Modifications of a viral coat protein to produce virus-like particles of various architectures

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Plant viruses represent an ideal starting point for fabricating structurally controllable nanoscale devices with novel functionalities. Viral particles can also be utilized in a non-infectious nucleic acid-free form, called a virus-like particles (VLPs), which often retain similar symmetry, shape and size as the native virus. As they can be precisely genetically and chemically modified, they are gaining importance in the field of nanobiotechnology and medical applications as drug nanocontainers, imaging agents, delivery systems or parts of biosensors and other nanomaterials. Our research group uses various biochemical, biophysical and structural approaches to study Potato virus Y (PVY), which belongs to the Potyvirus genus of flexible filamentous plant viruses. We recently determined cryo-EM structures of a the virus and the corresponding VLP filaments at near-atomic resolution [1]. In the viral particle, around 2000 coat protein (CP) units assemble in the left-handed helical symmetry (CPs) around the viral (+)ssRNA, while in VLP filaments, CP units form octameric rings that stack into up to 3 μm long "RNA-free" filaments, which are unique in architecture for PVY-based VLPs compared to other Potyvirus viruses. These structures now open up possibilities to produce VLP filaments of various architectures in solution by structural modifications of CP as a building block. In this poster, we will show how modifications of the primary structure of CP (deletions, insertions, point mutations) affect the three-dimensional structure of VLPs as well as their biochemical and biophysical features. In view of extending the spectrum of potential applications, tailored VLPs with the possibility of biomolecule conjugation on the filament scaffold will also be presented. [1] Kežar A. et al. (2019) Sci. Adv. 5(7), eaaw3808.

P-05.2-37
Supramolecular ribbon-like Congo red and their interactions with albumin and immune complexes for medical drug delivery purposes

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Drugs (D) used for chemotherapy are not selective enough. Carriers of D increase targeted delivery of carried cargo, lower the reactivity threshold and ensure its safe remove. Molecules which create supramolecular ribbon-like structures (S) of Congo red type (CR) undergo self-association in an aqueous solution and bind D by intercalation. Advantage of such structures is the possibility of protein (albumin and immune complexes) binding, enabling targeted transport. The aim of the study was to check whether the model carrier system made of albumin and CR efficiently binds doxorubicin (Dox) and release it at a reduced pH and at place of immune complexes existing. The following methods were used: electrophoresis, dialysis, gel filtration, spectral analysis and DLS. The research model constituted T24-bladder cancer cell line. Analysis of toxicity, proliferation and apoptosis were taken. Also heat aggregated immunoglobulins was analyzed as immune complexes model. Results: We described the large CR-Dox complex, that differs in properties from free CR. The presented results show that albumin binds both: free CR and CR-Dox. Various CR-Dox molar ratios: 5:1, 2:1 and 1:1 were analyzed. Albumin has been shown to compete with the immune complex for binding CR-Dox and may eventually donate it to the immune complex. Combined with the result of drug release from the BSA-CR-Dox triple complex at reduced pH and the results of in vivo CR donation into the formed immune complex, it supports the theory of using the BSA-CR complex as an effective drug carrier. The IC50 doses of Dox and CR-Dox have been established. Conclusion: The presented research is important due to the search for optimal solutions for the use of S in drug immunotargeting using the protective role of albumin as a nanocarrier. We acknowledge the financial support from the National Science Centre, Poland (grant no. 2016/21/D/NZ1/02763). K. K. acknowledges the support of InterDokMed project no. POWR.03.02.00-00-1013/16

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P-05.2-38
Attempts to develop nano-targeting of glioblastoma stem cells

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Malignant gliomas are the most common type of primary malignant brain tumor, with an annual incidence of 5.6 per 100,000 individuals. Among them, glioblastoma (GBM) is the most frequent and malignant histological type, accounting for 65% of gliomas. Despite a better understanding of tumor biology and the development of new treatments over the past two decades, it remains incurable with a poor prognosis, characterized by an average survival of only 15 months after diagnosis. Rapid reccurence of the tumor after surgery is due to the presence of higly proliferative cancer stem cells (GSCs) that remain after surgical resection and are resistant to chemo- and radiotherapy. Extensive research efforts are therefore focused on finding GSC-specific biomarkers as potential targets of developing new therapeutic approaches. In this context, we used a reverse proteomics approach using camellid nanobodies (Nbs) in combination with virtual screening of transcriptome datasets, where we identified 11 potential GBM or GSC biomarkers to date, including a membrane-specific FREM2, which shows high over-expression in GSCs compared to normal brain cells. Small and especially positively charged 2.5 x 4 nm Nbs have been reported to cross the blood-brain barrier and penetrate brain cells, which was also found in our experiments with GBM or GSC cell lines. Naked Nbs efficiently enter these cells, some of which exhibiting a significant cytotoxic effect, but to avoid extracellular Nb degradation and increase the specificity of GSC targeting, our experiments are designed to package naked Nbs or their genetic information in the form of expression constructs into appropriate carriers such as liposomal archaeosomes or extracellular vesicles. The incorporation of anti-FREM2 Nb into the membrane structure of these vectors offers the possibility of specific GSC targeting in the intended in vivo experiments with zebra fish or GBM rat models. *The authors marked with an asterisk equally contributed to the work.

P-05.2-39
Recombinase polymerase amplification combined with lateral flow assay for detection of potato spindle tuber viroid

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Potato spindle tuber viroid (PSTVd) infects the large number of plants and causes large-scale disease of potato and tomato. Its detection for diagnostic purposes is hindered by the fact that PSTVd is organized as high structured circular non-coding RNA molecule with double-stranded RNA regions. Reverse transcription followed by PCR is the conventional approach for the detection of PSTVd, but it is time consuming and demand special laboratory equipment. The aim of this study was to develop an analytical approach for rapid PSTVd detection in the field. We have chosen isothermal recombinase polymerase amplification (RPA) combined with lateral flow assay (LFA) for this purpose. According to the proposed concept, two primers (with fluorescein and biotin labels) complementary to single-strand RNA loop regions were used for reverse transcription and the following RPA. All these reactions proceed in one test-tube during 15 min at 37 °C and produce double labeled DNA product that is applied to a test strip. PSTVd RNA was obtained by in vitro transcription for development and optimization of our test system. Eight pairs of primers were designed and tested. Two 30 nt primers were chosen as well as their RT-RPA products demonstrated the absence of non-specific interactions on the test strip. We found that MMLV reverse transcriptase is more appropriate for the assay than AMV. To detect the DNA amplification product with fluorescein and biotin labels, the test strips contained conjugate of gold nanoparticles with anti-fluorescein antibodies applied on a fiberglass starting pad and streptavidin adsorbed at a test zone of the working nitrocellulose membrane. RT-RPA-LFA with the chosen primers provided sensitivity equal to 10^6 copies of PSTVd RNA in test-tube. The obtained results are significant for the development of the first RT-RPA-LFA for rapid PSTVd detection under non-laboratory conditions. This study was supported by the Russian Science Foundation (Grant 16-16-04108).

P-05.2-40
Redesigning the function of bacterial microcompartments

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Bacterial microcompartments (BMCs) are protein organelles involved in various processes in cells. One of the largest BMCs is synthesized by the pdu operon and is involved in 1,2-propanediol metabolism in *Salmonella enterica*. Several enzymes are selectively encapsulated into BMCs via the specific peptide carried by the enzyme. These packaging sequences can be linked to the protein of interest to direct it into BMCs. In this study, we constructed a plasmid carrying subunits required for Pdu microcompartment assembly under the arabinose-inducible pBAD promoter and the protein LacI fused to a peptide that allows LacI to be directed into BMCs, which was also linked to an enhanced green fluorescence protein (PduD-LacI-EGFP). To facilitate the isolation of BMCs, we equipped the major Pdu protein with affinity tag. We induced the transcriptional "program" of LacI packaging into BMCs in *Escherichia coli* cells with arabinose. Mass spectrometry and transmission electron microscopy analyses showed that we successfully isolated LacI-containing BMCs, and the fluorescence microscopy approach confirmed that PduD-LacI-EGFP was encapsulated in vivo. Because LacI is a DNA-binding protein, we predicted that specific DNA sequences might also be

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encapsulated into BMCs. Therefore, a second plasmid carrying five binding sites for LacI was co-transformed in *E. coli* cells. After extensive DNase treatment of the purified BMCs, we indeed detected that some DNA remained protected. To our surprise, high-throughput sequencing analysis indicated that only a segment of DNA carrying multiple LacI binding sites was protected from DNases, but not the flanking regions. We anticipate that by using a DNA carrying multiple target site sequences and enzymes linked to specific transcription factors, a scaffold to direct selected enzymes within the BMCs can be provided, to increase the yield of specific metabolites and sequester toxic metabolic intermediates in the BMCs lumen. *The authors marked with an asterisk equally contributed to the work.

P-05.2-41
De novo designed parallel heterodimeric coiled-coil peptide pairs with high affinity for use in mammalian cells

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Coiled coil (CC) peptides represent attractive building blocks for assembling modular protein structures. We designed three sets of orthogonal CC forming peptides with low Kd's. CC dimer formation is based on interactions between two alpha helices assuming a twisted super-coiled structure with a heptad repeat periodicity. Our group previously designed several orthogonal CC peptide pairs with Kd in the micro molar range. In order to extend the toolbox of CC-peptide we designed a set of tighter binders that would be more suitable for *in vivo* application. The original pairs were modulated by the number of asparagine residues along the hydrophobic seam. At the b, c and f position, alanine residues were placed in order to increase the stability of CC pairs. Furthermore, we tuned the stability of CCs by introducing a polar glutamine residue at the f position in all heptads or by introducing a second asparagine instead of isoleucine at the a position of the first or fourth heptad. The Asn at the a position in the fourth heptad reduced the thermal stability and reduced the likelihood of homodimers formation. Formation of CC dimers in vivo was measured by reconstitution of split luciferase fused to orthogonal peptides and split transcription factor, based on TALEN DNA binding domain and VP16 activation domain fused to coiled coil peptide pairs. Luciferase and TALEN activity was proportional to the stability of the coiled coil pairs. Peptide displacement of the reconstituted split luciferase could be controlled by the CC building blocks with the increased stability. Luciferase assay confirmed the traits of in vitro coiled coil characterization. The set of orthogonal peptides with different thermal stability of CC's extends the toolbox of peptides that can be used as regulators of enzymatic activity or transcription regulators.

P-05.2-42
Silver nanoparticles enhance the effectiveness of antibiotics

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Interest in nanoparticles is growing in the biological sciences due to their pluripotential of applications in various fields including biomedical science health care, food and feed, cosmetics, drug-gene delivery and environmental health. The present study aimed to modulate antibiotics (Kanamycin, Ampicillin, Cefazoline, Benzylpenicillin) with 2 nm silver nanoparticles (Nanoserebro Argitos, OOO NPP Sintek Nano, Russia). The *Escherichia coli* M17 (EC) and *Staphylococcus aureus* ATCC 6538(SA) strains were used as Gram-negative and Gram-positive models. The microdilution method was used to determine the minimal inhibitory concentrations (MICs) and minimal bactericidal concentrationsn (MBCs) of antibiotics and Silver nanoparticles (AgNPs). The Fractional inhibitory concentration (FIC index) was used to assess the synergy between antibiotics and AgNPs, with FIC = FICA+FICB; FICA = MIC'ATB/MICATB and FICB = MIC'AgNPs/MIC AgNPs . The FIC index was interpreted as follows: FIC $\leq$ 0.5, synergy; 0,5 $\leq$ FIC $\leq$ 1, addition of effects; 1 $\leq$ FIC $\leq$ 4, indifference and for FIC > 4, Antagonism. The MICs (in μg/mL) of Kanamycin, ampicillin, cefazolin, Benzylpenicillin and AgNPs were 32, 256, 4, 16 and 8 for *E. coli* M17 and 32, 4, <4, <4 and 128 for S. aureus ATCC 6538 respectively. Modulation was done on antibiotics with MIC > 16. The lowest concentrations of antibiotics, combined with 0.1MIC of AgNPs, for which FICs0 $\leq$ 0,5 were observed, were 16 μg/mL (Kanamycin, EC and SA), 128 μg/mL (Ampicillin, EC) and 8 μg/mL (Benzylpenicillin, EC). Thefore, low doses of AgNPs can significantly reduce the MICs of antibiotics ($P < 0.05$) and therefore enhance their activity.

P-05.2-43
Computational study of interactions between silver nanoclusters and pterin

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Metal nanoclusters (NCs) are ubiquitously used nowadays in diagnostics and bioimaging due to their excellent biocompatibility, small size, and sensitivity to the molecular environment. Silver NCs often possess intense fluorescence and photostability, which is in high demand during the detection of organic matter like, for example, pterins (Ptrs) which are biomarkers of oxidative stress, inflammation and immune system activation, cardiovascular diseases, and neurotransmitter synthesis. The possibility of Ptr detection by the adsorption on a silver colloid has been already experimentally shown. In this regard, studies of Ptr complexes with Ag NCs seem to be in great demand. We calculated the binding energy (E_b) between Ptr and Ag_n^z (n = 1–6; z = 0–2) NCs using dispersion-corrected density functional theory (DFT-D3) method, namely PBE functional with a 6-31G(d,p) basis set, effective core potential LANLTZ for Ag, and COSMO solvation model for water. We considered both neutral Ptr^0 and deprotonated Ptr^{-1} forms of pterin (pK_a = 8). Different binding sites have been found depending on the NC charge and size. Thus, for example, in Ptr^0 N5 is preferencial for both Ag^0 and Ag^+, whereas in Ptr^{-1} Ag binds to O and N5 simultaneously. The highest E_b was obtained for the complexes between the Ptr^0 and

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Ag_3^{2+} (72.8 kcal mol^{-1}), between the Ptr^{-1} and Ag_3^{2+} (95.9 kcal mol^{-1}), which means that these complexes are preferably formed in aqueous solutions under acidic and alkaline pH, respectively. The latter complex possesses a long-wave maximum at 541 nm and a major peak at 361 nm in the absorption spectrum. The results obtained can be used for the development of fluorescent and surface-enhanced Raman scattering (SERS) Ptr biosensors. The calculations were carried out using the facilities provided by Resource Center «Computer Center of SPbU» (http://cc.spbu.ru/en). The work was supported by the Russian Science Foundation grant 20-73-10029.

P-05.2-44
Encapsulated mutant form of methionine γ-lyase: steady-state kinetic and pharmacokinetic parameters of antitumor enzyme

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The methionine dependence is a well-known phenomenon in metabolism of cancer cells. Methionine γ-lyase (EC 4.4.1.11, MGL) catalyzes the γ-elimination reaction of L-methionine and thus effectively inhibits the growth of malignant cells. Therefore, the application of the MGL in enzyme anticancer therapy is relevant. To minimize several problems typical of therapeutic proteins including high plasma clearance, short half-life in the bloodstream, and high immunogenicity, the mutant form V358Y MGL with increased catalytic efficiency in the γ-elimination reaction of L-methionine was encapsulated in polyionic vesicles and steady-state kinetic and pharmacokinetic parameters of encapsulated enzyme were determined. The catalytic efficiencies of the encapsulated and naked enzymes in the γ-elimination reaction of typical substrates were comparable. The inclusion of V358Y MGL in polyionic vesicles allowed us to increase the stability of the enzyme in the blood stream by almost one order of magnitude compared to the naked enzyme ($\tau_{1/2}$ = 50.8 and 7.4 h, correspondingly). Thus, encapsulation of V358Y MGL in polyionic vesicles provides an improvement of the pharmacokinetic characteristics of the enzyme for further study as an antitumor agent for in vivo trials. Acknowledgements The work was supported by the Russian Science Foundation (project No. 20-14-00258).

P-05.2-45
Detection of tyrosine using metal nanoclusters

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Tyr is involved in the synthesis of neurotransmitters, catecholamines, thyroid hormones, etc. There is a number of pathologies associated with impaired Tyr metabolism: phenylketonuria, hypothyroidism, tyrosinemia, alkaptonuria, and vitiligo. We have shown the possibility of Ag nanocluster (NC) synthesis on Tyr. Ag NC can be applied for SERS and fluorescent Tyr detection. However, prior to development of Tyr biosensor one should understand the theoretical basics of interactions between Tyr and NCs. We calculated the binding energy (E_b) between Tyr and Ag_n^z (n = 2–8; z = 0–1) NCs using DFT-D3 method: PBE functional with 6-31G(d,p) basis set, effective core potential LANLTZ, and COSMO model for water. Since the growth of Ag NC occurs at pH 12.5 we studied both Tyr^{-1} and Tyr^{-2}: protonated and deprotonated through the side-chain (pK_a = 10.5). Ag_5^+ had the highest E_b equal to 87.3 kcal mol^{-1}. The absorption spectrum of the Tyr^{-2}/Ag_5^+ complex had a long-wave maximum 525 nm and a major peak at 394 nm. At high pH values, Tyr can reduce Ag^+ ions directly into Ag NCs or nanoparticles in the absence of additional reducing agents. In this case, the absorption peak at about 400 nm can be used to detect Tyr. The alternative strategy of Tyr detection is to use the already known Tyr RNA-aptamer (5'-GGGCAGUCAACUCGUAAGAUG GCCUUACAGCGGUCAAUACGGGGGUCAUCAGAUAGG GGAGGCC-3'). We performed a docking of Tyr and the aptamer using AutoDock Vina 4.2 and found E_b equal to 7.9 kcal mol^{-1} (K_d = 16 μM), whereas the experimental value is 7.6 kcal mol^{-1} (K_d = 22 μM). Tyr forms H-bonds with A19, U46, A48, and hydrophobic interactions with U20, G21, and C47. We designed the aptamer-conjugated Ag NCs to develop a sensor for Tyr detection. The research was carried out using the facilities provided by Centre for Chemical Analysis and Materials Research, Optical and Laser Materials Research Centre, and Computer Center of SPbU. The work was supported by the Russian Science Foundation grant 20-73-10029.

P-05.2-46
Stressed by hydrogel? Physiological response of yeast cells embedded in thermoresponsive Pluronic F127: a prerequisite for bioreactor development

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One class of "smart" materials are thermoresponsive polymers, macromolecules that change their physicochemical properties with temperature. Pluronic F127 is one such biologically-compatible, reversely-gelling polymer, i.e. a material that is liquid near 0°C but hydrogel at room temperature. Such properties make F127 and its functionalised derivatives interesting for 3D bioprinting, as they can be mixed with bacteria, yeast, algae, or mammalian cells to form next-generation bioreactors. However, while initially promising, cells embedded in such hydrogels are impaired quickly, which requires tricky and expensive coupling between hydrogel design and the start of the bioprocess. To investigate the cause of this deterioration, we characterised the physiological state and stress response of yeast cells trapped in the hydrogel. We constructed a set of *Saccharomyces cerevisiae* strains expressing GFP-based ratiometric biosensors that measure cells' cytoplasmic pH and energetic state. Moreover, we constructed strains expressing fluorescent proteins under the control of newly-designed promoters induced by a specific stress response. Our results provide insights into the interaction between yeast cells and hydrogels, allowing us to use functionalised F127 as a hydrogel matrix to construct yeast biosensors for the detection of common aquatic pollutants.

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P-05.2-47
Structural dynamics of cationic polymer are associated with the forming of aggregate of bacteria

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The biological utility of cationic polymer has been expanding by modifying its electrostatic and structural properties, such as an antimicrobial agent (bacterial and viral) and a biohazardous eluent treatment. We developed a unique pretreatment agent containing one of our proprietary cationic polymers, a polyallylamine hydrochloride (PAA) derivative, which realizes an effective and rapid collection of bacteria by forming the aggregates for a MS spectrometric identification of pathogen in a patient with suspected blood stream infection. Here, we analyzed the aggregation mechanism between *E. coli* and the cationic polymer, focusing on its thermodynamic property and hydrodynamic diameter. PAA (CAS: 71550-12-4) was utilized for the analysis whose pKa is 9.67. The isothermal titration calorimetry analysis revealed that dissociation constant of the aggregation reaction was 1.26 μM at pH 10. In addition, the values of enthalpy change and entropy term for the aggregation were -131 and 97.6 kJ/mol, respectively, indicating that the enthalpy favorably contributes to this aggregation. The amino group in PAA significantly loses its electrostatic charge under this basic pH condition. On the other hand, the negligible apparent heat in a calorimetry analysis and no aggregation reaction was observed at pH 4.0 despite strong positive charge of PAA. The analyses with dynamic light scattering showed that the hydrodynamic diameter of PAA is 0.88 nm and 2.53 nm at pH 10 and pH 4, respectively. Considering this significant size change of PAA between acidic and basic condition and the positive value of entropy term at pH 10, non-electrostatic interactions such as the structural dynamics of polymer in addition to residual electrostatic interactions play critical roles for the aggregation of E. coli. Our finding may serve an alternative development approach of biomedical cationic polymer and further the possible application to the field of bionanotechnology.

P-05.2-48
The influence of conditions of "green" synthesis of cobalt-containing micropowders on spectral characteristics of final products

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An important quality of plant extracts is polyphenols, carbohydrates and proteins in them, which contain various groups (phenolic, aldehyde, sulfhydryl) that can reduce metal ions. Based on this, the interest in plants as a source of biomaterial for "green" nanotechnology grow. In this work, cobalt-containing Co_3O_4 and $CoFe_2O_4$ powders obtained in aqueous extracts of *Petroselinum crispum* plants synthesized at different pH levels, which is described in early works. To identify the obtained samples, Fourier IR spectroscopy used in the wave range from 400 to 4000 cm^{-1} (KBr medium). An intense wide bandwidth recorded at 3450 cm^{-1}. It corresponded to valence fluctuations of metal hydroxyl (Fe-OH and Co-OH) in all samples. The peak 1630 cm^{-1} and its arm 1500–1600 cm^{-1} confirmed the presence of interlayer water (H-O-H coupling fluctuations). The tops in the 1100 cm^{-1} value corresponded to the valence fluctuations of Me-O-Ni. They strongly manifested in samples of cobalt oxide and cobalt ferrite synthesized in an acidic medium. In addition, bands 1320–1400 cm^{-1} characterized magnetite-like modifications of ferrihydrite and noted in a sample of ferrite obtained in an alkaline medium at 1416 cm^{-1}. Two main frequencies characteristic of ferrites were recorded: the transmission band, in the range of 600–500 cm^{-1}, due to valence oscillations of the tetrahedral Fe^{3+} (band 583.26 cm^{-1} of $CoFe_2O_4$ synthesized in an acidic medium) and the transmission band, in the range of 400–450 cm^{-1}, due to the octahedral metal Co^{2+} (band 432.32 cm^{-1} in the powder Co_3O_4). The change in the synthesis conditions (pH of the medium from acidic to alkaline) led to a shift in the Fe-O frequency towards higher frequencies: from 583.26 cm^{-1} for samples obtained in acidic medium to 668 cm^{-1} for samples synthesized at a pH of more than 10. Moreover, a decrease in the acidity of the medium resulted led to decrease in the intensity of characteristic peaks compared to similar micropowders obtained in an acidic medium. All this confirms the IR spectroscopy using prospect in an express selection of conditions for the biological synthesis of metal nano- and micropowders. The studies were performed in accordance with the plan of research works Federal Research Centre of Biological Systems and Agro-technologies of the Russian Academy of Sciences (No. 0761-2019-0004)

P-05.2-49
"Green" synthesis of metal-containing materials and modeling of their antioxidant activity

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Plants are used for "green" synthesis of nanoparticles as an alternative to physicochemical methods. For example, methods of producing gold, silver and iron nanoparticles of various morphology from salt-corresponding metals using plant extracts as a reducing agent implemented. In particular, extracts of plants belonging to various taxonomic groups can be used for these purposes. It is evident that the substances adsorbed on the nanoparticles and then they will exhibit biological properties which determined by the plant extracts composition. This phenomenon leads to the modulation of biological properties which is characteristic of a given metal or its oxide nanoparticles. Nano- and micro-powders of metals Cr_2O_3, CeO_2, MoO_3, Co_2O_3 and Fe_2O_3, ZnO, $ZnFe_2O_4$ (synthesis at pH = 2 and at pH = 10), $CoFe_2O_4$ (synthesis at pH = 2 and at pH = 10) synthesized in Parsley's aqueous extract of curly *Petroselinum crispum*. The antioxidant activity of nanomaterials was evaluated by the ability to remove the standard radical DPPH (2,2-diphenyl-1-picrylhydrazyl) *in vitro*. There are a solution of 0.135 mM DPPH in methanol took and mixed with aqueous p-frames of metal powders in a ratio of 1:1. The reaction mixture was thoroughly stirred and the absorbency of the mixture was measured at 517 nm. The results showed that some of the powders showed a

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sufficiently high antioxidant potential, neutralizing the free radical from 12 to 17% of Trolox (standard antioxidant). Thus, the high anti-radical activity coefficient of CeO_2 in dilutions from 10^{-1} to 10^{-5} M (2.7–3), 10^{-1} and 10^{-3} M $CoFe_2O_4$ (pH = 2) (3.8–4) and 10^{-3}-10^{-5} M $ZnFe_2O_4$ (2.3–3.3). The studies were performed in accordance with the plan of research works Federal Research Centre of Biological Systems and Agro-technologies of the Russian Academy of Sciences (No. 0761-2019-0004).

P-05.2-50
Improvement of polymeric shell for encapsulation of methionine γ-lyase

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Pyridoxal 5′-phosphate-dependent methionine γ-lyase (MGL, EC 4.4.1.11) is studied as antitumor enzyme and as antibacterial agent in enzyme prodrug therapy. A number of factors, including a short life-time in the bloodstream, limit its use in experiments in vivo. To overcome this limitation, MGL was encapsulated in polyionic vesicles composed of PEGylated poly-L-aspartic acid and poly-L-lysine (PICsomes). Effect of polymer length on the degree of MGL incorporation into PICsomes and other PICsomes characteristics were investigated. Polymers with chain units 20, 50, 70, 120, 160 were synthesized. The degree of polymerization was determined by ^{1}H NMR spectroscopy. The efficiency of encapsulation of MGL mutant forms (C115H MGL and V358Y MGL) labeled with rhodamine into PICsomes was estimated by fluorescence spectroscopy. The most promising proved to be PICsomes consisting of polymers with a number of chain links equal to 70 (PICsomes70) (the degree of inclusion 5 - 9%) whereas encapsulation into PICsomes consisting of polymers with a number of chain links <70 was less than 0.5%. The hydrodynamic diameter and surface potential for PICsomes consisting of polymers with different numbers of chain links with incorporated mutant forms were evaluated by dynamic light scattering. In the solutions of PICsomes70 there are two types of particles, the prevailing fraction with a size of about 52 nm and a small amount of large particles with a size of about 500 nm. PICsomes20, 50, 120, 160 mainly contain large particles with a diameter of 500–800 nm, which may be due to aggregation. The surface potential of PICsomes70 was equal to 3.45 ± 0.36 mV. Acknowledgement The work was supported by the Russian Science Foundation (project No. 20-14-00258).

P-05.2-51
HSA functionalized nanoparticles for photoacoustic imaging in breast cancer

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The development of new nano-platforms is an innovative approach for early diagnosis in cancer. The nanoparticles (NPs) presented here, namely MelaSil_Ag, are constituted by an external shell of silicon and a melanin-like compound (5,6-dihydroxyindole-2-carboxylic acid, DHICA) and a metallic silver core. DHICA and silicon have been selected for their biological compatibility and physical properties, the metallic silver core improves the photoacoustic (PA) effect, acting as a signal amplifier. To increase their hemocompatibility, biocompatibility and stability, MelaSil_Ag NPs were modified with Human Serum Albumin (HSA), using EDC/NHS chemistry. After HSA conjugation, an increase of cytocompatibility of HSA-NPs was observed in HS578T and MCF10a, compared to bare NPs. The same results were obtained in hemocompatibility assays. HSA interacts with SPARC protein (overexpressed in cancer cells) and can promote a selective HSA-NPs internalization in tumors. This data was confirmed by confocal microscopy and cytofluorimetric analysis. Higher levels of HSA modified NPs internalization in HS578T (SPARC positive) compared to MCF10a (SPARC negative) was observed. Moreover, experiments performed with specific endocytic inhibitors showed that HSA-NPs were uptaken by a clathrin-mediated pathway. Furthermore, the evaluation of photoacoustic signal (PAS) of MelaSil_Ag-HSA NPs internalized by HS578T was performed. The PA spectral analysis of HSA-NPs inside cells showed strong detectable PAS. Moreover, the HSA-NPs photostability (PHS) was evaluated under continuous laser irradiation. Results showed that internalized NPs are more photostable compared to NPs in the test-object, suggesting that the cellular environment protects the NPs from degradation. The future prospectives will be the improvement of theranostics applications by photothermal effect in combination with a chemotherapeutic drug (e.g. doxorubicin) and PA imaging.

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P-05.2-52
Towards multiscale tracking of stem cells with genetically encoded encapsulin nanocompartments

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Stem cell therapies are an emerging line of treatment options for metabolic, neurodegenerative, autoimmune, and trauma-associated diseases. Nevertheless, approaches for non-invasive monitoring of stem cell identity and function in the patient's body are still not well developed. In this work, we present encapsulin-based biomaterials as genetically controlled labels that may be used for non-invasive and multiscale stem-cell tracking. Genetically encoded encapsulins are self-assembling protein nanocompartments that can encapsulate various cargos such as fluorescent proteins or enzymes such as ferroxidases, which lead to iron oxide nanocrystal formation inside the nanoshell lumen. It has been shown that different encapsulin constructs from *Myxococcus xanthus* (MxEnc) [Sigmund F et al. (2018), Nat. Comm. 9: 1990] and *Quasibacillus thermotolerans* (QtEnc) [Sigmund F et al. (2019), ACS Nano 13, 8114−8123] can be successfully expressed in eukaryotic cells and detected across scales via optoacoustic and magnetic resonance imaging (MRI), as well as fluorescence and electron microscopy. We demonstrate here that mesenchymal stem cells (MSCs) also readily express MxEnc or QtEnc without affecting cell viability and that encapsulation of coexpressed fluorescent proteins allows for sensitive visualization of genetically labeled MSCs via fluorescent microscopy. We furthermore show that co-expression of encapsulin-targeted ferroxidases (MxBCD or QtIMEF) leads to effective iron accumulation as characterized by biochemical methods enabling detection via MRI. Our data provide the first basis for multimodal imaging of MSCs via genetically controlled and modular encapsulin nanocompartments that may also support future image-guided stem-cell-based therapies. We acknowledge support by Helmholtz-RSF Joint Research Group (HRSF0064), RSF grant № 194506302 (A.G., A.S., M.A.), an Alexander von Humboldt Research Fellowship for Postdoctoral Researchers (M.E.). and an ERC-CoG 865710 (G.G.W.). *The authors marked with an asterisk equally contributed to the work.

P-05.2-53
Quantitative evaluation of the superparamagnetic iron oxide nanoparticles release by mesenchymal stem cells *in vitro*

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Tuberculosis (TB) caused by *Mycobacterium tuberculosis* (Mtb) constitutes a major threat to the global health. Despite an integrated approach to the treatment of TB, patients often develop severe complications and death. Superparamagnetic iron oxide nanoparticles (SPIONs) functionalized with various bioligands and directed to Mtb-associated antigens could be applied for the early diagnosis and therapy of TB. Another approach could be based on the delivery of nanoparticles by mesenchymal stem cells (MSCs) to the site of inflammation. However, the release of SPIONs from the nanoparticle-loaded MSCs currently remains insufficiently studied. In the current study the FetMSCs obtained from Cell Culture Collection (Institute of Cytology of the RAS, St. Petersburg, Russia) were cultured for 24 hours with SPIONs at various Fe concentrations (50, 100, 150 and 300 μg/mL). At designated time points (from 1 to 7 days) SPION-labeled cells were cultivated in the fresh culture medium. Quantitative analysis of the iron concentration in the medium was performed employing thiocyanate method. Culture medium with fetal serum and a mixture of antibiotics was used as a control. When SPIONs were applied at concentration of 50 μg/mL, no differences of the iron content in the medium compared to the control were detected. A slight increase of iron concentration (up to 10%) in medium from SPIONs-loaded cells was detected when nanoparticles were used at 100–150 μg/mL. The highest nanoparticle release from cells (up to 24% in the period of 7 days of cultivation) was observed when SPIONs were applied at 300 μg/mL. This data indicates that at low concentrations of SPIONs only a tiny fraction of nanoparticles is released into the medium, while an increase in particles concentration corresponds to the active release of nanoparticles from MSCs. The work was supported by a grant from the RFBR №19-58-45012 and the Ministry of Science and Higher Education within the State assignments. *The authors marked with an asterisk equally contributed to the work.

P-05.2-54
Comparative study on the effects of silver nanoparticle and silver ions on disturbance of antioxidant system in roots of tobacco plants

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Antimicrobial properties of silver and enhanced reactivity when applied in the form of nanoparticles (AgNPs) led to increased utilization of AgNPs in consumer products. Their release into environment directly affects biological systems and indirectly human health, as AgNPs can be transported through plants into the food chain. AgNPs can impose detrimental effects on plants, mainly through excess generation of reactive oxygen species (ROS), leading to induction of oxidative stress. In this work, *in vitro* grown tobacco (*Nicotiana tabacum*, L.) plants were exposed to AgNPs stabilized with cetyltrimethylammonium

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bromide (CTAB) or polyvinylpyrrolidone (PVP) coating and to ionic silver ($AgNO_3$), applied in the same concentrations (25, 50 and 100 μM). The aim was to compare AgNP and $AgNO_3$ impact by investigating ROS generation and subsequently changes in the activities of enzymes catalase (CAT), ascorbate (APX) and pyrogallol peroxidase (PPX), and superoxide dismutase (SOD) and their isoform and expression patterns in roots of tobacco plants in correlation to Ag accumulation upon exposure to ionic- and nano-silver. Presence of nanoparticles observed with electron microscopy and Ag accumulation in roots were correlated to increased ROS levels, which were most prominent after exposure to AgNP-PVP. Among aforementioned enzymes, the most significant changes were observed in SOD activities after all types of treatments, compared to control. The form of applied silver seemed to affect the expression of different isoenzymes, with the most notable differences observed in APX and PPX isoform patterns when comparing effects of Ag^+ ions and AgNPs. Finally, immunoblotting assay showed higher abundance of antioxidant enzymes in treated compared to control plants. Obtained results suggest that oxidative stress is associated with AgNPs toxicity in plants, and cannot be ascribed only to dissolution of Ag^+.

P-05.2-55
New possibilities for cancel cell tracking using genetically encoded encapsulin proteins

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Robust cell labels for noninvasive whole-organism imaging techniques will contribute to a better understanding of tumor biology and open new cancer treatment avenues. Genetically encoded reporters are contrast-selective for viable cells and are not diluted by cell division. In this work, we present iron-filling encapsulin proteins as genetically controlled labels for multimodal cancer cell detection. Encapsulins, bacterial protein nanocompartments, can contain various guest molecules inside their lumen. These include fluorescent proteins or ferroxidase leading to iron oxide's biomineralization inside the encapsulin nanoshell [Sigmund F et al. (2018), Nat. Comm. 9: 1990; Sigmund F, Pettinger S, Kube M et al. (2019), ACS Nano 13, 8114−8123]. For the first time, we implemented heterologous expression of encapsulin systems from *Q. thermotolerans* using the fluorescent reporter protein mScarlet-I and the iron-biomineralizing protein IMEF in the human hepatocellular carcinoma cell line HepG2 [Efremova MV et al., Pharmaceutics, under revision]. Successful expression and assembly of encapsulin nanocompartments with functional cargo proteins was confirmed by transmission electron microscopy, fluorescence microscopy, and gel electrophoresis. Iron oxide nuclei with an average diameter of 28 ± 4 nm were detected. The cell viability was proved by the LDH assay. Co-expression of encapsulin nanoshells, IMEF ferroxidase, and iron transporter Zip14 increased the T_2-weighted contrast in magnetic resonance imaging of HepG2 cells. The results demonstrate that the encapsulin: cargo system may be suitable for cancer cell tracking by MRI and optical techniques. We acknowledge support by the RSF grant number 19-45-06302, Helmholtz-RSF Joint Research Group (HRSF0064), an Alexander von Humboldt Research Fellowship for Postdoctoral Researchers, and an Add-on Fellowship for Interdisciplinary Life Science provided by the Joachim Herz Foundation (M.V.E.), as well as an ERCCoG 865710 (G.G.W.).

P-05.2-56
The effects of size, shape and surface functionality on *in vivo* biodistribution and toxicity: a multiparametric biochemical study of gold nanomaterials

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Gold nanomaterials have a widespread use due to their superior physical and chemical properties. Gold nanoparticles and nanorods are very popular and stable metallic nanomaterials which can be synthesized easily in various sizes and surface chemistry and their surfaces can be functionalized by desired polymers. They have a wide range of uses in medicine, electronics, pharmacy, chemistry, biology and biomedical fields. Gold nanomaterials are often preferred for cell and tissue imaging, photothermal therapy, targeting studies, RNA/drug delivery, biosensors, detection systems and hyperthermia fields [1,2]. The aim of this study, the synthesis of gold nanoparticles and gold nanorods functionalized with polyethyleneimine (PEI) and polyethylene glycol (PEG), multiparametric investigation of their *in vivo* toxicological effects and biodistributions in mice. Within the scope of this study, it is aimed to synthesize and perform surface functionalization of gold nanomaterials that can remain in the body in sufficient time/amount by creating minimum toxic effects on the tissues. Gold nanoparticles (AuNPs) of two different sizes (20 nm and 50 nm) were synthesized and the nanorods (AuNRs) were successfully produced by extending them up to 15–90 nm. Surface functionalization of the nanomaterials was carried out using PEI and PEG. ICP-MS analyzes were performed to determine the time-dependent biodistribution of different gold nanomaterial groups in different tissues and organs (liver, kidney, spleen, heart, blood and brain) of mice. *In vivo* analyzes were performed for multiparametric biochemical parameters. Results we obtained at the end of the study coincided with the goals. Gold nanomaterials have been developed that have the potential to be used in systemic teranostics and their effects have been evaluated with a wide range of multiparametric analyzes and their potentials have been revealed. PEI and PEG surface coating increased both biocompatibility and biodistribution at the in vivo levels. The shape and size of the gold nanomaterial are important parameters for potential applications. Surface functionalization processes provide superior properties to the gold nanomaterials in terms of nanotoxicological parameters and the potential in vivo teranostic applications in the future studies. "This study was supported by The Scientific and Technological Research Council of Turkey (Grant no: 217S135)." REFERENCES: [1] Khlebtsov, N. and Dykman L., Chem Soc Rev., 40 (3), 1647–1671, 2011. [2] Wang, X., et al., Journal of Nanoparticle Research, 15 (9), 2013.

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P-05.2-57
Selection and characterization of DNA aptamers to glutamic acid

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Glutamic acid is a key neurotransmitter involved in learning process and memorizing. Disturbance of its levels in tissues is associated with various neural diseases. Monitoring of glutamic acid is an actual task for biomedicine. ssDNA aptamers to glutamic acid were selected using Capture-SELEX. ssDNA library containing 60 random nt region was used for selection process against 1 mM of glutamic acid. 11 selection cycles were followed by Sanger sequencing. Among the 168 sequences, 10 candidate aptamers were selected based on cluster analysis and screened for the ability to bind the target. An aptamer to glutamic acid named Glu1d04 was recognized. Aptamer binding ability and specificity to the target were confirmed with target-specific elution of the oligonucleotide hybridized onto magnetic beads. In cultural medium, the effective K_D for the oligonucleotide Glu1d04 was calculated to be 12 ± 6 μM using fluorescent analysis. The aptamer-target complex was characterized with K_D value of 0.6 ± 0.1 μM and the stoichiometry of 1:2 using ITC. The samples of ssDNA pools obtained at different selection cycles (#3, 5, 7, 9 and 11) were retrospectively subjected to high-throughput sequencing (HTS). The discovered aptamer Glu1d04 was the most abundant sequence at selection cycles 3 and 5, then vanished and appeared again at the last cycles at low enrichment rate. Three other aptamer candidates picked after Sanger sequencing were overrepresented at the last few selection cycles while did not display target binding. These results demonstrate that the aptamers can be identified in HTS-assisted SELEX at early selection cycles, and artifact and biased sequences become predominant with the progression of SELEX driving out the target-binding species. The selected DNA aptamer Glu1d04 demonstrates the potential for the development of aptasensors for the detection of glutamic acid. The work was supported by Russian Ministry of Science and Higher Education (project #0591-2021-0002).

P-05.2-58
An effect of peptide ligand on the "stealthy" properties of PEGylated magnetic nanoparticles

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Possibly the most commonly used approach to make the nanoparticles invisible for mononuclear phagocyte cells is PEGylation – modification of nanoparticles with polyethylene glycol (PEG). However, there is important question about influence of targeted peptide or antibody on the PEGylated nanoparticles surface on the "stealthy" properties of PEGylated nanoparticles. Fluorescent-labeled, PEGylated iron oxide magnetic nanoparticles (MNP) were synthesized by co-precipitation method and covalently conjugated with peptides [1]: pH-low-insertion peptide (pHLIP) or their noninserting counterpart pHLIP-K [2]. For investigation of MNPs uptake by blood cells, human blood samples were mixed with MNPs, incubated on rotator, lysed and stained using antibodies to CD14, CD45, CD66b, CD19, CD3, then analyzed by flow cytometry. To investigate MNPs uptake by blood cells *in vivo* BALB/c mice were intravenously administered with MNPs, blood was collected in definite intervals after MNPs injection and stained for flow cytometry analysis using antibodies to CD45, Ly-6G, Ly-6C, CD11c, CD11b. Human monocytes were isolated from PBMCs fraction by magnetic sorting using CD14 MicroBeads. The transmission electron microscopy analysis of the monocytes after incubation with MNPs was performed according to the procedure reported in ref. [3]. For protein corona analysis, MNPs were incubated in 90% human serum, separated from unbound proteins using MACS magnetic separator and samples were analyzed by LC MS/MS. The flow cytometry analysis data showed that monocytes uptaken MNPs more avidity in comparison with other blood cell type. It was found, that peptide-conjugated MNPs are internalized in monocytes more effective in comparison with parent PEGylated MNPs. A donor-dependent effect of serum on the internalization of MNPs in human monocytes has been observed. To summarize, the peptide ligands strong affect interaction of PEGylated MNPs with mononuclear phagocyte system.

P-05.2-59
Abstract withdrawn

P-05.2-60
Testing polyacrylamide gel as the carrier for yeast biosensors

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Genetically modified yeasts *Saccharomyces cerevisiae* are currently widely applied as biosensors to various substances in environmental and medical samples. Different applications require them hydrogel carriers with different properties; however, polyacrylamide gel (PAAG), which is used for many tasks, almost was not tested for this application. Here we examined PAAG as the yeast carrier for analyzing molecules in extracted animal blood. We tested the influence of solutions with different concentrations of PAAG components on yeast mortality and division (acrylamide: 2.5–15%; ammonium persulfate, or APS: 0.05–0.2%; TEMED: 0.04–0.29%); mortality was assessed by staining with 0.4% trypan blue. All components of PAAG demonstrated dose-dependent toxicity during 30 min incubation. The highest toxicity was observed from acrylamide (up to 40% of cells) and the lowest from APS (up to 10%). Also, the acrylamide monomer stopped cell division. Based on the obtained results, 5% of acrylamide (the lowest concentration keeping shape), 0.08% TEMED, and 0.1% PSA was chosen as the optimal recipe for yeast immobilization. After polymerization of yeasts in 5% PAAG, the mortality after 16 hours was ~20%. To verify the metabolic activity in PAAG, copper-sensitive yeast was used, and immobilized yeast showed the reaction to the trigger at least 20 hours after polymerization. There was no significant increase in yeast mortality or decrease in cell multiplication during at least 30 min incubation in the diluted and intact blood plasma of rats and fish. Thus, animal plasma does not have the fungicidal activity to yeast, and such biosensor can be used to identify blood parameters. This work was funded by the Russian Science Foundation (#20-64-47011, the multidisciplinary project performed

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P-05.2-61
Optimization of human adenocarcinoma HT-29 cell culture in a biomimetic tumor-on-a-chip platform as a prospective drug-delivery systems screening tool

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Colorectal cancer (CRC) is one of the most prevalent types of cancers, being the second cause of cancer-related deaths worldwide. These alarming statistics highlight the need for improving current CRC therapy by developing novel antitumoral agents or drug-delivery systems for conventional chemotherapy to overcome chemoresistance and reduce the severe side-effects. However, the development of better anticancer therapies is hindered by the lack of appropriate in vitro models that recapitulate the architecture and physiology of colorectal tumors. In vitro 3D models partially overcome these limitations, but still, fail to recreate the dynamics of the tumor niche. In this context, the microfluidic tumor-on-a-chip (ToC) systems have emerged as powerful tools to emulate the 3D hierarchical complexity and microenvironment of in vivo tumors. Our study aimed to optimize the cell culture parameters for HT-29 human adenocarcinoma tumor cells in dynamic multichannel microfluidic chips to generate functional ToC models for colorectal cancer. In this view, HT-29 cells were seeded in different extracellular matrix (ECM) formulations at different initial densities, on microfluidic chips. At various time points, HT-29 cell metabolic activity was measured by MTT assay as an indicator of cell viability and proliferation,while cell morphology and distribution was investigated by fluorescence microscopy after labeling tumor cells with phalloidin-FITC and DAPI. Consequently, HT-29 cell seeding density and ECM compositions were optimized to select the best experimental setup for culturing colorectal tumor cells in microfluidic chips. The optimized protocol for generating biomimetic systems that mimic colorectal cancer will be further employed for screening novel drug-delivery systems and investigating their mechanisms of action. This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project number PN-III-P1-1.1-PD-2019-0955, within PNCDI III

P-05.2-62
Nanoemulsion-encapsulated magnetic nanocarriers for cancer theranostics

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Superparamagnetic iron oxide nanoparticles (SPIONs) due to their unique physicochemical properties could be employed for diagnosis and therapy of cancer. However, SPIONs are known to form aggregates, that could significantly limit their application in vivo. A promising method for modifying a magnetic carrier is encapsulation of nanoparticles in nanoemulsion media. Synthesized via coprecipitation method SPIONs (with a diameter of 50 nm, Z-potential –23 mV) underwent in situ precipitation in an inverse emulsion media that contained tridecane, mineral and peach oils as hydrophobic components. Based on the observation of the behavior of nanoemulsion samples during long-term storage, three-phase diagrams were compiled showing the location of the experimental points and the monophase zones. Electron microscopy of nanoemulsion samples loaded with SPIONs demonstrated a significant amount of particles inclusion into micelles. The nanoemulsion samples were in fact of the reverse type (water-in-oil) with different micelle morphology depending on the phase ratio. Measurement of low field NMR spectra of both emulsion compositions and their components was possible by recording high-resolution spectra without using the technique of line narrowing and magnetization transfer. The latter indicated the liquid-phase nature of the formed compositions without the formation of elements of the liquid-crystal structure, which are characterized by a strong slowdown in the molecular movement of groups, especially in the region of polar surfactant head residues. The interaction of proton spins with large total magnetic moments of microspheres of iron oxide nanoparticles increased the rate of NMR relaxation indicating the MR contrast enhancing properties of the nanoemulsion. In conclusion, obtained nanoemulsion-encapsulated magnetic carriers can be applied for non-invasive cancer diagnostics and targeted drug delivery. The work was supported by a grant from the RFBR №19-08-00024. *The authors marked with an asterisk equally contributed to the work.

P-05.2-63
Neutralization of reactive oxygen species by Prussian blue–lipid nanoparticles for siRNA delivery

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Excess of reactive oxygen species (ROS) is associated with the development and progression of many diseases and interferes with drug efficacy. Combination of two approaches: antioxidant defense and RNA interference could be helpful for the treatment of liver pathologies. This project aims to develop a hybrid delivery system based on Prussian blue (PBNP) and lipid nanoparticles for siRNA delivery to the hepatocytes. First, we synthesized PBNP with different diameters (38 ± 4 nm, 57 ± 6 nm, 98 ± 10 nm). It was shown that the bigger size of PBNP correlates with

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the increase of its catalytic activity (from 610 ± 50 s^{-1} for 38 nm to 5371 ± 80 s^{-1} for 98 nm) and affects the resulting diameter of PBNP-lipid nanoparticles. Lipid nanoparticles with embedded 38 nm PBNP have a resulting diameter of about 160 nm, while the incorporation of 57 nm PBNP increases the size of lipid NP up to 250 nm. It was shown that the size of PBNP did not affect its cytotoxicity (PBNP did not exhibit any cytotoxicity at AML12 cells), while PBNP-lipid nanoparticles with bigger diameter showed decreased cytotoxicity, probably to the less cell accumulation. Using flow cytometry, we demonstrated that developed PBNP-lipid nanoparticles could decrease the ROS level in mouse hepatocytes (AML12). Moreover, it was shown that both types of nanoparticles (160 nm and 250 nm) successfully accumulate in the liver, as was determined by IVIS Spectrum CT. Thus, we synthesized novel hybrid PBNP-lipid nanoparticles that could effectively neutralize ROS and deliver siRNA to the liver. This work was supported by the grant of the Russian Scientific Foundation 20-74-00116. *The authors marked with an asterisk equally contributed to the work.

P-05.2-64
Preparation and characterization of basil seed mucilage/alginate biocomposite hydrogels

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Hydrogels are structures consisting of homopolymer or copolymer characterized by water insolubility, which can be defined as three-dimensional hydrophilic polymer networks. In biomedical and pharmaceutical applications, the use of polysaccharide-based hydrogels as a non-toxic and biocompatible drug carrier has made a significant contribution to solving the biocompatibility problems. Basil seed (*Ocimum basilicum* L.) is easily available and has a high swelling capacity pectin layer with a significant amount of unesterified galacturonic acid. In this study, hydrogel synthesis was carried out by crosslinking basil seed mucilage and Alginate. Optimization and characterization studies have been carried out to design the synthesized polymers as a drug transport system. The process of separating the mucilage from the seed were mixed the pH: 8 0.2N NaOH solution from the basil seeds (weight ratio of 50 to 1) with a magnetic stirrer at 65°C 600 rpm for 24 hours. The pure mucilage obtained was dried in the oven at 62°C for 10 hours. It was mixed for 15 minutes at 200 rpm in the presence of 1% $CaCl_2$ of 1%, 1.25%, 1.5% and 1.75% Alginate and 1% basil seed mucilage solutions to ensure polymerization. Hydrogel synthesis was carried out under the same conditions at 0.1%, 0.25%, 0.5%, 0.75% and 1% $CaCl_2$ concentrations by selecting 1.75% Alginate concentration. The swelling behavior of hydrogels was investigated in deionized water, pH: 4, pH: 7 and pH: 8.5 buffer solutions. The size and morphology of the surface of the mucilage, alginate and the synthesized hydrogels, were analyzed using a scanning electron microscope (SEM). Hydrogels prepared with 1.75% Alginate showed the highest swelling behavior. The highest swelling behavior to different crosslinker concentrations was detected in hydrogels containing 0.75% $CaCl_2$. Concentrations of hydrogels exhibiting high swelling behavior were consistent with SEM images. In the next step, these hydrogels will be used in drug release analysis.

P-05.2-65
Through new platforms for arsenic biosensing: self-assembling arsenate reductase chimeras

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Arsenic (As) is a toxic metalloid component of the Earth crust widespread in soil, water and air, harmful to humans and the environment. It is released into the environment by the consumption of arsenic-containing products such as insecticides, pesticides, and chemotherapeutic drugs and is usually found in in two oxidation states, As(III) and As(V) and in inorganic and/or organo-metalloid forms[1]. The development of biosensors to monitor these forms is a hot topic in biotechnology, since the actual procedures require specialized and expensive instruments. For the set-up of functional biosensors, crucial steps are the stability of the biomolecular component, as well as its correct immobilization on surfaces. In this work we describe the production of two versions of a chimeric protein, namely ArsC-Vmh2 and Vmh2-ArsC, which combined the self-assembling properties of Vmh2, a hydrophobin from *Pleurotus ostreatus*, with that of TtArsC, a thermophilic arsenate reductase from *Thermus thermophilus*. Their enzymatic activities were characterized in comparison to that of the native TtArsC and their immobilization properties tested on polystyrene and gold. Both chimeras were also immobilized on gold electrodes, and a procedure to detect As(III) was set up. The results demonstrate that gold-immobilized ArsC-Vmh2 and Vmh2-ArsC can be exploited as electrochemical biosensors to detect As(III)[2]. References: [1] Mandal, B. K. & Suzuki, K. T. Arsenic round the world: A review. Talanta 58, 201–235 (2002). [2] Puopolo, R. et al. Self-assembling thermostable chimeras as new platform for arsenic biosensing. Scientific Reports 11, 2991 (2021).

P-05.2-66
Investigating the biotin-streptavidin binding dynamics on DNA origami nanostructures with high-speed atomic force microscopy

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Atomic force microscopy (AFM) is a powerful tool, which allows the comprehensive study of mechanical properties and interactions with nanometer resolution. The ability of AFM to obtain three-dimensional topography images of biological molecules and complexes under near-physiological conditions make novel high-speed AFM developments a perfect tool for investigating dynamic biological processes. DNA origami nanostructures (DONs) have emerged as excellent molecular pegboards for the immobilization of ligands on surfaces to study early signaling events in adherent cells. The bottom-up self-assembly of such supramolecular architectures can be harnessed to create bio-instructive materials, such as, nanocomposites for cell receptor stimulation [1] or biosensor surfaces for investigation of nanoscale effects on early cell signaling [2]. These applications take

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advantage of the effective linkage between receptor ligands and the DONs through high-affinity biotin-streptavidin bridges. Here, we present high-speed AFM (HS-AFM) data obtained from DONs containing biotin binding sites, imaged in fluid in the presence of streptavidin at 20 ms per frame. The occupation of each binding site is analyzed revealing details on the binding properties and dynamics, which could be tailored by changing the chemical nature of the nanoscale binding sites [3]. Citations: [1] Y. Hu et al., Angew. Chem. Int. Ed. 59 (43), pp. 19016–19020, 2020. [2] P. Lanzerstorfer et al., Biomolecules 10 (4), p. 540, 2020. [3] C. M. Domínguez, A. Kraus, C. M. Niemeyer, H. Haschke (in preparation). *The authors marked with an asterisk equally contributed to the work.

P-05.2-67
Selective cytotoxicity of redox-responsive iron oxide magnetic nanoparticles towards cancer cell lines

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Redox-responsive systems (RRS) are used for triggered drug release and usually designed on the basis of disulfide bonds that dissociates in the cells and subsequently trigger drug release or other effect. At the same time iron oxide nanoparticles (ION) are widely used for magnetic field directed drug delivery, hyperthermia and MRI. Combination of magnetic properties RRS can gain the drug delivery systems enhanced functionality. However, ION itself as a source of oxidizing Fe^{3+}-ions may affect cells redox-environment and viability. Here we report a study of dual-functionalized magnetic RRS and its cytotoxicity toward PC3, U87 and 4T1 cell lines. The system was synthesized by covalent grafting of PEG on the ION surface via SS-bonds. The obtained IONs had average size of 6 nm with Ms≈63 $A{\bullet}m^2/kg$. The PEG conjugate structure with disulfide was confirmed by ^{1}HNMR. Redox-responsive ION (R-ION) loses colloidal stability in dithiothreitol medium due to desorption of PEG shell, while the control pegylated ION (C-ION) retain stable. The R-ION dynamic of internalization and reduction were studied by Cy3 and Cy5 dyes that covalently linked with and without SS-bond, respectively. The difference in fluorescence was detected due to delayed Cy5 desorption from ION quenching fluorescence via FRET-effect. Cytotoxicity studies at 50–800 μg/mL per ION showed: the C-ION and R-ION are non-toxic for the PC3, U87; R-ION is toxic for 4T1 at ≥200 μg/mL while C-ION didn't show significant cytotoxicity. Glutathione – the main cell reducing agent – has different concentration in cells depending on its type and compartments. The R-ION containing both Fe^{3+} and disulfide can greater deplete glutathione level compared to C-ION with only Fe^{3+}. This leads to the 4T1 cell death due to its presumably less redox-activity. The described selective cytotoxicity may be used in antitumor therapy. The reported study was funded by RFBR, project number 20-03-00967.

P-05.2-68
Perspective micromycetes producers of fibrinolytic enzymes with the properties of proteins of haemostasis system proteins

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According to the World Health Organization, cardiovascular diseases have become a serious problem. Therefore, the search for new highly active drugs among micromycetes enzymes is a very promising direction. As a result of a preliminary study, two enzyme complexes were selected, formed by the micromycetes *Aspergillus ochraceus*, *A. raperi* and *Sarocladium strictum*. The obtained preparations were separated by the method of isoelectric focusing. Their ability to cleave chromogenic peptide substrates with the properties of proteins of the hemostasis system was investigated. The fibrinolytic preparation formed by *A. ochraceus* was a complex of three proteins AO-1, AO-2, and AO-3 with iso-points 5.1, 6.0, and 6.8. Proteinase AO-1 had a high activity against the substrates of thrombin and plasmin of 41.5 and 76.2 nmol pNA/mg × min, respectively, and did not act on other substrates. Proteinase AO-2 was able to activate plasma protein C (53.1 nmol pNA/mg × min). Proteinase AO-3 cleaved the substrates of thrombin, plasmin, and urokinase: 94.3, 129.6, 22.7 nmol pNA/mg × min, respectively. The micromycete *A. raperi* forms a complex preparation, which includes two proteinases with isoelectric points 4.4 and 5.7. These proteinases were able to hydrolyze thrombin, plasmin and urokinase substrates with high efficiency, as well as other chromogenic peptide substrates. This drug is a broad-spectrum complex. The preparation formed by *S. strictum* was also a complex preparation containing three proteinases I, II, and III with iso-points 4.5, 7.2 and 11.8. Proteinases I and II had a high ability only to activate plasminogen via the urokinase pathway, and their urokinase activity was 133.7 and 26.5 nmol pNA/mg × min. Proteinase III was capable of cleaving small amounts of thrombin and plasmin substrates, and was also active against tissue plasminogen activator and urokinase. Thus, the enzymes formed by these micromycetes could be of great interest to clinical practice. *The authors marked with an asterisk equally contributed to the work.

P-05.2-69
Engineered outer membrane vesicles from *E. coli*: a platform for immunotherapy and drug delivery systems

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Synthetic nanodevices such as liposomes, polymers and metal-based nanoparticles have long been studied for pharmacological applications such as immunotherapies and drug delivery systems. However, the correct production and loading of these structures might be tricky and inefficient. Bacterial outer membrane vesicles (OMVs) are bacterial non-replicating extracellular small-sized proteo-liposomes (20–250 nm) continuously discharged by several bacteria and hold a great potential to be used as engineerable biological carriers. Noteworthy, extracellular vesicles possess

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adhesion molecules on their surface that render their binding and internalization into target cells highly specific and appealing to be fine-tuned for the regulation of specific ligand-receptor recognition. In this framework, the use of OMV-based vaccines is an upcoming topic in latest years and different OMVs engineering approaches for vaccine development were described so far. In this work, we describe the isolation of OMVs from different commercially available strains of *E. coli* (BL21(DE3), JM109, DH5α). Currently, no hypervesiculating *E. coli* strain is commercially available; therefore, a comparative analysis of OMVs obtained from these commercial strains is of particular interest. Their size and proteomic profile were characterized, respectively, by DLS (dynamic light scattering) and proteomic mass spectrometry analysis. OMVs were preliminary engineered by overexpressing in the corresponding recombinant *E. coli* strain a mutated form of a bacterial membrane protein, the cytolysin A (clyA/hlyE). ClyA enrichment in OMVs was confirmed by mass spectrometry analysis. Beneath the future perspective of this project there is the subsequent modification of clyA for the generation of a recombinant fusion protein that could expose human tumor-specific antigens on OMVs surface.

Designed regulatory circuits and genome editing

P-05.3-01
Ultrafast circulating breast tumor DNA detection in blood by CRISPR/dCas9 biosensor

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Extracellular nucleic acids, a biomarker evaluated in the context of liquid biopsy, are small nucleic acids that are not associated with the cell or cell fragments. The increase in the latter, called the circulating tumor DNA (ctDNA), can be used for diagnostic purposes in blood. Till recently, extracellular DNA was analysed with quantitative real-time polymerase chain reaction (PCR)-based, fluorescent-based or spectrophotometry-based approaches. We aimed to develop an electrochemical biosensor based on a very specific and innovative measurement approach that targets the ctDNA sequence in plasma. Biosensors are a combination of a biorecognition receptor and a physicochemical transducer. The hallmark in our study is the use of a very specific biorecognition receptor, CRISPR/dCas9 complex, that specifically binds to the mutated sequence in the ctDNA carrying genetic mutations. To develop the biosensor, gold electrodes were modified by Cysteamine (Cys) to form a self-assembly monolayer. Then, PAMAM-G5 dendrimers were covalently immobilized on Cys layer via glutaraldehyde. The next step is the immobilization of dCas9 on AuE-Cys-PAMAM modified electrode via glutaraldehyde followed by the immobilization of sgRNA on the dCas9 layer. All modifications were characterized by electrochemical impedance spectroscopy (EIS). EIS parameters were set as 10 000–0.05 Hz, 10 mV AC and 180 mV DC current. Afterwards, a calibration curve was prepared between 10 to 640 pM ($R^2 = 0.9811$). LOD and LOQ were calculated as 8.19 pM and 24.8 pM, respectively. The newly developed biosensor with the CRISPR/dCas9 complex based biorecognition receptor can determine ctDNA in 25 seconds successfully. With its highly selective, practical and cost-effective characteristics, this biosensor is a promising tool for the determination of ctDNA in cancer patients.

P-05.3-02
The role of Tyrosyl-DNA-phosphodiesterases in the repair of DNA-protein crosslinks *in vivo*

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DNA protein crosslink (DPC) is a type of DNA lesion which occurs when protein becomes irreversibly covalently bound to DNA. If not repaired, DPCs interfere with all DNA transactions, thus causing genomic instability which can lead to cancer, accelerated aging and neurodegeneration. The orchestration of DPC repair pathway is still unknown, especially in vivo. Recently, it has been shown that metalloproteases Wss1 in yeast and SPRTN in mammals play central role in the repair of DPCs. These proteases cleave crosslinked proteins, thus leaving protein remnants of unknown size in the DNA backbone. While proteases act ubiquitously, cleaving wide variety of general DPCs, different set of proteins are involved in the repair of enzymatic DPC. Enzymatic DPCs are formed when proteins which are reversibly bound to DNA in order to preform their function become crosslinked due to endogenous and/or exogenous inducers. Tyrosyl phosphodiesterase 1 and 2 (TDP1 and 2) play crucial role in the removal of these enzymatic DPCs. TDP1 has been shown to remove protein remnant of TOPO1cc by separating DPC from DNA backbone through esterase activity, most probably after SPRTN mediated proteolytic cleavage of TOPO1 crosslinked to DNA. TDP2 can act (a) downstream of SPRTN mediated proteolysis of TOPO2 DPC or (b) together with ZATT protein to remove TOP2 DPC independent of SPRTN proteolysis. We aim to show the function of TDPs in DPC removal *in vivo* using the zebrafish model using CRISPR/Cas9 mediated mutagenesis of the TDPs active site via knock-in technology and fluorescent reporter. We have identified zebrafish TDPs and compared them to human orthologs in regard to phylogenetics, synteny and mRNA and protein expression, while functional studies are on the way. Our study will reveal actual contribution of TDP1 and 2 in the DPC repair pathway at the organismal level.

P-05.3-03
Designing novel calcium-dependent transcription factors

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Synthetic systems for regulation of gene expression are often developed based on known cell signalling pathways. Changes in intracellular concentration of calcium ions trigger many biological processes, including transcriptional regulation. Based on known calcium-dependent signaling pathway calmodulin-calcineurin-NFAT (nuclear factor of activated T cells), we designed novel synthetic calcium-dependent transcription factors for

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regulation of target gene expression. Transcription factors were designed based on regulatory domain of calcium-dependent transcription factor NFAT, containing phosphorylation sites rendering the protein responsive to changes in intracellular calcium. These domains were fused to DNA binding domains from TALE proteins which allow targeting and transcriptional regulation of specific target genes. Confocal microscopy of cells transfected with designed transcription factors fused to BFP was used to follow translocation from the cytosol into the nucleus after stimulation with calcium ionophore. Furthermore, activation of reporter gene expression was tested with luciferase assay. We have investigated different triggers of calcium influx (e.g. temperature, ultrasound). We have shown that designed modular transcription factors respond to changes in intracellular calcium concentrations and can be used to regulate expression of target genes in mammalian cells. *The authors marked with an asterisk equally contributed to the work.

P-05.3-04
Knock-in genome targeting risks: comprehensive analysis is essential for precision chromosome-editing identification

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Within a short time, CRISPR-Cas9-mediated genome editing has emerged as a state of the art approach for precise gene modification in a wide range of model organisms, including mouse and human. CRISPR-Cas9 became a powerful technology for generation of animal models to investigate different human diseases. Despite the advantages of CRISPR-Cas9 based genome editing, a number of potential problems such as genome rearrangements and off target effects still impede the CRISPR-Cas9 technology for use in biomedical research and further efforts are necessary to overcome these hurdles. Our study examines problems that affect direct knock-in genome targeting. During generation of conditional knock-out (cKO) mouse models, we discovered that frequently (sometimes solely) homology-directed repair and/or non-homologous end-joining mechanisms caused multiple unwanted head-to-tail insertions of donor DNA templates. Observed systematic obstacles were associated with single stranded (ss)DNA, double stranded (ds)DNA or single stranded oligodeoxynucleotides (ssODNs) templates injection during "one-step" cKO mouse models generation. Disturbingly, conventionally applied PCR analysis - in most cases - failed to identify such multiple integration events. We caution that comprehensive analyses of modified alleles are essential, and suggest experimental approaches to correctly identify precisely edited chromosomes (Skryabin et al. (2020) Sci. Adv. 6, eaax2941). In addition we suggest possible methodological solutions for successfully generation of cKO-targeted alleles to minimize unwanted multiple integration events. Our findings are important to unlock the full potential of the CRISPR-Cas9-mediated genome editing protocols for the generation of custom designed gene variants for biomedical research and gene therapy.

P-05.3-05
Engineering transgenic T cells to study mechanisms of HIV-1 cell-to-cell transmission

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HIV can spread as a cell-free virus or via intercellular contact called virological synapse (VS). Cell-to-cell transmission is an efficient mechanism that help virus to escape from neutralizing antibodies and to increase multiplicity of infection, but has been difficult to quantify. To accurately measure the level of HIV cell-to-cell infection, earlier we have developed replication-dependent reporter vectors inLuc and inGFP. However, this method required transient co-transfection of T cells with the packaging, reporter, and Env expressing vectors making it costly. The goal of this study is to generate human T cell lines Jurkat and CEM that would stably express indicated vectors and, ones mixed with target Raji/CD4 cells, will enable measuring VS formation and cell-to-cell infection using flow cytometry. The VS will be assessed by formation of red-blue conjugates between RFP+ producer cells and BFP+ target cells. For this purpose, we introduced BFP expression cassette into AAVS1 locus of Raji/CD4 target cells using zinc finger nuclease (ZFN). The RFP expression cassette was combined to inGFP/Luc construct in order to label producer cells and co-select the silent inGFP/Luc reporter, which becomes active only in target cells after completing one cycle of HIV-1 replication. HIV-1 packaging construct was modified by adding CD5HA2 marker (Zotova A et al. (2019) Sci Rep 9, 3132) at the C-terminus of Nef for isolation by FACS sorting. To avoid cell cycle arrest, we generated and tested Vpr(-) and Nef(-) packaging constructs and found that the first one is suitable for stable transgenesis. Among the three gene delivery strategies exploiting AAVS1-directed ZFN or CRISPR-Cas9 knock-in, FRT recombination and transposition, we selected transgenesis with Sleeping beauty as the most efficient. In summary, we prepared a genetic platform to create a cellular system for HIV-1 cell-to-cell transmission evaluation. This work was supported by RFBR grant 18-29-07052. *The authors marked with an asterisk equally contributed to the work.

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P-05.3-06
Creation of mice with constitutive and inducible expression of mutant Polg

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Aims. Polymerase-γ (Polg) is an enzyme that plays a key role in mitochondrial DNA replication. The inclusion of "incorrect" nucleotides without subsequent correction leads to the accumulation of mitochondrial mutations and mitochondrial dysfunction. Many pathologies are associated with mitochondrial mutations, such as Leber's hereditary optical neuropathy, MELAS syndrome, chronic fatigue syndrome, atherosclerosis, cardiomyopathy, etc. So, our main aim is creation of mice with constitutive and inducible expression of mutant PolG for basic research and preclinical trials. Results. We replaced the GCT instead of the GAC at position 4574–4576 in the mouse genome to create a model with constitutive expression of the mutant Polg using CRISPR/Cas9 technology. This replacement leads to a mutation of D257A in the Polg protein, leading to a change in conformation in the N-terminal "correcting" domain, which deprives the enzyme 3'->5' of exonuclease activity. The mutant protein Polg-D257A is not able to correct polymerization errors, which leads to the accumulation of mutations in the mitochondrial genome. Using random integration technology, we also inserted the cDNA of the mutant gene Polg surrounded by LoxP sites into the mouse genome for subsequent crossbreeding of these mice with mice with endothelial specific expression of cre-recombinase. So, we plan to obtain mice in which the mutant Polg protein is expressed only in the endothelium. Transplantation into 29 mice to recipients of 217 embryos with an integrated genetic construct led to the birth of 5 mice, of which 4 were transgenic. Conclusions. Our model animals with an increased number of mutations in mitochondrial DNA can be used as a precision test system to study the effectiveness of mitochondrial-oriented drugs. This study was supported by Russian Science Foundation (Grant #17-75-20249).

P-05.3-07
CRISPR interference of *Mycobacterium tuberculosis* adenylate cyclase

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The research is devoted to the investigation of the CRISPR interference method (CRISPRi), which suppresses transcription of target genes using the CRISPR/dCas system. Here, we present a modification of the pRH2521 vector of the pRH2502/pRH2521 system for CRISPR-dCas9-mediated RNA interference. We modified the system to facilitate the selection of colonies using the blue-white screening by inserting the gene of the β-galactosidase α-peptide into the cloning vector. The modification provided an increase of the cloning efficiency of guide RNA spacers. The ability of the modified pRH2502/pRH2521 system to suppress the transcription of certain genes was evaluated using the genes of adenylate cyclases (ACs), the possible virulence factors of *Mycobacterium tuberculosis*. The interference of the following ACs was demonstrated: Rv2212, Rv1320, Rv1319, Rv1318. The last three form an operon. The results indicate that the pRH2502/pRH2521 system allowed partial inhibition (43%) of Rv2212 gene and no significant suppression of the genes expressed in the operon. The possible reason could be connected with the low basic level of expression of the operon genes in vitro. The results also revealed the limitations of using described system. The process of interference was complicated due to the difficulty of choosing a PAM in correct region. In conclusion, a CRISPRi system for inhibiting target genes was studied using AC of *M. tuberculosis*; the pRH2502/pRH2521 system appears to be efficient but has its limitations of usage. The work was partially supported by RFBR 19-015-00149-A

P-05.3-08
Development of the CRISPR/Cas13-based posttranscriptional gene regulation system in *Listeria monocytogenes*

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The RNA-targeting Clustered Regularly Interspaced Short Palindromic Repeats/CRISPR-associated (CRISPR-Cas) systems have proven very useful tools both in studying role of bacterial genes as well as modifying their expression for biotechnological purposes. *Listeria monocytogenes* is a Gram-positive, human pathogen. Due to its intracellular life cycle, it is considered as a model organism of intracellular infection and a candidate for a vector in gene therapy. Here, we develop the system for post-transcriptional gene regulation based on CRISPR/Cas13 from the *Listeria seeligeri*. The native CRISPR array was extended with the artificial fragment where additional restriction sites were introduced to facilitate incorporation of desired spacer sequences. To validate the system, we decided to target two listerial genes: hly and cadA responsible for hemolytic activity and heavy metal resistance, respectively. These were chosen due to easy to measure physiological effects. We first analyzed the crRNA processing with northern blot. Then, the effect of anti RNA activity of Cas13 on silenced genes was measured by appropriate in vitro assays. In addition to that, their mRNA levels were measured using RT-qPCR method. What is more, the influence of active Cas13 on overall fitness of the bacteria was assessed by growth curve analysis. Taken together our results show that CRISPR/Cas13 system from *L. seeligeri* shows potential for its utilization in other listerial species. However, further studies are required to fully characterize this system. Acknowledgements: This work was supported partially by a grant no. 2016/21/B/NZ6/00963 from the National Science Center, Poland and by the Ministry of Science and Higher Education through the Faculty of Biology, University of Warsaw (intramural grant DSM no. 501-D114-01-1140400).

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P-05.3-09
Creating a transgenic mouse model of neurological pathologies with a mutation in the Grin2A gene

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Aim. The Grin2A gene encodes a subunit of the N-methyl-D-aspartate receptor (NMDAR). Glutamate NMDA receptors play a crucial role in memory, learning, and in the synaptic plasticity of neurons. NMDA receptors are both ligand-gated and voltage-dependent, and are involved in long-term potentiation, an activity-dependent increase in the efficiency of synaptic transmission thought to underlie certain kinds of memory and learning. Mutations in this gene lead to a change in the NMDAR subunit. Sequencing of the entire genome has led to the discovery of a relationship between mutations in the Grin2A gene and a wide range of neurological diseases, including epilepsy, mental retardation, autism spectrum disorders, developmental delay and schizophrenia, the spectrum of cognitive, language disorders. This makes the problem of creating murine genetic models carrying mutations in the Grin2A gene relevant for further study of the consequences of these mutations and the development of possible therapy. Results. Using CRISPR/Cas 9 technology, we first obtained mice with a deletion of thymine at position 418252 of the Grin2A gene. This mutation leads to a shift in the reading frame and the premature appearance of a STOP-codon. As a result of this deletion, the mutant protein is synthesized 88 amino acids shorter than the native protein and has a modified C-terminal domain. In the resulting mice, we expect to see the phenotype described above. Conclusion. The mutation model in the Grin2A gene in mammals was first obtained. In the future, we plan to identify phenotypic manifestations of the resulting mutation in mice and develop genetic therapy. This study was supported by Russian Science Foundation (Grant # 17-75-20249).

P-05.3-10
Creating a murine model of atherosclerosis with increased expression of neuraminidase in blood plasma

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Aim. According to WHO, cardiovascular disease is the leading cause of early mortality and disability in the world. Atherosclerosis, one of the best-known CVDs, is a chronic arterial disease accompanied by impaired lipid and protein metabolism and characterized by an accumulation of lipoproteins and cholesterol in the vessel wall. One of the atherosclerosis occurrence hypotheses is the hypothesis of lipoproteins desialization by plasma neuraminiadases, which changes their physical and chemical properties and leads to their increased atherogenicity. Our work is aimed at creating a murine model with increased expression of neuraminidases in blood plasma, which contributes to the desialization of lipoproteins and cells of the endothelial layer of blood vessels. Results. The genetic construction for producing transgenic mice is based on random integration technology. The Neu gene, obtained from total mouse liver RNA by a reverse transcription reaction, with an extracellular transport signal, was cloned under a stop cassette surrounded by loxP-sites. Such Neu-tg mice alone do not have the phenotype of interest to us, but after crossing them with mice with specific expression of Cre-recombinase in blood cells, we expect to obtain mice with modified atherogenic lipoproteins. In the future, we plan to cross such Neu-Cre mice with homozygous ApoE -/- mice to obtain a more vivid picture of the atherosclerotic phenotype. Conclusion. The study of atherosclerotic lesions of the first transgenic mice we received brings us closer to a more complete understanding of the mechanisms of atherogenesis. This study was supported by Russian Science Foundation (Grant #17-75-20249).

P-05.3-11
Use of CRISPR/Cas9 gene targeting to conditionally inactivate immunological checkpoint Tim-3 in mice

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We utilized CRISPR/Cas9 system to create a strategy to generate mice with a "floxed" allele of Havcr2 gene encoding immunological checkpoint protein Tim-3. This mouse strain can be used to inactivate Tim-3 in a tissue-specific manner with Cre/LoxP recombination system. We first tested single guide RNAs (sgRNAs) flanking exon 4 of Havcr2 gene and single-stranded donor DNA oligonucleotides containing loxP sites and short homology regions flanking sgRNA binding sites. Donor DNAs and pX330 plasmids expressing Cas9 and sgRNAs were injected predominantly into the male pronuclei of fertilized mouse eggs. Of seven live pups, mutations around the Cas9 cleavage sites were found in only one surviving mouse. Analysis of F1 progeny will be carried out to more accurately determine the genomic changes. To increase the probability of obtaining mice with "floxed" Havcr2 allele, we also designed an alternative strategy for flanking the exon 4 with loxP sites in one step using sgRNA directly targeting exon 4 and long DNA donor containing loxP sites and extended homology regions. This study was supported by grant 19-14-00341 from Russian Science Foundation.

P-05.3-12
New RNA chemical modifications improve genome editing using the CRISPR-Cas9 system

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Over the past few years, CRISPR-Cas9 system has become a popular and fairly effective tool for genetic engineering. The CRISPR-Cas9 system requires recognition of the target DNA by the RNP based on the complementarity of a relatively short oligonucleotide sequence. Thus, efficiency and specificity of gRNA – DNA binding is especially important for the therapeutic use of this system. However, the selectivity of this system is still

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insufficient for widespread use in therapy. Uncontrolled insertions and deletions that occur in non-targeted regions of genomic DNA can lead to cell transformation after *ex vivo* or *in vivo* genetic engineering. Here we used a combination of already known and new RNA modifications to improve the performance of the CRISPR-Cas9 system. New modifications resulted in RNA stabilization, as well as decreased toxicity compared to phosphorothioate modifications, which are used to prevent RNA degradation *in vivo*. We investigated the influence of these chemical RNA modifications *in vitro* and *in vivo* on the efficiency and specificity of genome editing and found that the effect strongly depends on the position of modification. The same modifications can both drastically reduce and increase the effectiveness of DNA cleavage. We also analyzed the effect of new modifications on the efficiency and selectivity of the CRISPR-Cas9 system, in which mutant versions of the protein were used, which have greater specificity compared to the wild type of Cas9 protein. This work was supported by the Russian Foundation for Basic Research grant № 19-04-00298 (synthesis of modified gRNA, analysis of efficiency and specificity) and in part by Russian Science Foundation grant no. 19-73-00356 (design and synthesis of fluorescently labeled probes for *in vitro* screening of CRISPR-Cas9 efficiency). *The authors marked with an asterisk equally contributed to the work.

P-05.3-13
A method for efficient non-marked insertion of target genes into the mycobacterial chromosome

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Tuberculosis is the leading cause of death from infectious diseases worldwide. Therefore, vaccine strains with improved protective efficiencies are needed. As they could be constructed through genome editing, the development of corresponding efficient tools for mycobacteria is of high importance. We have developed a method of rapid insertion of target genes into the mycobacterial chromosome, in particular, that of BCG vaccine strains, as a part of an integrative construct, followed by plasmid backbone removal with a two-component CRISPR system. The latter consists of FnCpf1-encoding plasmid pSBY1_FnCpf1cg (Addgene #104622) and pRH2521 vector (Addgene #84380), which was modified to coincide with FnCpf1 nuclease and bear dual synthetic gRNA for the elimination of the extended plasmid DNA fragment. The construction of pMVG_SL series plasmids was based on the pMV306hsp vector (Addgene #26155) and included 2 steps. Firstly, pMVG_N plasmids were constructed by replacing a gene for kanamycin resistance with a gene for the gentamycin resistance and inserting a certain amplicon of interest (N). Secondly, the amplicon containing sacB and lacZ genes was obtained by PCR using pGoal19 vector (Addgene #20190) as a template and was cloned into pMVG_N vector. The proposed CRISPR vector system allows efficient removal of the integrative plasmid backbone with the antibiotic resistance gene included without affecting the target gene due to FnCpf1-induced two double-stranded breaks and DNA repair through nonhomologous end joining (NHEJ) mechanism. The use of two selectable markers sacB and lacZ allows an easy selection of the desired clones after NHEJ. The developed method makes the target genes insert into a mycobacterial genome without any antibiotic resistance genes needed. It could be extremely useful for the construction of new recombinant vaccine strains. The work was partially supported by RFBR grants 19-015-00149-A and 18-29-07021.

P-05.3-14
SORTS is a novel method for selection of gene-edited cells: advantages and new areas of application

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We have recently described SORTS (Surface Oligopeptide knock-in for Rapid Target Selection). This method relays on CRISPR-Cas9-based targeted integration of a short cell surface expressed GPI-anchored epitope tag that is delivered in the context of CD52 molecule and under control of endogenous promoter (Zotova A et al. (2019) Sci Rep 9, 3132). The small size of coding and terminating sequences facilitates targeted integration, while the surface expression of marker allows quick isolation of live cells with a complete knockout or modification of genes encoding intracellular or secreted proteins. The purpose of this study is to demonstrate new advantages and applications of SORTS. First, we compared functionality of cells isolated by cloning or by SORTS. To estimate function in general, we used two human retroviruses HIV-1 and HTLV-1, which replication is dependent on many cellular genes and growth kinetics. We found that despite the targeted gene was not involved in the infection, viral replication in clones was significantly impaired. In contrast, in polyclonal population obtained by SORTS both viruses replicated normally as in parental cells. This suggests that cells with minimal off-target damage of genome outgrow severely damaged cells and that does not occur in clones. Secondly, we modified SORTS by replacing epitope tags in CD52 with fusion inhibitory peptides derived from HR2 domain of gp41 and selected two the most active ones, MT-C34 and 2P23, that completely protected T cells from infection with a broad range of HIV-1 isolates. Thus, this new area of SORTS application demonstrates that the originally developed method has a great potential for different modifications, including adaptation to HIV gene-based therapy. This work is supported by the grant 18-29-07052 of Russian Foundation for Basic Research.

P-05.3-15
Restoring of wild-type activity level of high-fidelity SpyCas9 forms

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Prokaryotes use CRISPR/Cas systems to degrade foreign genetic material. These systems are repurposed for genome editing of various organisms, and the CRISPR/Cas system of II-A type from *Streptococcus pyogens* is the most widely used. CRISPR/SpyCas9

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system has several advantages among other CRISPR/Cas systems that are harnessed for the genome editing technology, but its relatively high off-target activity hampers its application in medicine. To improve the CRISPR/SpyCas9 system, several strategies were developed, including the mutagenesis of SpyCas9. Mutant high-fidelity SpyCas9 forms are generally more specific than the wild-type protein, but show decreased on-target activity, possibly due to increased sensitivity to the chromatin structure. To improve known high-fidelity SpyCas9 variants, we introduced additional mutations that increase DNA hydrolyzing activity in the chromatin context (D147Y and P411T) together with random mutations using a low-fidelity polymerase. The most active SpyCas9 variants were selected by their ability to disrupt the ADE2 gene and generate red colonies of the yeast *Saccharomyces cerevisiae*. Using this approach, we have obtained derivatives of such high-fidelity forms as eSpCas(1.1), HypaCas9, and Sniper-Cas9 that are as active as the wild-type protein. Novel SpyCas9 forms may further improve genome editing technology. This work was supported by the Russian Foundation for Basic Research projects no. 18-29-07015 and 18-29-07021.

P-05.3-16
CRISPR-Cas9 mediated knockout of host restriction factors allows increasing influenza A virus replication in human cell line

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The aim of presented work was exploring the CRISPR-Cas9 mediated knockout of several genes as a way to overcome the host restriction barrier and generate human cell line permissive to influenza infection. A preliminary transcriptome analysis was performed to reveal molecular pathways activated in intact cells during influenza infection. In addition to the upregulation of antiviral response pathways, RNA-Seq showed the activation of cholesterol metabolism, cell cycle regulation and chromatin remodeling pathways, as well as the downregulation of RNA modification processes. Therefore, we selected IRF-7 and AnxA6 factors which were earlier described as antivirus system component and cholesterol metabolism regulator in human cells, respectively. By CRISPR-Cas9 genome editing we obtained several clones of 293FT cells with individual knockout of IRF-7 and AnxA6. The suppression of target gene expression levels was confirmed with RT-PCR, western-blotting and RNA-Seq analysis. We compared replication dynamics of several influenza viruses in original and mutated cells in growth curve experiments. Using TCID50 assay, flow cytometry and immunofluorescence staining we showed that accumulation of influenza A virus in the mutant 293FT-ANXA6-/- and 293FT-IRF7-/- cells significantly exceeded the virus titer in the original 293FT cells. The individual knockout of host cell factors allows describing their role in virus replication control. We propose that multiple gene knockout could provide the desired level of influenza virus replication in adherent 293FT to use this cell line for influenza virus isolation and characterization. (Previously partially published in: Komissarov A. B. et al. (2019) Russ J Bioorg Chem 45, 749–757.) This work was supported by Russian Science Foundation grant 18-75-10069. *The authors marked with an asterisk equally contributed to the work.

P-05.3-17
Identification of potential enhancers contributed to the schizophrenia pathogenesis

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Hereditary factors contribute significantly to the development of schizophrenia. However, the genetic architecture and mechanisms of schizophrenia development are not well understood. Genome-wide analyses of genetic associations in non-coding regions of the genome point out to enhancers as one of the loci associated with an increased risk of schizophrenia. Using modified chromosome conformation capture Hi-C technique, we have identified new potential enhancers containing schizophrenia-associated polymorphisms and promoters contacting with them in neuronal cell lines. We have chosen several genes that may be involved in the neuron metabolism and encoding transcriptional factors for further studies. To find out how dysfunction of these genes affects neuronal cell function and make a possible contribution to the schizophrenia pathogenesis, we used CRISPR/dCas9-repression system. In our hands, the CRISPR/dCas9-KRAB repression system was unable to inactivate enhancers in the SK-N-SH neuronal cell. Then we have constructed more powerful CRISPR/dCas9 repressors by adding MeCP2 methyl-CpG binding protein and DNMT3a-3L fusion of de novo methylase. Additionally, we targeted new repressors not only to enhancers but also to the promoters of genes of interest. Moreover, we used CRISPR/Cas9 endonuclease system to affect promoters' activity by deletion of their potential enhancers found by Hi-C. The effects of CRISPR/Cas9-mediated repression/disruption of regulatory regions on neuronal cell fitness and metabolism related to the schizophrenia pathogenesis are discussed. This work was financially supported by the Russian Science Foundation projects no. 17-29-02164 and 19-015-00501.

P-05.3-18
The reporter cell line to study the expression of immunoproteasome subunit β5i

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The degradation of intracellular proteins is a dynamic and tightly regulated process. Most of such substrates is hydrolyzed by the proteasomes. Different forms of proteasomes are known. The role of immunoproteasomes (iPs) and intermediate proteasomes (intPs) is emerging. Here, using human colon adenocarcinoma cell line SW620 and CRISPR-Cas9 nickase technology we obtained SW620B8-mCherry cell line expressing proteasomes with mCherry-tagged β5i subunit, which is a catalytic subunit of iPs and intPs. Importantly, the chimeric gene expression in generated cells is under control of the endogenous regulatory mechanisms and was increased following IFN-γ or/and TNF-α stimulation. Efficient expression and integration of the chimeric protein into the assembling proteasomes was demonstrated. Moreover, using proteasome activity probe, we have shown that

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the tagged subunit is catalytically active within the assembled proteasomes. Fluorescent proteasomes were distributed within the nucleus and cytoplasm of the modified cells. The obtained cell line is a convenient platform to study the immunoproteasome gene expression, localization of iPs and intPs, association of proteasomes with other proteins and many other aspects of proteasome biology under the influence of various drugs and conditions in real time and in living cells. The study was supported by the Russian Foundation for Basic Research grant # 20-34-70106.

P-05.3-19
Influence of modified nucleotides on the activity of the CRISPR/Cas9 system *in vitro*

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Currently, the development and implementation of variously modified nucleotides into single guide RNAs (sgRNAs) or complementary interacting crRNAs and trans-activating RNAs (tracrRNAs) is a promising direction for improving genome editing systems, especially the CRISPR/Cas9 system. Many researchers offer their options for constructing effective modified guide RNAs. In this work, we investigated the effect of N6-methyladenosine (m6A), 5-methylcytosine (m5C), and pseudouridine (Ψ) on the activity of CRISPR/Cas9 *in vitro*. It was shown that the incorporation of modified monomers in the structure of sgRNAs affects the cleavage efficiency of DNA substrates by the CRISPR/Cas9 system. It was established that the cleavage efficiency depends on the sgRNA modification and decreases in the following order 5-methylcytosine, pseudouridine, and N6-methyladenosine. To investigate the influence of the modifications on the binding of gRNA to the Cas9 protein, the modified monomers were inserted into trans-activating RNAs. Based on the data obtained, the similarity of their effect both in sgRNA and in trans-activating RNAs on the activity of the CRISPR / Cas9 system was shown. It was found that the introduction of 5-methylcytosine into sgRNAs increases the specificity and does not significantly change the efficiency of hydrolysis of the DNA substrate compared with unmodified sgRNA. The main advantage of using modified guide RNAs is a decrease in the level of immunostimulatory and cytotoxic activity during their transfection into human cells. The work was partially supported by the State Budget Program (0245-2019-0001).

P-05.3-20
Knock-in of fusion inhibitory peptides into CXCR4 gene confers a strong resistance of lymphocytes to HIV-1

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Antiretroviral therapy has drastically improved outcomes for people with HIV, but it is associated with high toxicity and has to be taken lifelong. Its effectiveness is further compromised by emerging HIV drug resistance indicating an urgent need for novel therapeutics. One treatment option is fusion inhibitors that prevent conformational changes of gp41 and abrogate viral fusion with the host membrane. In this study, we generated HIV-1-resistant lymphocytes that express HR2-based inhibitory peptides on their surface. In contrast to previous studies, we used CRISPR/Cas9 technology to achieve peptide expression from an endogenous promoter after knock-in (KI) at the start-codon of HIV coreceptor CXCR4. To initiate KI, we electroporated CEM cells with Cas9- and gRNA-encoding plasmids together with the donor DNA, which encoded for either MT-C34 peptide placed in the context of CD52 molecule or 2P23 peptide, to express as GPI-anchored or fused to CXCR4 N-terminus, respectively. Using antibodies against peptides and co-receptor we demonstrated an efficient peptide KI with unaffected CXCR4 level of expression. Moreover, co-transfection of two donors above allowed to isolate cells with biallelic KI. These cells were protected from HIV-1 stronger than cells with individual peptide KI, and displayed survival and expansion during acute viral infection. Thus, gp41 fusion inhibitory peptides embedded in CD52 or N-terminally fused to CXCR4 represent potent inhibitors of HIV 1 entry and can be adapted for the gene therapy of HIV infected individuals. This work was funded by the RFBR grant 18-29-07052.

P-05.3-21
Development of a Cas9-based tool for the genome editing of the hyperthermophilic bacterium *Thermus thermophilus* HB27

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The progress of the genome editing techniques and systems biology provides new tools to engineer microbial genomes. Hyperthermophiles are microorganisms able to thrive in extreme environments and for their intrinsic properties, they are attractive hosts to be used as industrial platforms; however, their use is limited by the low number of suitable tools for their genetic manipulation. Among thermophilic bacteria, *Thermus thermophilus* HB27 is a good candidate as a chassis for metabolic engineering due to its high growth rates, high biomass yields, constitutive expression of a very efficient natural competence and metabolic versatility [1]. Here we describe the set-up of a highly efficient hyperthermoactive-Cas9 editing tool for the manipulation of the T. thermophilus HB27 genome [2]. Based on our previous knowledge on the arsenic resistance system of *T. thermophilus* HB27 [3], we developed a whole-cell biosensor for the monitoring of arsenic. The bioreporter is a genome modified strain, containing the gene encoding a thermotolerant yellow fluorescent protein integrated under the control of an arsenic-responsive promoter; it is able to detect concentrations of arsenic ions as low as 0.5 μM, a sensitivity much higher than that obtained with more conventional reporter systems. The developed genetic system is active up to 65°C and this is the highest temperature reported to date for a Cas9-based editing tool; we expect that the system can be easily adapted for editing the genomes of other thermophilic bacteria, significantly boosting fundamental and metabolic engineering in hyperthermophilic microorganisms. References [1]. Henne, A. et al. Nat. Biotechnol. (2004) https://doi.org/10.1038/nbt956. [2]. Mougiakos, I. et al.

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P-05.3-22
Cell regulation using synthetic calcium dependent transcription factors

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Meticulous control over gene expression is an important tool in design of synthetic gene networks, biomanufacturing and development of precision therapeutics. An important feature of synthetic circuits is external control of cellular processes through physicochemical signals. Various stimuli can activate membrane ion channels, so calcium-dependent regulation is an attractive approach to achieve pinpoint control of gene expression. We created a highly tunable, versatile, engineered calcium dependent transcription factors for programmable control of gene expression in mammalian HEK293 cells and in vivo. To achieve calcium-dependent activation we employed regulatory domains of NFAT (nuclear factor of activated T-cells) protein that is used in calcium-dependent gene circuits. Tight control of gene expression was achieved by splitting transcription factor, where NFAT was bound through coiled coil to transcription activation or repression domains, while CRISPR/Cas9 (Clustered Regulatory Interspaced Short Palindromic Repeats) or TALE (Transcription Activator-Like Effectors) fused with complementary coiled coil served as programmable DNA-binding proteins with which we targeted different genes. This adaptable synthetic calcium-dependent signalling pathway will make it easier for researchers to construct customizable transcription control systems and can be widely used in clinical and diagnostic applications. *The authors marked with an asterisk equally contributed to the wor

Plant biotechnology

P-05.4-01
Nature of active phytochemicals of crude extracts of some herbs from Armenian flora responsible for their antimicrobial activity

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Study of plant materials as a source of new antimicrobials can play an important role for the solution of antibiotic resistance problem. The goal was to explore qualitative and quantitative phytochemical constituents of methanol extracts from *Agrimonia eupatoria* L., *Hypericum alpestre* subsp. *polygonifolium* (Rupr.) Avet. & Takht., *Rumex obtusifolius* Willd and *Sanguisorba officinalis* L., which possess high antimicrobial activity, as shown previously. We also aimed to analyze the nature of bioactive constituents of the plants methanolic extracts. For the detection of the active antimicrobial bands, TLC-contact bioautography technique was used against *Staphylococcus aureus* MDC 5233 strain. Photochemical analysis revealed the presence of wide range of secondary metabolites. The highest total phenolic, flavonoid and tannin content was detected in methanol extracts of *A. eupatoria* (358.9 mg GAE/g DW) and *H. alpestre* (78.08 mg QE/g DW and 27.78 mg CE/g DW). In TLC analysis different mobile phases (chloroform:methanol (27:0.3), (19:1), chloroform:glacial acetic acid:methanol:water (32:17:6:4), butanol: water:acetic acid (12:2:1) and ethyl acetate:acetic acid (20:4)) were used in order to make assumptions about the nature of active antimicrobial compounds. It was revealed that polar compounds could have considerable contribution to the antimicrobial activity of methanol extracts of *H. alpestre* and *R. obtusifolius*. It was hypothesized, that complex compounds can be responsible for the antimicrobial action of extracts of *A. eupatoria* and *S. officinalis*. Thus, phytochemical analysis and TLC-bioautography techniques allowed to get some insight about nature of the active phytochemicals of some Armenian herbs responsible for their antimicrobial activity. High content of phenolic compounds including flavonoids and tannins was found compared to reported data which can be result of environmental factors typical to Armenia. *The authors marked with an asterisk equally contributed to the work.

P-05.4-02
Stress specific regulation of a monocot chromatin factor under inducible and constitutive promoters in Arabidopsis

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Chromatin dynamics is one of the key factors funneling cellular regulation during stress in plants. Manipulations in an abiotic-stress responsive chromatin factor is thus promising to reach sustainable plant production levels. Yet, its modulation might differ under different functional promoters while plants processing environmental cues. Here we characterized the metabolic changes of ABA inducible (pAsr11875) and constitutive (CaMV p35S) promoters in drought and salt stressed Arabidopsis transgenics over-expressing an actin depolymerizing factor (ADF) of a Louisiana native monocot halophyhte *Spartina alterniflora* ADF2. Transgenic lines bearing both p35s and pAsr1 which is carrying a combination of cis-regulatory elements showed improved physiological and phenotypic response under salt and drought stress attributed to healthy siliques with robust shoot systems, better germination capacity, water homeostasis and membrane stability. The growth of the transgenics expressing the gene under pAsr1 was better compared to p35S under non-stressed control conditions affirming the idea that using inducible promoters maximize the benefits of transgenes and avoid responses that may adversely affect the plant performance under specific stresses. Since dicot halophytes like Arabidopsis wild relative *T. halophila* differs extensively than monocots due to the different morpho-physiological features, elucidation of stress adaptations by manipulating transcription factor expressions using wild relatives of monocot crops such as rice is also advantageous to transfer stress inducible promotor knowledge to other cereal species.

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P-05.4-03
Chrysanthemum SEP3-like gene CDM44 overexpression does not affect transgenic tobacco flowering, whereas enhances its tolerance to cold stress

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In plants, the MADS domain transcription factors of the SEPALLATA3 (SEP3) clade are well known for their decisive role in specifying the identity of floral organs and the development of fruits and seeds. In addition, these proteins can also participate in the plant's response to various abiotic stresses. The present study attempted to elucidate the function of *Chrysanthemum morifolium* MADS-box gene CDM44, which is a close homolog of *Arabidopsis thaliana* SEP3, *Capsicum annuum* MADS-RIN and *Solanum lycopersicum* RIN. Structural homology suggests functional similarities. The yeast two-hybrid GAL4 analysis showed that CDM44 is able to interact with sunflower C-type MADS-proteins HAM45 and HAM59. To investigate the possible participation of CDM44 in the development of plant and flower/inflorescence meristem, two types of Nicotiana tabacum transgenic plants were generated: with overexpression of a single transgene CDM44 and three transgenes CDM44, HAM45 and HAM59 at once under the control of the enhanced 35S CaMV promoter. The ectopic expression of CDM44 did not affect the flowering time or the inflorescence and flower morphology of both non-transgenic control and HAM45/HAM59ov plants. The only important thing to note is that the CDM44ov capsules matured much more slowly than the control. This effect can be explained by silencing SEP3 genes; therefore, CDM44 may be able to act in fruit ripening similarly to LeMADS-RIN. During long-term treatment with low temperatures (three weeks; +4°C), CDM44ov plants showed higher resistance to stress than wild type control tobacco plants; the latter had visible symptoms of leaf damage (wilting) and a decrease in the level of chlorophyll in the leaves. The study was supported by the RFBR #18-29-07007 and Ministry of Science and Higher Education of the Russian Federation.

P-05.4-04
Constitutive expression of tomato TF LeMADS5 gene in tobacco plants alters flower morphology

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MADS-domain transcription factor (TF) SEPALLATA3 (SEP3) plays the key role in specifying floral organ identity, including fruit and seed development. In tomato genome, two SEP3 paralogs are known. One of them, RIPPENING INHIBITOR (RIN) is well-characterized, whereas LeMADS5 data are limited. In present study, LeMADS5 cDNA from the tomato cv. Silvestre recordo was amplified, sequenced and used for yeast two-hybrid GAL4-analysis of possible protein-protein interactions with known tomato MADS-proteins, as well as for obtaining transgenic *Nicotiana tabacum* plants with overexpression of LeMADS5 under the control of 35S CaMV promoter. LeMADS5 protein alignment revealed its sequence identity with RIN (54.5%), CaMADS-RIN (32.9%) and Arabidopsis SEP3 (72.2%). It was found that LeMADS5 is able to activate gene transcription, but does not interact with MADS-TFs TAGL1 and FUL2, as it was previously shown for RIN. Tobacco-independent transgenic clones constitutively expressing LeMADS5 in sense (16 lines) and antisense (3 lines) orientation were generated. 35S:: as-LeMADS5 lines showed no visible morphological changes, whereas four 35S::s-LeMADS5 lines flowered 1.5–2.0 times later than WT, formed double thickened stem and flowers with curved, magenta corolla in contrast with regular shaped, light pink WT petals. T1 progeny of L16 and L17 lines flowered in average 1.5 times later, formed internodes 1.23 times shorter and produced 1.18 times less seeds than WT. Late flowering was due to an ongoing reversible meristem transition from reproductive to vegetative state. Expression patterns of LeMADS5 and genes of tobacco homologs of TFs LFY, WUS, AP1 and SEP3, were determined in leaves and meristems (vegetative and reproductive). The study was supported by the RSF grant 19-16-00016 and Ministry of Science and Higher Education of the RF.

P-05.4-05
New plant virus platform with adjuvant properties

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Plant viruses are promising tools for designing of new vaccines and adjuvants. Structurally modified spherical particles (SPs) from tobacco mosaic virus (TMV) were applied as an effective adjuvant [1]. We have also shown, that Althernantera mosaic virus (AltMV) and virus-like particles (VLPs) from AltMV coat protein demonstrate immunostimulating properties [2]. Despite morphological similarities AltMV virions and VLPs differ in structure [3]. The present work addresses the structural remodeling of AltMV virions and VLPs into SPs. We established the differences in formation conditions of SPs from virions and VLPs that in accordance with the structural data (on AltMV virions and VLPs) [3]. SPs originate from AltMV virions through an intermediate stage as it was known for SPs from TMV and potato virus X. Nevertheless, we did not detect the same intermediate stage during AltMV VLPs structural remodeling. According to the biochemical analysis, AltMV SPs consist of protein and do not include RNA. We revealed that AltMV SPs can form complexes with target antigens and identified that the model antigen preserves the antigenic specificity in the complexes. The complexes induce a significantly higher immune response toward the model antigen than the protein itself confirming the adjuvant activity of AltMV SPs. Moreover, some part of antibodies after the immunization by the complexes was produced against SPs. It allows to use SPs as a component for marker veterinary vaccines. The presence of antibodies against SPs will enable to identify how an animal was immunized – by contact with a wild pathogen or by vaccination. The work was funded by RFBR grant №20-34-70023. References: 1.Trifonova E.A. et al. 2017. Antiviral Research. V. 144. P. 27-33. 2.Donchenko E.K. et al. Advances in virology. 2018. 3.Donchenko E.K. et al. PLos ONE. 2017.

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P-05.4-06
Inheritance of heterologous genes in first and second generations of transgenic tomatoes

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Nowadays, development of transgenic plants - biofactories of "therapeutic proteins" is a promising area. Production of the human interferon (INFalpha-2b protein), encoded with HuINFalpha-2b gene is of great demand for its application in treatment of some tumours and viral diseases like hepatitis. The aim of this investigation was to analyse inheritance of HuINFalpha-2b gene and nptII (selective gene of neomycin phosphotransferase) in tomato plants *Solanum lycopersicum* cv. Shedevr during two generations. Methods of plant cultivation *in vitro* and *in vivo*; PCR and statistical analyses were used in this investigation. Transgenic tomato lines carrying the HuINFalpha-2b and nptII gene were obtained by Agrobacterium-mediated transformation. T_1 and T_2 plant generations, successfully grown from T_0 plants, were analysed by PCR for HuINFalpha-2b and nptII genes presence. PCR analysis of DNA from T_1 generation of tomato plants revealed that 87.5% of plant lines were positive for HuINFalpha-2b gene. In T_2 generation 268 plants were analysed. PCR analysis of T_2 generation confirmed presence of the HuINFalpha-2b and nptII genes in 216 plants (80.59%). Inheritance of the transgenes was observed in T_2 tomato lines in a ratio of 3:1 due to the gene splitting according to the second Mendelian law. The reliability of the obtained data was confirmed using X^2 distribution criterion. We can propose insertion of a single T-DNA copy into primary T_0 plant from the results of the genetic analysis.

P-05.4-07
Investigation of toxic liver cirrhosis with the proposed new approach and its prevention with combination herbal drugs

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Due to its anatomical-physiological features, the liver plays an important role in the regulation of body homeostasis. A healthy liver is a guarantee of a healthy life. Various internal (emotions and etc.) and external (poisons, drugs, viruses and etc.) factors can lead to liver dysfunction resulting in cirrhosis. Modern medicine tends to describe cirrhosis as a dynamic process, so it is imperative to develop new tactical mechanisms for the study of cirrhosis pathology, along with the study of the effects of new hepatoprotective compounds. Currently, in medical practice, widely used plant extracts in the prevention and treatment of liver cirrhosis. From this point of view, we have been a task to develop a new approach for the earlier diagnosis of liver cirrhosis, for the study of its dynamics and prevention. The planned studies will allow preventing the development of the disease in earlier stages, moreover, develop more effective treatment approaches using the new combined Flamin / Silymarin cure. The aim of this work is to investigate the dynamics of progressive liver disease to cirrhosis with a new approach (by studying the liver-lung-kidney functional system), also develop the treatment tactics with Flamin / Silymarin mixture. The Flamin and Silymarin were obtained from *Helichrysum rubicundum* and *Silybum marianum* plants growing in Armenia. The varying ratios of Flamin /Silymarin (1:1 or 1:2) mixture were administered orally for 8 weeks in fixed dosage (300 mg/kg) after CCl4-induced liver toxicity. Histopathological investigations have shown that due to toxic failure of the liver, the symptoms of lung and kidney dysfunction were revealed before the development of cirrhosis. Further in-depth studies will reveal the dynamics of the development of liver cirrhosis using a new approach. The latter will allow predicting the pre-cirrhotic condition and developing more effective prevention and treatment strategies.

P-05.4-08
The use of extracts *Tilia cordata* flowers and *Tripleurospermum inodorum* flowers against *Candida albicans* biofilms

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The search for new agents to control pathogens of human and animal diseases continues, and plant extracts and individual products of their metabolism are worthy of attention as promising means of combating biofilms. They can act without destroying microbial cells, as antibiotics do, but selectively changing their behavior, which is achieved primarily by interacting with the quorum sensing (QS) system. This is quite enough to prevent the chronization of the inflammatory process due to the accumulation of pathogen cells in biofilms, which is necessary for full recovery without complications after the disease. It is known that *Tilia cordata* flowers contain Farnesol, which is a QS molecule that reduces the growth of biofilms in a certain concentration. We prepared extracts from air-dried flowers of *Tilia cordata* and *Tripleurospermum inodorum* in a feedstock: extractant ratio of 1:10, using an aqueous alcohol solution with an ethanol concentration of 70% as an extractant by extraction with reflux. *Candida albicans* is a dimorphic fungus and also an important opportunistic pathogen. One of the virulence factors of *C. albicans* is its ability to form biofilms, which are associated with chronic infections. *C. albicans* (concent. 4 McFarl.) was added to the wells of a 96-well of plate and cultivated on Nutrient Broth at 37°C, 48 h alone (control); with 1 extract or both of them. Optical Density (OD) of biofilms was measured by the degree of crystal violet binding at a wavelength of 580 nm in an Immunochem-2100 microplate photometric analyzer (HTI, USA). The results were: OD control = 0.503 ± 0.06; OD experiment 0.342 ± 0.07 *Tilia cordata*; 0.392 ± 0.06 *Tripleurospermum inodorum*; 2 extracts 0.273 ± 0.08. The effects of plant metabolites and their ability to effectively block fungi QS allow us to consider them as promising weapons in the fight against fungi biofilms. This paper has been supported by the RUDN University Strategic Academic Leadership Program and Research project №031622-0-000

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P-05.4-09
Monitoring of genetically modified food turnover in the Russian Federation

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Modern agricultural production is characterized by biotechnology widespread use for creation of new genetically modified plant varieties (GMO). In the Russian Federation the use of GM food is under strict legislative regulation, including the ban on unregistered GM lines use; sanitary-epidemiological and molecular-genetic examination in framework of GMO state registration; mandatory labeling of food with more than 0.9% of GMO; and the ban on GMO use for the certain food types production. The above requirements are reflected in a number of intergovernmental legislative acts, such as technical regulations of the Customs Union (TR CU 015/2011, TR CU 021/2011, TR CU 022/2011, TR CU 023/2011, TR CU 024/2011, TR CU 027/2012, TR CU 029/2012, TR CU 033/2013, TR CU 034/2013). In Russia the control over these acts implementation is carried out as a part of GM food turnover monitoring by the Federal Service for Surveillance on Consumer Rights Protection and Human Wellbeing. Since 2003 there have been conducted more than 350,000 laboratory tests of food aimed on GMO detection. The detection, identification and quantification of GMO was performed by polymerase chain reaction. The monitoring results show that manufacturers have abandoned the use of biotechnological raw materials: while in 2003–2004 GMO were found in 11–12% of products, in the following years their fraction decreased significantly (2014 – 0.14%, 2015 – 0.09%, 2016 – 0.05%, 2017 – 0.07%, 2018 – 0.08%). The data summarized by Federal Research Centre of Nutrition and Biotechnology show the same pattern, according to which the part of GM-containing products on the Russian market over the past 10 years is less than 1.0%. This work was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation (theme No. 0529-2019-0056).

P-05.4-10
The destruction of lignocellulose by actinobacteria

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The aim of the study was to assess the ability of actinobacteria of genus Streptomyces sp. to the destruction of hardly-biodegradable plant material. Actinobacteria were cultivated on different nutrient-rich media (1) and nutrient-poor media (2) containing a lignocellulose. The ability to the synthesis of natural products and biodestruction were carried out both in liquid and in solid-state cultivation. At the end of the experiment, the extraction of natural compounds, analysis of biological activity and mass-spectrometric analysis were performed. It was found that only 50% (n = 26) of all analyzed cultures (n = 52) were capable to growth on semi-dried lignocellulose (visual assessment). Seventy percentage (n = 36) of strains characterized by antibiotic activity. We revealed that the solid-state cultivation of the strain Streptomyces sp. IB15-31A on lignocellulose led to the elimination of natural products with masses [M + H] such as 514.12 Da (RT - 3.6 min.), 255.09 Da (RT - 6.9 min.), 943.58 Da (RT - 10.2 min.), 797.54 Da (RT - 10.5 min.), 522.48 Da (RT - 14.1 min.), 1036.84 Da (RT - 18.7 min.). At the same time, other natural compounds were found "as appeared" (synthesized) in the crude extracts after solid-state biodestruction of lignocellulose. These masses [M + H] were presented as 546.60 Da (RT - 14.3 min.), 567.44 Da (RT – 15.6 min.), 512.67 Da (RT - 15.9 min.), 696.55 Da (RT - 16.3 min.), 703.63 Da (RT - 16.5 min.), 540.69 Da (RT – 16.7 min.), 629.58 Da (RT - 18.2 min.). Thus, it was shown that the strains were able not only to grow on various nutrient media containing hardly-biodegradable plant material but also to synthesize the natural products and antibiotics. The study was carried out with the main financial support of the Russian Foundation for Basic Research grant 18-29-05051, and with the partial financial support of the Russian Science Foundation grant 18-74-00018 and Ministry of Science and Higher Education of the Russian Federation.

P-05.4-11
Protective properties of the *Monarda menthaefolia* extract on the example of a model object of *Drosophila melanogaster*

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Due to the high prevalence of oncological diseases, there's an active search for new protectors to reduce the toxic impact of anticancer drugs in therapy. In this aspect, the plant of the genus Monarda *Monarda menthaefolia* growing in the Ural region is of great interest. The method we applied is based on the use of the cytostatics of etoposide, which together with *M. menthaefolia* extract, reduces the toxic effect of the model object Drosophila melanogaster. The data were obtained on the composition of the phenolic complex of *M. menthaefolia* by the method of high performance liquid chromatography on an analytical HPLC system. 70% ethanol extract from the above-ground part of *M. menthaefolia* contains at least 35 phenolic compounds. Comparison of the retention times of the peaks of substances and spectral data on the chromatograms of the analyzed samples with the retention times of the peaks of standard samples made it possible to identify luteolin flavones, luteolin 7-glucoside and chlorogenic acid. 3.90 % flavones, 14.68 % tannins, 0.26 % catechins, 0.97 % pectins, 8.62 % protopectins, 102.73 % carotenoids, 7.18 % saponins were found in the above-ground parts of *M. menthaefolia* plants (from the air-dry mass of raw materials). Groups of compounds found in *M. menthaefolia* have antioxidant and antitoxic effects. To assess the overall toxic effect, 1 % and 10 % of *M. menthaefolia* extract together with etoposide 800 μg / kg of medium were added to the wild-type Oregon-R *D. melanogaster* nutrient medium. The data obtained indicate a positive effect of 1 % extract in relation to anti-toxicity when using the antitumor agent etoposide, namely, general mortality and viability indices of individuals of the Oregon-R *D. melanogaster* strain. When using 10% concentrated extract, a similar effect was not observed. Completed within the framework of the state task of the Botanical garden UB RAS.

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P-05.4-12
New proposals for updating the Russian framework for risks assessment of the genetic technologies products

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Biosafety issues have played a dominant role in the field of genetic engineering in Russia for over the last 25 years and led to the fact that on the one hand Russia is "GMO-free" country, in which the release of GMOs into the environment is prohibited, and on the other hand Russia lags in genetic technologies from the world level. Our analysis of the Russian framework for GMOs potential biological risks assessment showed that in the field of Agriculture this system was not formed in a coordinated manner. As a result, a high level of risk assessment methodology was achieved only for the use of GMOs as Foods, whereas for GM Feeds science-based methodological base remained incomplete till now. It was shown that new genetic technology (genome editing, synthetic biology) was outside of scope of existing thesauruses, regulatory acts, and guidelines. In order to update the potential risks assessment system a number of proposals were developed: to shift the focus of the risk assessment system to the identification and the elimination of risks at the earliest possible step of the construction of a new biological element, product, technology or material. The following new elements were proposed to proactively identify risks: a) the formation of agreed "prohibited" lists / databases of sequences DNA, other genetic elements for a proactive risk assessment on the stage of planning / design of product; b) the inclusion of innovation developers, regulatory bodies, risk assessment experts and other stakeholders in the process in the early stages of development - in a declarative manner, c) the involving manufacturers of the appropriate equipment to create a database of organizations which are producers of gene-edited organisms or organisms with synthetic genomes, d) creation and implementation of a culture of responsibility and safety in genetic engineering at the stage of the education of future biotechnologists. The work was partly supported by the grant RFBR 182914067\18. *The authors marked with an asterisk equally contributed to the work.

P-05.4-13
Effect of chitosan hydrolysate on tomato seed germination and seedling growth

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It was shown that chitosan and its derivatives are effective plant immunity elicitors, inhibitors of the growth of fungal plant pathogens, and affect the content of several secondary metabolites, stimulating plant development. The purpose of this work was to study the effect of chitosan hydrolysate (CH) on seed germination and the growth of seedlings of tomato cultivars (*Solanum lycopersicum* L.) Cherry rozovyi and Viking. Systematic research was performed using various concentrations (0.002–5 mg/mL) of CH with an average molecular weight of 33 kDa (Ip = 2.1) dissolved in a nitrogen-containing (N-solution - 0.2N Nitric acid; the pH was adjusted to 5.5 using 12.5 % NH4OH). The experiment consisted of two parts. In the first, the seeds were germinated at different concentrations of CH in N-solution. In the second, seeds were pre-soaked in CH solutions for 22 hours, and then germinated on filter paper soaked in water. In both cases, distilled water and N-solution were used as controls. On the 4th day in the first experiment, a variant CH at a concentration of 5 mg/mL demonstrated an inhibitory effect on seed germination, while all other variants in both experiments, including controls, showed the same germination index. In both experiments, seedlings were measured on the 7th day. Seedlings obtained from tomato seeds previously soaked in CH 5 mg/mL followed by transfer to water had on average the longest hypocotyl and root. In general, the length of hypocotyls and roots upon pre-soaking at 0.02–5.00 mg/mL CH concentrations was higher than the size of seedlings in the first part of the experiment and in all controls. The results suggest that CH 33 kDa has a positive effect on seed germination and seedlings growth at a concentration of 5 mg/mL in the case of seed pre-soaking. The study was partially supported by the RFBR grant #20-016-00205. *The authors marked with an asterisk equally contributed to the work.

P-05.4-14
Evaluation of the adaptive potential reducing model within reprotoxicological experiment: approach to plant biontechnologies safety assessment

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The use of adaptive potential reducing models is considered to be the promising course of preclinical studies of potentially toxic objects. The model of rats' adaptation potential reducing is based on the modification of diets' vitamin-salt mixtures composition. The purpose of the experiment was to assess the effectiveness of the model in reprotoxicological study on rats. The Wistar rats of the control groups received AIN-93 diet with different content of vitamins B1, B2, B3, B6 and minerals Fe and Mg: 18–19% content (C-18 group), 75% content (C-75). Animals of the test groups got the similar diets (T-18 and T-75) and also received glyphosate per os. In order to impregnate females, they were cohoused with males in 2:1 ratio for 7 days (32 females and 16 males from each group). Postnatal progeny development was being assessed during the first month of life by counting the number of alive and dead pups, dynamic of body weight and length, physical developmental landmarks. Postnatal development of the C-75 and T-75 rats had no significant differences (the total number of pups in these groups was 252 and 255, with an average litter value of 10.8 ± 0.9 and 10.8 ± 0.6, respectively), while groups C-18 and T-18 were characterized by pronounced differences in these indicators (the total number of pups was 168 and 9, with an average litter value of 6.9 ± 1.6 and 4.5 ± 2.5, respectively). Thus, the introduction of glyphosate against the background of reduced availability of B vitamins, Fe^{3+} and Mg^{2+} salts led to significant changes in reproductive function, while against the background of normal availability of essential substances the toxic effect of glyphosate was not observed. The obtained data allow us to recommend this model for the study of reprotoxicity, e.g. during the safety assessment of plant biotechnology food. This work was supported by the Ministry of Science and Higher Education of the Russian Federation (research project No. 0529-2019-0056).

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P-05.4-15
Cytokinin as an important factor for the onset of potato tuber formation and germination

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Cytokinins (CKs) are thought to be positive regulators of tuberization in potato. Our study was aimed at elucidating the role of CK related genes in onset of tuber formation. For that we performed large-scale transcriptomic studies, *in silico* and *in vivo* promoter analysis, using potato cv Desirée as the object of interest. Also we employed molecular modeling and bioinformatic approaches; for the latter transcriptomic data on potato tuberosum group RH89-039-16 (http://solanaceae.plantbiology.msu.edu/) were used. According to this database, CK receptors StHK2/3 predominate in leaves and flowers, StHK4 in roots. Among phosphotransmitters, expression of StHP1a predominates. Pseudophosphotransmitters that suppress CK effects in Arabidopsis are not expressed in potato. Among RR-B transcription factors, StRR1b is expressed most uniformly in all organs, StRR1a much weaker. StRR11 has maximum expression in roots. Apart triggering tuber formation, CKs contribute to their germination. CK receptor genes are strongly expressed in tuber sprouts, where StHK4 transcripts prevails over other receptor genes. Among B-type RR genes, StRR1b, StRR11 and StRR18a are actively expressed. By cluster analysis prevailing CK-signaling chains were identified in leaves and flowers, StHK2/3→StHP1a→StRR1b/+; shoot apical meristems, stolons, and mature tubers, StHK2/4→StHP1a→StRR1b/+; stems and young tubers, StHK2/4→StHP1a→StRR1b/11/18a; and roots and tuber sprouts, StHK4→StHP1a→StRR11/18a. CK synthesis isopentenyl transferase genes StIPT3/5 and StCYP735A are expressed mainly in roots, but almost not in stolons or tubers. Tuber sprouts are also sites of rather active CK synthesis. A special group of CK-activation genes StLOGs are active in stolons, and StLOG3b expression is restricted to this organ. Thus, CK effect on tuber initiation seems to be realized via the activation of StLOG1/3a/3b/8a genes in stolons. Supported by a grant of the Russian Science Foundation No. 17-74-20181.

P-05.4-16
Engineering of TMV coat protein termini allows formation of stable virions useful for conjugation of target proteins

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Genetically modified tobacco mosaic virus (TMV) particles can serve as a carrier for chemical conjugation of target proteins. Recently we constructed several viral vectors carrying different inserts, including reactive lysine codons, at either N- or C-terminus of the coat protein (CP) open reading frame (Petukhova et al., 2013, 2014; Gasanova et al., 2016). TMV-N-lys mutant forms stable complexes with influenza conserved epitope M2e (Gasanova et al., 2020). TMV-C-lys vector was partially characterized previously (Koroleva et al., 2019) and our new data demonstrated that accumulation of recombinant virions may be evaluated as 6 g per 1 kg of fresh non-inoculated leaves of *Nicotiana benthamiana* plants. TMV-NC-lys construct contained lysine triplets at the 5'-terminal and 3'-terminal regions of the CP gene. We assumed that these residues in the engineered CP molecule should be exposed on the particle surface in order to improve stability of putative protein-virion complexes. Agroinfiltration of *Nicotiana benthamiana* plants with TMV-NC-lys caused symptoms (curling of upper leaves, yellow chlorosis) similar to TMV-C-lys within 30–45 days post inoculation (d.p.i.). Accumulation of TMV-NC-lys particles was lower than TMV-C-lys and reached the level ~2.6 g/kg. Time course of accumulation was tested for three weeks (10 – 31 d.p.i.) and we did not notice significant differences from the TMV-C-lys. Rigid rod-shaped particles were purified from systemic leaves (25 d.p.i.). Unlike TMV-N-lys virions, curved particles were not observed. In this report we describe for the first time that successful mutagenesis of both ends of TMV coat protein is possible and leads to formation of stable virions suitable as a platform for bioconjugation of foreign proteins.

P-05.4-17
SSR analysis of soybean (*Glycine max* (L.) Merr.) genotypes genetic diversity

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Soybean (*Glycine max* (L.) Merr.) is one of the world's most important economic legume crops, and there is increased demand for cultivars such as productivity and resistance to various abiotic and biotic stresses. Development of soybean varieties with improved qualities, such as high yield, adaptability, disease resistance, nutritive content, and maturity date, is carried out mainly through breeding works, and their successes depend on the availability of promising genetic resources with high genetic potential and genetic diversity. Therefore, the goal of our study was to assess the genetic diversity of 29 genotypes using nine microsatellites (SSR – Simple sequence repeat) markers. A collection of 29 samples of DNA was isolated for PCR analysis and the optimal annealing temperature of the nine primer pairs was determined. The nine SSR primer pairs were able to amplify polymorphic SSRs from all of these genotypes with 76 allelic variants detected, and an average of 8.4 alleles per locus. The polymorphic information content (PIC) among genotypes had an average of 0.73, and the genetic diversity index of Nei – 0.75. Some unique allelic variants were identified in the studied loci mainly in the wild genotypes. The Jaccard similarity coefficient ranged from 0 to 0.73, indicating a diversity among studied genotypes. Moreover, based on the Jaccard similarity coefficient, these nine SSR markers grouped the genotypes into four clusters. Our results show the effective use of SSR markers assessment and could provide useful information for efficient utilization of genetic resources, especially for genetic improvement. This paper has been supported by the RUDN University Strategic Academic Leadership Program

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P-05.4-18
Stress-induced activation of the tobacco pectin methylesterase gene promoter

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Plants release many volatile organic compounds in response to stress. Among these substances, gaseous methanol, which is formed as a result of demethylation of cell wall pectin by stress-activated pectin methylesterases (PME), plays an important role. Plant-emitted gaseous methanol up-regulates so-called methanol-inducible genes involved in defense reactions in the cell. One of these genes encodes tobacco aldose-1-epimerase-like protein (NbAELP) that was shown to regulate the expression of the PME gene by a feedback mechanism (published in: Shehukova et al. (2017) Front Plant Sci 8, 1646). To assess the influence of abiotic and biotic factors on the activity of tobacco PME gene promoter (PrPME) we have obtained transgenic *Nicotiana tabacum*, carrying the PrPME-GUS expression cassette, in which the *E. coli* uidA gene encoding β-glucuronidase was under control of PrPME. Control plants were transformed with 35S-GUS plasmid that contained a constitutive 35S promoter. PrPME-GUS plants are phenotypically indistinguishable from both wild-type and 35S-GUS plants. The transgenic plants were transiently transformed with 35S-NbAELP plasmid or a binary vector pBIN19 as a control. It turned out that PrPME was induced in both variants regardless of which plasmid is delivered to plant cells, while 35S was insensitive to agroinfiltration. Modeling of abiotic stress was carried out by incubating transgenic plants in the dark for 96 hours, after which the level of emitted methanol that reflects the activity of PME was estimated. The methanol emission by PrPME-GUS transgenes after incubation in the dark was 3 times higher than that for the wild-type plants kept under the same conditions. Thus, we can conclude that stress effect leads to the immediate emission of methanol, and simultaneously to the PrPME activation leading to an increase of PME synthesis and methanol emission. This study was performed with the financial support of the Russian Science Foundation (project No. 19-74-20031).

P-05.4-19
Obtaining transgenic tobacco plants containing and simultaneously expressing the Δ12-acyl-lipid desaturase gene of cyanobacterium *Synechocystis* sp. PCC 6803 and the thaumatin II gene

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Currently, it is quite relevant to study the mechanisms of adaptation of plants to abiotic and biotic stresses. One of the mechanisms of increasing the adaptation of plants to abiotic stresses (frost, drought, UV and others) is the increase in the proportion of unsaturated fatty acids in the composition of membrane phospholipids. Desaturases are enzymes that promote the formation of double bonds in fatty acids and thereby convert saturated FA into unsaturated FA. Thaumatin is a protein produced by plants of the genus *Thaumatococcus* in response to an attack by viral pathogens. Genetic transformation method obtained tobacco plants *Nicotiana tabacum* containing in their genome and expressing the gene Δ12-acyl-lipid desaturase cyanobacterium *Synechocystis* sp. PCC 6803 and the thaumatin II gene, the sequence of which was cloned from the plant genome of *Thaumatococcus daniellii*. The work is supported by a grant 0120U100130 from the National Academy of Sciences of Ukraine

P-05.4-20
Test systems for the identification of polymorphisms in FAD3A and FAD3B genes that associated with the ratio of fatty acids in flax oil

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Flax (*Linum usitatissimum* L.) is an important crop. The ratio of fatty acids in linseed oil determines the direction of seed use. Fatty acid desaturases FAD3A and FAD3B catalyze the conversion of linoleic (LIO) fatty acid into linolenic (LIN) one that mainly determines the composition in flax oil. Two key single nucleotide polymorphisms (SNPs) in FAD3A and FAD3B genes associated with the content of LIO and LIN were revealed by us using deep sequencing. For fast and cost-effective identification of these SNPs, we developed test systems based on the HRM (high-resolution melting) analysis: primers for amplification of FAD3A and FAD3B regions with target SNPs were designed, and PCR (polymerase chain reaction) conditions were optimized. Temperature shift in melt curves was distinguished clearly within FAD3A and FAD3B standards, which were present by homozygotes with reference or alternative SNPs and also heterozygotes. To evaluate the accuracy of our test systems, we performed targeted deep sequencing on the Illumina platform and HRM analysis of FAD3A and FAD3B genes for F_2 flax hybrids obtained from varieties that differed in the SNPs associated with the content of LIO and LIN. The results of SNP identification in FAD3A and FAD3B genes based on deep sequencing and HRM analysis were in good concordance. Thus, the developed HRM-based test systems will be useful for the marker-assisted selection of flax cultivars with targeted oil characteristics. This work was funded by RFBR according to the research project 17-29-08036.

P-05.4-21
Expression profiling of shattering and non-shattering accessions of common buckwheat

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Common buckwheat (*Fagopyrum esculentum*) is an important non-cereal grain crop. Its wild variety *F. esculentum* ssp. ancestrale, has an unfavorable trait, the seed shattering, while

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cultivated varieties are non-shattering. It was earlier shown to be determined by a single gene; shattering is a dominant trait and non-shattering is recessive. The genetic control of shattering is extensively studied in grasses, in contrast, almost nothing is known about buckwheat, which belongs to another lineage of flowering plants. We investigated this question using a transcriptomic approach. To do this we collected pedicels of shattering and non-shattering plants at two developmental stages: small seeds (early stage) and large ripening seeds (late stage) and analyzed them using RNA-seq. Analysis of expression profiles showed that differential expression is more pronounced for the younger stage (224 down-regulated and 198 up-regulated genes under fold change threshold = 2 and threshold for FDR = 0.05). For the stage of ripening seeds, these numbers are 111 and 70, respectively. 58 and 37 transcripts are up- and down-regulated on both stages. The genes that are up-regulated in the pedicels of shattering plants show significant (FDR < 0.05) enrichment with Gene Ontology categories related to sugar metabolism and extracellular protein localization. Consideration of individual genes also indicates on the genes associated with the cell wall (for example, genes encoding expansins, plasmodesmata callose binding protein PDCB3, beta-xylosidase). This is in congruence with the results in other species (in particularly in rice - Ji et al. 2006) which indicate that the reorganization of a cell wall plays an important role in seed shattering.

P-05.4-22
The seed proteome of pea (*Pisum sativum* L.) lines different in their response to beneficial microorganisms highlights the different strategies for seed production in plants

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Plants of the Fabaceae family possess the ability to form beneficial symbioses with rhizobial bacteria (Rh) and arbuscular mycorrhizal fungi (AM). Different pea (*Pisum sativum* L.) lines were observed to differ in their response to the microsymbionts, some increasing their productivity more than others. Two pea lines with were chosen for this study, as contrasting in their efficiency of interaction with soil microorganisms (EIBSM), high for K-8274 and low for K-3358. Analysing the pea seed proteomes of the lines both with, and without combined microbial inoculation we were able to identify 111 differentially expressed proteins in the two lines (previously published in: Mamontova et al. (2019) Molecules 24(8), 1603). The effect of plant genotype on seed proteome signatures turned out to be more pronounced, than that of inoculation with BSM, with proteins important for seed maturation being more abundant in the seed of K-8274. The high-EIBSM line K-8274 responded to inoculation by up-regulation of proteins involved in cellular respiration, protein biosynthesis, and down-regulation of late-embryogenesis abundant proteins. In contrast, the low-EIBSM line demonstrated lower levels of cell metabolism proteins. The line K-8274 demonstrating prolonged seed filling under optimal conditions, and the line K-3358, that completes the seed development as fast as possible are reminiscent of the K- and R-strategies characteristic for various organisms. Taking into consideration the findings about the retardation of pea plant senescence caused by inoculation with AM (previously published in: Shtark OY et al. (2019) PeerJ 7, e7495), it is reasonable to assume, that the AM component of the inoculant plays an important role in the extension of the vegetation period in the lines with high EIBSM, and shortening it in those with low EIBSM. This finding might be useful in the selection of pea varieties with specific traits. This work is supported by the RSF grants (17-76-30016,16-16-00118).

P-05.4-23
Physiological and biochemical states of winter wheat donor plants as factors affecting gametophytic haploid production

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Haploid technologies, in particular induced androgenesis, stand out among other biotechnological methods in plant breeding. However, the widespread use of them for the production of new cereal varieties is largely limited. In this work, we investigated the changes of energy metabolism and fatty acid composition in flowers – anther donors – after cutting the spikes and 7 or 14 days after the start of cold pretreatment of winter wheat variety Irkutskaya. The analysis of biochemical parameters was carried out using flowers from the middle third of the spike – exactly the part that is used for the anthers extraction for an induced androgenesis. The cytochrome pathway activity showed high stability – it was about 30 nmol O_2 / (min * g fresh weight) regardless of the presence and duration of cold exposure, while the activity of the alternative pathway decreased after 7 days of cold treatment from 30 to 15 nmol O_2 / (min * g fresh weight). High respiratory activity during cold exposure led to a 2-fold decrease of the content of water-soluble carbohydrates in flowers after 14 days of exposure. It was also found that changes of the total lipids fatty acid composition, indicating the successful hardening (an increase in the content of unsaturated fatty acids and a decrease in the content of saturated ones), occurred only after 7 days of cold pretreatment of plants. An increase of the cold treatment period led to a sharp decrease of the unsaturated fatty acids content. So, an increase of the cold pretreatment period may led to a sharp decrease of energy supply necessary to maintain adaptation processes and negatively influenced on haploid production. Acknowledgment: The reported study was funded by RFBR, project number 20-34-80003. The research was done using the collections of The Core Facilities Center "Bioresource Center" and the equipment of The Core Facilities Center "Bioanalitika" at The Siberian Institute of Plant Physiology and Biochemistry SB RAS (Irkutsk, Russia).

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P-05.4-24
Transcription factors MYB60, CPC and MYBL2 are involved in anthocyanin accumulation and formation of root hairs in *Brassica napus*

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The ability of anthocyanins to increase plant resistance to stress and quality of human nutrition have been widely discussed in the recent years. Genes of MYB transcription factors, namely MYB60, CPC and MYBL2, were reported to suppress anthocyanin accumulation in *Arabidopsis thaliana*. Therefore, the abundance of anthocyanins in plants can be increased by the knockout of these genes using CRISPR/Cas system. Important crops and close relatives of *A. thaliana*, such as *Brassica napus* and *B. oleracea*, are the objects of choice for such experiments. MYB genes also have other functions. For example, strong expression of MYBL2 inhibited trichome formation in the leaves, and CPC is considered a positive regulator of root hair (RH) development. The role of MYB60 in formation of RH and trichomes was never previously studied. Plasmids for the knockout of target genes were created by designing and cloning gRNA spacer sequences into the pDIRECT_22A vector (Addgene plasmid # 91133). The accumulation of anthocyanins in the leaves of *B. napus* was observed after agroinfiltration with *Agrobacterium tumefaciens* carrying these plasmids. However, there was no anthocyanin pigmentation in mutant hairy roots, generated using *Agrobacterium rhizogenes*, the length and density of RH were affected. Control hairy roots had approximately 100 hairs per mm with average length 0.3 mm. In CPC mutants, RH density was 50% lower, however the length of RH increased up to 0.68 mm. There was no significant change in RH density in MYB60 mutants, but RH length increased by 30%. In MYBL2 mutants, RH density increased by 70%. Therefore, all studied MYB transcription factors are probably involved in formation of RH not only in normal roots, but also in hairy roots. Hairy roots can be used as a model system to study the efficiency of CRISPR genetic constructs. The reported study was funded by Russian Science Foundation according to the research project №20-74-10053. *The authors marked with an asterisk equally contributed to the work.

P-05.4-25
Application of self-sustained luminescence for monitoring plant physiology

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Fluorescence-based biosensors for monitoring the concentration of calcium and other metabolites have limited application in heavily pigmented plant tissues. In addition to the background fluorescence of these indicators, high autofluorescence of most plant tissues, a "nightmare for fluorescence imaging" (Pawley 2010), hampers their performance and limits their use in plant biology. Unlike fluorescence-based approaches, by not requiring light excitation luminescence-based imaging has essentially no background signal and no phototoxicity, no photobleaching, and light-induced physiological effects. Here, we report our progress on engineering a luminescent calcium indicator based on the bioluminescent system from fungi (Kotlobay et al., PNAS 2018). By creating a calcium-dependent version of the bioluminescence system, we plan to develop the first substrate-free luminescence imaging technology suitable for monitoring calcium concentration in plants and other multicellular organisms. This work was supported by the Russian Foundation for Basic Research project 21-54-52004 and partly by the President grant MK-5405.2021.1.4.

P-05.4-26
Cannabinoid content in hemp (*Cannabis sativa* L.) phenotypes of two different varieties

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Hemp (*Cannabis sativa* L.) is a multi-purpose crop (food, oil, fibre, medicine) (Andre et al., 2016). The plant contains many different secondary metabolites such as cannabinoids, terpenoids, steroids, flavonoids, lignans, alkaloids, etc. The most important compounds are cannabinoids and terpenes, both found in hemp inflorescences especially in trichomes (Janatová et al., 2018). The aim of our study was to determine the composition of cannabinoids in two different phenotypes of the Italian Carmagnola CS (CI and CII) variety and four different phenotypes of Finola selection (FI, FII, FIII, FIV). CS was grown on the field in Ljubno ob Savinji and Finola selection was grown in Žalec, Slovenia in 2019. The inflorescences of the plants were collected in the phase of maturity, dried and stored in refrigerator until analysis. Cannabinoid content was analyzed by HPLC. All five phenotypes of Finola selection had the highest content of CBD-A (6.36% - 6.59%) in comparison to CS phenotypes. The highest content of CBG-A was in phenotype CI (1.62%), CI had also the highest content of Δ-9-THC-A (0.91%) while the highest proportions of Δ-9-THC were in phenotype FII (0.11%). Based on almost the same ratio between CBD and Δ-9-THC and CBD-A and Δ-9-THC-A, phenotype CI could be classified in chemotype II, while CII and all phenotypes of Finola selection could be classified in chemotype III. According to PCA analysis for the averages of phenotypes, differences between Finola selection and CS can be seen, and also differences between CS phenotypes are noticeable, while there were no evident differences between individual phenotypes of Finola selection. As it is known, cannabinoids in combination with terpenes have a synergistic effect on human health. Based on the obtained results we will be able to assign the most suitable phenotype for further investigations in pharmacy. *The authors marked with an asterisk equally contributed to the work.

P-05.4-27
Screening assay for proteins conferring desiccation tolerance

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Intrinsically disordered protein 106094 from tardigrade *Hypsibius dujardini* is responsible for the ability of this organism to survive desiccation. However, the mechanism of action of the protein 106094 and the sequence-to-function relationship within this protein is not well understood. Here, we report our progress on the

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development of a high throughput assay for testing functional performance of proteins conferring desiccation tolerance. The assay will be used to test the performance of mutants of the tardigrade protein 106094. This work was supported by the Russian Science Foundation project 19-74-10102.

P-05.4-28
Tomato transgenic plants for phytoremediation

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Phytoremediation is the elimination, neutralization or conversion of pollutants to a less toxic form with the help of plants. This method is often used for heavy metal (HM) contaminated soils. It is important to select suitable plants – hyperaccumulators of HM to restore the biological productivity of ecosystems. Tomato is widely cultivated in agriculture. In recent years, studies on the accumulation of HM in tomato plants and the potential use of tomatoes for phytoremediation have become popular. It is known that phytochelatins protects plant cell from HM harmful effect and results in accumulation of metals in plant in low toxic form. Phytochelatins contain a gamma peptide bond, therefore, the use of pseudophytochelatins with an alpha peptide bond is relevant in biotenology. Vector constructs for the transformation of plants with the pseudophytochelatin pph6 gene were obtained. Artificially synthesized the pph6 gene assembled from complementary blocks 5'ATGGAATGCGAATGTGAGTGCGAG TGCGAGTGCGAATGTGGC-TAA3 'and 5'TTAGAGA-CACTCTCGCACTCGCACTCGCAT. After stitching these blocks, the sequence was cloned into a vector for plant transformation pCambia 1301 under the control of the 35S promoter of the cauliflower mosaic virus and and later used to obtain transgenic tomato plants by agrobacterium transformation method. Then, the positive effect of the expression of the pph6 gene on the resistance of transformed plant tissue cultures to the effects of heavy metals (Cd^{2+} and Ni^{2+}) was shown. Thus, a system of agrobacterial transformation of the industrial variety of tomato plants Gruntovy Gribovsky 1180 with the pseudophytochelatin pph6 gene was developed, which made it possible to obtain plants that would potentially efficiently accumulate heavy metals for further use in phytoremediation. This work was performed as a part of State Assignment no. №AAAA-A21-121011990120-7; it was financially supported by the Russian Foundation for Basic Research, project no. 18-34-20004 mol_a_ved.

Immunotherapy

P-06.1-01
Cytotoxic recombinant antibody specific to galectin-3 as a candidate for the treatment of malignant tumors

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Immunotherapy is based on activation of humoral or cell immunity for induction, intensification or suppression of immune response. In cancer therapy, this way of treatment is actively developing. Antigen-dependent cell-mediated cytotoxicity (ADCC) based on ability of T-cells to emit cytotoxic substances in response to binding of Fc-receptors of specific antibody. Human galectin-3 (Gal-3) participates in wound healing, adhesion, apoptosis and angiogenesis. High concentration of galectin-3 in serum is most often found at malignant tumors (Danguy et al. Biochim. Biophys. Acta, 2002). We have obtained four recombinant monoclonal antibodies with affinities from 33 to 0.12 nM against galectin-3 by the phage display method. To check antigen-dependent cytotoxicity of our antibodies, we used MCF-7 cell line since it contains Gal-3 on the surface. Although we used G7015 kit (Promega, USA). The principle of the method is based on the ability of genetically modified Jurkat cells to express luciferase in response to binding of the test antibody complex to the antigen on the surface of the target cells. Luminescence of antibodies was stronger that of non-specific immunoglobulin but not sufficient for developing a medicine. We applied afucosylation of antibody with highest ADCC activity based on preliminary experiments (Kanda et al., Glycobiology, 2007). Afucosilation results in significant enhancement of antigen-dependent cytoxicity of the antibody. EC50 (half of the effective concentration) for our antibody was 2.9 nM. Rituximab (a positive control) was EC50 70 pM in our experiment. Weak ADCC activity may be mainly due to the property of galectin-3 to induce apoptosis of cells which contain the CD45 receptor on the surface, including the effector cells of the Jurkat line, which also contain such receptor. For perspectivity of creating drug based on the B3 antibody, this property should be studied further for successful creation of a functional drug based on the B3 antibody.

P-06.1-02
Influence on protein conformation as an approach to immunotherapy

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Structure of proteins contributes significantly to their function and its changes could have crucial role in the biological effects. For instance, conformational conversion of a cellular prion protein into a misfolded isoform is a key factor for prion disease pathology. In therapy, allosteric drug binding induces protein conformational rearrangements that may lead to notable adjustments in an organism. Thus, finding approaches to a specific change in the conformation is an important issue. We chose antibodies, one of the most specific molecules, and studied high dilutions of them (HD-A) as medicines. For HD-A to interferon-γ (IFNγ), which antiviral activity has been shown before, it was suggested the possible influence on the target protein structure. Using 1D proton NMR spectroscopy of IFNγ in the presence of HD-A-IFNγ (not a control), changes in the local environment of some peaks in backbone and side-chains of amino acids of the molecule were found. The 2D ^{1}H-^{15}N HSQC spectrum for IFNγ showed a total of 13 peaks had shifted after addition of HD-A-IFNγ (not a control). By the method of time-resolved fluorescence, a decrease in IFNγ conformational transition temperature

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in the presence of HD-A-IFNγ almost on 1°C vs control ($P < 0.05$) was shown. The changes in interaction between target molecule IFNγ and complementary molecules in the presence of HD-A-IFNγ also could be a consequence of the protein conformation changes. Thus, HD-A-IFNγ influence Ag-Ab binding statistically significantly vs control ($P < 0.05$) on the surface plasmon resonance biosensor. The treatment of U-937 cells with HD-A-IFNγ resulted in a significant increase up to 29% ($P < 0.05$) in the specific binding of [^{125}I]IFNγ to IFNγ receptors vs control. Given interesting results for HD-A to other proteins, it can be assumed that the nature of HD-A effects could be connected to the influence on conformation of target proteins, so a novel approach to protein conformation changes by therapy is presented.

P-06.1-03
Mixed adjuvant formulation based on peptide hydrogel and desmuramyl peptides: preparation and influence on specific immune reaction

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Adjuvants are essential for enhancing vaccine potency by improvement of the humoral and cell-mediated immune response to vaccine antigens. Adjuvants of new generation are adjuvant systems composed of various combinations of classical adjuvants designed to adjust the adaptive immune responses against pathogens. The challenge for this strategy is to define an effective and safe formulation in which individual components can synergize with one another to elicit a more robust immune response. Self-assembling peptides have a great potential for applications in bionanotechnology and it has been shown that certain peptides possess immunostimulatory activity [1]. Muramyl dipeptide (MDP) is a synthetic immunoactive peptide consisting of N-acetyl muramic acid attached to a dipeptide L-Ala-D-isoGln. It was first identified in bacterial cell wall peptidoglycan as the smallest fragment possessing adjuvant activity. Desmuramylpeptides have been extensively studied in an attempt to increase adjuvant activity and boost the immune response effectively for clinical use in the treatment of cancer and other diseases. We report here the preparation and characterization of adjuvant system based on supramolecular hydrogel of a self-assembling tripeptide Ac-L-Phe-L-Phe-L-Ala-NH_2 with built-in desmuramylpeptide immunopotentiators [2,3]. Recently, a series of novel acyl tripeptides mimicking MDP were identified as potent nanomolar NOD2 agonists. The most potent derivatives were incorporated into hydrogel and tested in the mouse model of adjuvancy. The obtained results show that adjuvant system based on a hydrogel of a self-assembling tripeptide Ac-L-Phe-L-Phe-L-Ala-NH2 with built-in desmuramylpeptides elicits stronger specific immune response in comparison with antigen and adjuvant alone. References [1] Zichao L et al. (2016) Adv Mater https://doi.org/10.1002/adma.201601776 [2] Pospišil T et al. (2016) Biomater Sci 4, 1412–1416. [3]. Gobec M et al. (2018) J Med Chem 61 (7), 2707-2724.

P-06.1-04
Single-domain antibodies (sdAbs) against influenza A viruses (IAV) with potential broadly neutralizing activity

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Influenza is one of the major issue in global healthcare. Seasonal epidemics lead to 3–5 millions of severe illness and up to 650,000 lethal cases annually. IAV, which can cause pandemics, are of high epidemiological importance. The main problem in development of universal vaccine and novel antiviral therapy is antigenic drift. One approach to resolute the task is synthesis of molecules targeted to conservative epitope of hemagglutinin (HA), which is the major surface antigen of IAV. SdAbs, derived from Camelidae family heavy-chain IgG, appear to be a promising agent due to its unique structure. The aim of this work was isolation of sdAbs, binding with different types of IAV. We have used phage display technology for screening the specific sdAbs from peripheral blood of alpaca, immunized with the Grippol® plus influenza vaccine (containing HAs of H1N1, H3N2 and B) + H5 (H5N1) and H7 (H7N9) recombinant HA. There are two strategies we have chosen: 1) selection of sdAbs, binding with highly conservative stem domain of HA; 2) selection of sdAbs, targeting to conservative epitopes of globular domain. To implement the first strategy stem domain (H1N1) was obtained in stable trimeric conformation and three rounds of phage panning were performed on stem trimer. In the second variant three rounds of panning were carried out on H1N1 or H3N2 viruses. After selection, pDNAs coding 190 sdAbs were exposed to Sanger sequencing and according to its CDR3 sequence were divide into 17 groups in total. Individual sdAb-phages were tested for binding to different IAV in ELISA. Eventually we selected four stem binding sdAbs (H1.2, G2.3, 2F2, B6.2) and two head specific sdAbs (H10.2, 3D7). H1.2, G2.3, 2F2 and B6.2 clones bind to group 1 IAV, whereas H10.2 and 3D7 are able to bind both group 1 and 2 IAV. Further work is planned on the characterization of the selected sdAbs and *in vivo* and *in vitro* virus neutralization assays for subsequent construction of bispecific sdAbs and fusionbody.

P-06.1-05
The study of hemolymph antimicrobial activity of Baikal endemic amphipods *Eulimnogammarus verrucosus*

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The natural products with antibiotic activity are important part of invertebrate innate immunity. Humoral immunity of crustaceans includes proteins and their complex, which recognize molecular patterns of pathogens. The antimicrobial peptides are one of the main components of the innate immune defence. They provide resistance against Gram-positive, Gram-negative bacteria and fungi. The aim of the study was to estimate the hemolymph antimicrobial activity of Baikal endemic amphipods *Eulimnogammarus verrucosus* (Gerstfeld, 1858) and to do a preliminary screening of antimicrobial peptides. Amphipods were collected in the littoral zone of lake Baikal near Listvyanka village. The acclimation of animals was carried out to laboratory conditions for 4 days at the temperature of 6°C in well-aerated aquaria. The

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hemolymph was collected from the central blood vessel using the glass capillaries. Antimicrobial activity of hemolymph was estimated within the disk diffusion method. We used 5 bacterial and 2 fungal cultures for antimicrobial testing. Hemolymph was concentrated and loaded on the paper disks. Disks were dried and placed on Petri dishes with test cultures. The antibiotic effect was observed after 24 hours. Antibiotic activity of hemolymph samples was shown against *Escherichia coli* and *Saccharomyces cerevisiae*. The presence of antimicrobial peptides can explain the antimicrobial effect of hemolymph. Several low molecular weight proteins were found after electrophoretic separation of hemocyte fraction. Thus, in the performed study, it was first found the antimicrobial activity of hemolymph of Baikal endemic amphipods. The study was carried out with the main financial support of the Russian Science Foundation grant 18-74-00018, Russian foundation for basic research grant 18-29-05051, Grant of President of Russian Federation MK-1245.2021.1.4 and Ministry of Science and Higher Education of the Russian Federation.

P-06.1-06
Automation of LC-MS multi-attribute method sample preparation in biopharmaceutical development

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Liquid chromatography-mass spectrometry (LC-MS) multi-attribute method (MAM) is an emerging methodology, which has been developed to monitor multiple product quality attributes (PQAs) of biopharmaceuticals in parallel. The MAM applies the principles of LC-MS peptide mapping and has the potential to provide site-specific quantitative information missed by traditional chromatographic and electrophoretic methods currently used for the monitoring of PQAs. Due to the complexity of the LC-MS MAM sample preparation workflow, its automation is of great importance for a successful implementation of MAM. In this study, we developed a fully automated platform for MAM sample preparation using a Tecan automated liquid handling system equipped with a liquid handling arm that enables precise pipetting of small volumes, a manipulator arm, and incubators. The objective of the protocol was to obtain two identical sets of injection-ready samples prepared by automated sample preparation and fully comparable to manual sample preparation. A biopharmaceutical was used to optimize the automated sample preparation procedure. Further on, LC-MS analyses of the prepared samples were performed using high-resolution MS Orbitrap technology. The automated sample preparation was verified by evaluating system precision and intermediate precision for relative quantification of glycan structures, oxidation and deamidation. In addition, carbamidomethylation was set as fixed modification and monitored to evaluate the efficiency of alkylation achieved during sample preparation.

P-06.1-07
Effects of statins in cultures of human CD4+ lymphocytes and macrophages

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Along with major lipid-lowering functions, statins possess immunotropic activity. As we have shown treatment of patients with CVD and HF with atorvastatin was associated with the increase of circulating regulatory T-cell numbers and decrease of proinflammatory cytokine levels. In this study, we performed an analysis of the effects of statins on cell proliferation, motility, apoptosis, cytokine production in cultures of human CD4+ lymphocytes and macrophages. Methods. Mononuclear leukocytes were isolated from donor blood by gradient centrifugation; CD4+lymphocytes and CD14+monocytes were obtained by subsequent immunomagnetic separation. As inducers of activation/differentiation PHA, IL-2, GM-CSF, LPS and INFγ were used. Cell proliferation and apoptosis were measured by CFSE dilution and active caspase-3 staining, respectively. Cell migration was assessed using Transwells. The analysis of intracellular and membrane proteins was performed with fluorescently labeled Mabs and Flow cytometry. Cytokine expression was assessed by RT-PCR (mRNA content) and ELISA (protein levels in supernatants). Results. Dose-dependent inhibition of CD4+T-cell proliferation accompanied by the increase of Foxp3+cell percent by statins was detected. Statins impaired spontaneous and chemokine-induced lymphocyte migration while not affecting surface chemokine receptor exposition and chemokine-induced Akt, p38, ERK1/2 kinase phosphorylation. Pretreatment with statins inhibited cytokine production by activated macrophages, while the level of cytokine mRNA in cells did not decrease. Statins did not affect Akt, p38, ERK1/2 kinase phosphorylation in activated cells. Active caspase-3 expression was down-regulated. Cellular effects of atorvastatin were manifested at lower concentrations compared to rosuvastatin. Conclusion. Immunomodulatory properties of lipophilic statins may be realized primarily due to the suppression of effector T-cell proliferation and motility and macrophage cytokine production.

P-06.1-08
Proteus bacteriophage augments specific and non-specific immunity against host bacterial pathogen

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Lytic bacteriophages have long been known for their capacity to precisely target and kill their bacterial host. While this is the basis for bacteriophage therapy of antibiotic-resistant bacterial infections, properties other than lytic capacity, such as phage immunogenicity, may contribute to the successful outcome of phage therapy. That study questioned whether modeled phage therapy can induce a bacteria-specific immune response. To show this possibility, Balb/c mice were administrated intraperitoneally with NaCl 0.9%, a non-lethal dose of *Proteus mirabilis*, its specific phage PM16, or both *P. mirabilis* and PM16, two times at a

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two-week interval. Blood samples were collected at different time points and corresponding sera were analyzed for the presence of antibodies against PM16 by ELISA and against *P. mirabilis* using FACS and Confocal microscopy high content analysis. Immunization with PM16 alone neither led to the induction of specific antibodies nor to cytokine production. Meanwhile, immunization with *P. mirabilis* gained antibodies against *P. mirabilis* just moderately. Alternatively, PM16 introduced after *P. mirabilis* resulted in increased both Proteus-specific and PM16 serum antibody levels. Interestingly, PM16-specific antibodies augment the engulfment of bacteria/phage/antibody complexes by macrophages in vitro. To test whether phage-specific immunity can influence the bacteria-specific response, mice were pre-immunized with PM16 using complete Freund's adjuvant. Compared to non-immunized mice, these mice showed increased Proteus-binding capacity of sera. Given data demonstrated that phage and its specific bacteria can mutually augment the immune response to each other. Presumably, newly formed phages can serve as baits for the innate and adaptive immune system to stimulate immune surveillance and allow efficient recognition of bacterial pathogen, thus providing the basis for novel antimicrobial immunotherapy. The work is supported by RSF grant 19-75-00065. *The authors marked with an asterisk equally contributed to the work.

P-06.1-09
Intracellular PD-1 interception for adoptive immunotherapy against PD-L1 positive cancers

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Currently, methods of immunotherapy are becoming more widespread in the treatment of cancer. They are a relatively new group of cancer therapeutics and include monoclonal antibodies, immunotoxins, and various options for cellular immunotherapy. One of the promising approaches is the use of T cells by modified a chimeric antigen receptor (CAR) has shown noticeable results for B-cell malignant. However, one of the urgent problems at present is the insufficient efficiency of adoptive cell therapy due to immunosuppression by tumor cells. One of the key points of immunosuppression is the interaction of the inhibitory receptor PD-1 presented on T cells with its ligand PD-L1, which can be expressed at a high level by tumor cells. The clinic now uses only therapy with antibodies specific to one of these proteins. A significant drawback of this method is the nonspecific blocking of the regulation of the activity T-lymphocytes, which leads to the risk of developing various complications, including an autoimmune response. An alternative to the administration of antibodies in conjunction with adoptive cell therapy can be the use of genetically modified tumor antigen-specific T cells with proteins that block the inhibitory PD-1 / PD-L1 interaction. We combined CAR through a self-cutting site T2a with single-chain variable fragment specific for PD-1 with the protein PEBL to retain it intracellularly. We tested these genetic constructs on reporter cells with overexpression of PD1 and found that there was much less PD1 on the surface. The activation will be shown in a model of immunosuppression mediated by PD-1 / PD-L1 interaction, where we have already created tumor cells overexpressing CD19 and PD-L1. Such an approach would have to cope with the therapy-negative tumor immunosuppression via PD-1 / PD-L1 interaction. This study was supported by the Ministry of Education and Science of the Russian Federation project №. 075-15-2020-773

P-06.1-10
A cheap immunoassay for determination of immune response to SARS-CoV-2 vaccine in capillary blood samples

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The coronavirus disease 2019 (COVID-19) caused by SARS-Cov-2 virus represents public health emergency of international concern. The recent development of SARS-CoV-2 vaccines offer great hope to fight against this worldwide pandemic. However, there are many unknowns moving forward, like how long immunogenicity lasts or how many doses are required to immunize a person. High-quality serology is a key tool in understanding the immunity to viruses, and the relevance of protection. The measurement of specific COVID-19 antibodies can be used and currently very necessary to verify the effectiveness of the different vaccines in the population. Here, we present an optimized ELISA-based serology protocol that can be applied in serum and in capillary blood. Validation was performed using serum collected from 113 hospitalized patients in Principe de Asturias University Hospital. Ninety-eight of them were diagnosed with COVID19 by a positive rRT-PCR result and fifteen samples were negative in rRT-PCR. We used an indirect ELISA assay, using the receptor binding domain (RBD) of the recombinant spike protein of SARS-Cov-2 to cover the plate. In addition, all patients were tested by commercial ELISA to compare with our cheap immunoassay. Results show that our ELISA assay is suitable for capillary blood samples and has a sensibility significantly higher than commercial ELISA. This study demonstrates the validity of our cheap immunoassay for the measurement of specific IgG in the serum of patients, which allows the safe and economic testing of all currently vaccinated people to ensure immunity in the population and the efficacy of vaccination. Surprisingly our assay works on capillary blood samples, which facilitates the process of obtaining the sample and can be carried out quickly and safely by the patients themselves. Therefore, our cheap immunoassay may be useful to provide population-based data on SARS-CoV-2 seropositivity, infection and immunity generated by the vaccines. *The authors marked with an asterisk equally contributed to the work.

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New frontiers in medicinal chemistry

P-06.2-01
DSC of blood plasma: a complementary technique for following efficacy of disease treatment

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Clinicians exploit the human plasma proteome routinely as an indicator of patient health and as a source for biomarkers of human disease. A number of FDA-approved plasma /serum diagnostic assays are routinely used. These include serum plasma electrophoresis and a variety of immunochemical assays that can monitor the concentrations of specific proteins in plasma. Recently, it was suggested that differential scanning calorimetry (DSC) could be used as a new disease-monitoring application. DSC is a quick and simple technique, which does not require a complicated sample preparation. Surveys already proved that DSC can distinguish between plasma from healthy person and sick person. Not only that the thermograms of blood plasma obtained from sick individuals are different from the thermograms of blood plasma obtained from healthy individuals but also several types of diseases display a characteristic thermogram. Our goal was to evaluate potential correlation between changes in DSC profile of blood plasma and treatment progress of patients with multiple myeloma (MM). For each included patient we performed DSC analysis of blood plasma for several patients that were diagnosed with MM for the first time or had a relapse. Alongside DSC analysis, we obtained the information on heavy chain class (IgG, IgE, IgA, IgD, and IgM) and the light chain proteins together with major diagnostic and prognostic markers (b2 microglobulin, lactat dehydrogenase, serum albumine). During and after the intensive treatment of patients, we analyzed blood plasma in regular time intervals (every 2 or 3 days) and evaluated correlation between DSC thermogram profile changes and monoclonal protein/clonal free light chain concentration. The collected data suggests that changes in DSC thermograms are in correlation with changes in biochemical tests (usually the main marker is M spike – heavy chain class). We will discuss these results in more detail.

P-06.2-02
Effects of cold atmospheric plasma on cancer and normal cells *in vitro*

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Cold atmospheric plasma (CAP) has been shown as potential anticancer tool. CAP is an ionized gas consisting of charged particles, active uncharged particles, an electric field and UV radiation. Here, we analyzed the cell death mechanism after CAP treatment using various cell lines. The biological effect of plasma-activated cultural medium on the epidermoid carcinoma cells A431, and normal human embryonic kidney cells HEK-293T has been investigated. The medium was exposed to CAP irradiation generated in argon gas for 2–8 min at a voltage of 4.9 kV and then added to the cells. The proliferation of the treated cells in real time mode was measured using iCELLigence RTCA. The rate of apoptotic and necrotic cells was analyzed by the flow cytometry. The levels of intracellular reactive oxygen (ROS) and nitrogen (RNS) species, which are known to be the main CAP affectors, were measured with 2',7'–dichlorofluorescin diacetate fluorescent dye. To revel the conditions when the selectivity of CAP against tumor cells can be achieved we used a pair of lung cell lines: adenocarcinoma cells A549 and normal fibroblasts Wi-38. The viability of A549 and Wi-38 cells after direct CAP treatment were examined. Under optimized CAP conditions (duration 60 c, voltage amplitude 4.2 kV, 3 L/min in helium) Wi-38 stayed alive and A549 cancer cells were killed. The expression profiles of treated cells were evaluated by using RNA-seq. Functional analyses were employed to reveal the difference in normal and cancer cell response. The data obtained could be a basis for the development of selective CAP treatment of cancer cells. This study was supported by the RSF grant # 19-19-00255 and by the RFBR grant # 20-34-90021.

P-06.2-03
Anticancer peptide RL2 – a promising tool for intracellular delivery of therapeutics and diagnostic molecules

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Understanding of mechanisms by which biologically active compounds penetrate into cells is necessary for development of antitumor drugs and overcoming multidrug resistance. RL2 is a fragment of human kappa-casein, which induces apoptosis of cancer cells and suppresses tumor growth in vivo. It efficiently penetrates into cancer and normal cells, but it has no cytotoxic effect towards the latter. However, the mechanism of RL2 penetration into cells remained unclear. We have shown that RL2

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efficiently enters into cells partly through lipid raft-mediated pinocytosis and partly by direct penetration through the plasma membrane which described for Cell-penetrating Peptides (CPPs) – peptides are known for their ability to effective deliver cargo molecules into the cells. The study of RL2 structure by circular dichroism and NMR spectroscopy showed that RL2 is an intrinsically disordered peptide, capable of partial folding of 31-40 a.a. fragment in hydrophobic environment. Such folding is also usual for CPPs, it is believed to allow them to penetrate cell membranes. Thus, RL2 structure and cell interaction characteristics allow to classify this peptide as CPP. So far as RL2 is CPP, it was interesting to investigate its ability to form noncovalent complexes with nucleic acids and deliver these molecules into human cells. We found that RL2 is capable of delivering different nucleic acids (plasmid DNA expressing the green fluorescent protein EGFP, siRNA against EGFP and cytotoxic small nucleolar RNA) as non-covalent complexes as well as covalently bound paramagnetic label into human cells. So, anticancer peptide RL2 is a promising tool for intracellular delivery of therapeutics and diagnostic molecules.

P-06.2-04
Is mitochondrial complex 1 inhibition responsible for metformin action in muscle?

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Metformin is an antidiabetic drug, widely used in clinical practice for over 60 years. Despite of this, the molecular mechanisms by which it exerts its effects are not agreed upon. Many studies state inhibition of complex 1 of the mitochondrial respiratory chain as a key mechanism of action, but a variety of concentrations and models used in these studies lead to inconsistent conclusions about the mechanism and properties of this inhibition. Metformin concentrations used in most studies are significantly higher than plasma concentrations found in vivo. The aim of this study was to compare the effect of different metformin concentrations, ranging from therapeutic (50 μM) to high concentrations used in most in vitro studies (5 mM), on cell viability, mitochondrial function and energy metabolism of muscle cells. C2C12 mouse myoblasts showed a slight, dose-dependent decrease of cell viability when treated with metformin concentrations higher than 200 μM, and 5 mM metformin decreased the proliferation rate, compared to untreated and cells treated with 50 μM metformin. As expected, 5 mM metformin treatment caused a decrease in intact cell respiration and respiration using complex 1-linked substrates, while complex 2 respiration was unaltered. This was accompanied by mitochondrial membrane depolarisation and an increase in superoxide production, observed by flow cytometry (JC-1 and DHE staining). 50 μM metformin treatment caused no changes in mitochondrial respiration or any of the other effects observed when using the high metformin concentration. The lowest concentration of metformin that caused a decrease in complex 1-linked respiration is 1 mM, but intact cell respiration was unaltered by this treatment. This confirms the need for further investigation into the molecular mechanisms of action of this drug, to see if the therapeutic, glucose-lowering effect of metformin can indeed be attributed to complex 1 inhibition.

P-06.2-05
New synthesized ferrocenyl trizole nucleobase derivatives induced cell death by mitochondria- mediated apoptosis in tumor cells *in vitro*

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Taking into consideration the biological importance of ferrocene and nucleoside analogues, the synthesis of twenty mono- and bis-ferrocene conjugated nucleobases that are bridged by triazole linker was undertaken with the aim to evaluate their antioxidant potential and activity against various tumor cell lines. Radical scavenging capacity, measured by 1,1-diphenyl-2-picrylhydrazyl (DPPH) and expressed as a % of DPPH reduction after 30 and 60 min, was between 62 and 90 %. Antiproliferative capacities of ferrocene derivatives were tested by MTT test on adenocarcinoma, leukemia, lymphoma, and normal fibroblast like cells. According calculated IC50 values, selectivity index, and no cytostatic effect on normal Madin-Darby canine kidney fibroblast like cells (MDCK1), four derivatives were selected for further testing on effects on intracellular ROS accumulation, mitochondrial membrane potential dysfunction, as well as apoptosis and autophagy induction in treated tumor cells. Slight changes in ROS intracellular accumulation and activated autophagy were observed. Tested new synthesised derivatives induced disruption in mitochondrial membrane potential ($\Delta\Psi m$) and mitochondrial dysfunction, resulting in mitochondria swelling accompanied by activation of apoptosis in treated cells. Early apoptosis induction was determined in colon adenocarcinoma (CaCo-2), Burkitt lymphoma (Raji) and acute lymphoblastic leukemia (CCRF-CEM) cells after treatment with selected derivatives. In conclusion, obtained results contribute to better understanding of antiproliferative activity of new ferrocenyl triazole nucleobase derivatives. Key words: bis-ferrocene conjugated nucleobases, antiproliferative activity, apoptosis, mitochondrial potential disruption, ROS accumulation

P-06.2-06
The use of computational methods for design of polyamine-targeted synthetic compounds with antitumor activity

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Polyaminoxidase (PAO) participates in biodegradation of polyamines, catalyzing their oxidative deamination. The cytotoxicity of oxidation products of polyamines - iminoaldehydes, is

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considered to cause apoptotic cell death, since being carbonyl agents, they are able to conjugate with proteins and nucleic acids. The decrease in the activity of polyamine oxidase was observed in histochemical study of malignant tumor tissue of animals and humans, in nitrosodiethylamino-induced hepatomas, in Guerin carcinoma, in neuroblastomas and human lymphomas and other types of cancer. The oxidative deamination of polyamines is practically absent in tumor cells. Therefore, activators of polyamine catabolism may be potential anticancer agents. The effect of aniline, azafluorene, benzimidazole and dioxaborininopyridine derivatives on oxidative deamination of putrescine, spermidine and spermine in a cell-free test system from the regenerating rat liver was studied. The derivatives of azafluorene and aniline were found to be activators of polyamine catabolism. To investigate their antiproliferative activity several cancer cell cultures were used in viability test with Alamar blue. The quantitative correlation analysis of the structure-activity system according to the ChemicDescript program showed a high correlation of cytotoxicity with several topological indices. Docking with the active site of PAO, murine enzyme 5LFO (Syatkin et al., 2019), was carried out with the use of Molegro Virtual Docker 6.0 with flexibility in torsion angles of ligands. Positions with the lowest energy level for each compound have been described by the interaction energies of a ligand with different enzyme amino acids. Asp211 and Tyr204 were found to be the most significant active site residues for the differentiation of the tested compounds into activators and inhibitors. These results can be helpful in design and synthesis of new activators of polyamine catabolism with antitumour activity.

P-06.2-07
The illumination dependent synthesis of novel natural products in Actinobacteria

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The aim of this study was to perform a screening of natural products produced by Actinobacteria related to *Nocardiopsis* and *Streptomyces* genera under the cultivation in conditions of excess daylight, in the absence of light, and in case of aggressive UV(A) radiation. The cultivation of microorganisms was performed at a temperature of 28° C of 7 days. Metabolites from cultural liquid and cell biomass were extracted with ethyl acetate and a mixture of acetone:methanol, respectively. Extracts were evaporated and dissolved in methanol:DMSO mixture. Samples were initially analyzed within high-resolution LC-MS and dereplication technique using the Dictionary of Natural Products database. Data were collected and analyzed within Bruker Compass Data Analysis software. We identified 2 compounds (members of antibiotic family Sannamycin) known for the *Streptomyces* sp., when this strain was cultured in the liquid medium SG under conditions of excess daylight. The cultivation of strain in the conditions of absence of light led to the biosynthesis of 10 new natural products. The growth of *Streptomyces* sp. strain under the conditions of aggressive UV(A) radiation led to the appearance of 1 new amphiphilic natural product with mass 219.1730 Da. Besides the number of natural products, the rate of illumination influenced on the average mass of natural products produced by the studied strain *Streptomyces* sp. At the same time, the parameters of cultivation were less effected of the strain of *Nocardiopsis* sp. while UV (A) radiation led to the death of strain. Thus, the cultivation of the *Streptomyces* sp. strain in the absence of light makes it possible to obtain new natural products. The study is carried out with the main financial support of Russian Science Foundation grant 18-74-00018 with the partial financial support of Russian Foundation for Basic Research grant 18-29-05051, Grant of President of Russia Federation MK-1245.2021.1.4, and Ministry of Education and Science.

P-06.2-08
New cathepsin V inhibitors as antitumor agents

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Cathepsin V is a lysosomal cysteine peptidase. It is closely related to cathepsin L; however, it is not ubiquitously expressed while its expression is mainly limited to thymus, testis and corneal epithelium. It has also an important role in immune cells and is expressed during the maturation of macrophages. In pathological processes its increased expression was observed in colorectal and breast carcinomas, but not in the normal colon or mammary tissue. Cathepsin V is functional equivalent of mouse cathepsin L and its ability to degrade elastin leads to the degradation of extracellular matrix, a prerequisite for tumor development. Because of its role in diseases progression, targeting cathepsin V with selective inhibitors opens new opportunities for therapeutic treatments. Therefore, for this purpose we selected compounds from commercial libraries, by their molecular docking into the active site of cathepsin V. Their inhibition of cathepsin V was evaluated by using enzyme kinetics and microscale thermophoresis. Best performing compounds were further tested for their selectivity for cathepsin V, compared to other cathepsins, and their effect on cell viability and cell proliferation. The most potent new cathepsin V inhibitors were ureido methylpiperidine carboxylate derivatives decreasing significantly cell proliferation. Our results suggest on one hand the important role of cathepsin V for tumor cell proliferation as well as identify new selective cathepsin V as possible antitumor agents.

P-06.2-09
Progestins affect expression of inflamatory response genes in a model cell line of peritoneal endometriosis 12-Z

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The endometriotic microenvironment is characterized by increased concentrations of inflammatory molecules produced by immune cells and endometriotic tissue, which contribute to disease-associated chronic inflammation. Endometriosis is also characterized by diminished progesterone action, and therefore current treatments also include progestins, synthetic progestagens that mimic the effects of progesterone. While the anti-inflammatory effects of progesterone and progestins have already been reported in endometriosis, their influence on the global expression of inflammatory genes has not yet been examined. The aim

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of this study was to evaluate the effects of three commonly used progestins, medroxyprogesterone acetate (MPA), dienogest and dydrogesterone, as a comparison to progesterone, at the molecular level, in 12Z model cell line of peritoneal endometriosis using PCR arrays. The majority of the investigated inflammatory genes were expressed in 12Z cells and three genes, IL1R1, TNFRSF11B, and IL10RA, were significantly affected by treatment with progestins. Progesterone, MPA and dydrogesterone down-regulated IL10RA, MPA up-regulated IL1R1 and down-regulated TNFRSF11B, while dienogest showed no significant effects on gene expression. Analysis of gene interconnections indicates that progesterone, MPA, and dydrogesterone can act via IL10 signaling pathway, while MPA can affect IL1, OPG/RANK/RANKL and OPG/TRAIL signaling pathways. These pathways can be associated with known positive effects, e.g. reduced lesion size, and known side effects, e.g. mineral bone density loss. The results from the present study clearly indicate that progesterone and the progestins regulate the expression of inflammatory genes in distinct manners, and the association with known signaling pathways explains some of the activities that have been reported for individual compounds. Validation at the protein levels is currently in progress. *The authors marked with an asterisk equally contributed to the work.

P-06.2-10
Molecular docking of the C(6)-substituted purine derivatives into the active site of *Mycobacterium tuberculosis* glutamine synthetase

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Previously, we have demonstrated that purine derivatives, including those containing the fragments of amino acids and short peptides, exhibit high antimycobacterial activity against both laboratory strains (*M. tuberculosis* H37Rv, *M. avium, M. terrae*) and multidrug-resistant (MDR) strain [Previously published in: Krasnov VP et al. (2016) Bioorg Med Chem Lett 26, 2645–2648; Krasnov VP et al. (2017) Pharm Chem J 51, 769–772]. *Mycobacterium tuberculosis* glutamine synthetase (MtGS) was considered as a potential biological target due to the structural similarity of the obtained compounds to known inhibitors of this enzyme. In silico studies into the active site of MtGS were performed using the structures of reference ligands and appropriate PDB codes extracted from the RCBS PDB database. Molecular docking was carried out using Schrodinger Suite Release-2018-4. It was found that for some compounds (2-amino-6-chloropurine, its N-Form and N-Ac derivatives, 2-acetamidopurine conjugates with glycine and (S)-alanine, purine and 2-aminopurine conjugates with glycyl-(S)-glutamic acid), significant antimycobacterial activity (MIC 0.7–1.5 μg/mL) correlated with high affinity and reproducibility of the reference ligand binding modes. So, MtGS seems to be a correctly selected biological target for these compounds. On the other hand, there was not similar correlation in the cases of purine and 2-aminopurine conjugates containing 1,2-ethylenediamine, phenyl or (2-hydroxyethoxy)methyl fragments. Despite significant antimycobacterial activity (MIC 0.7–3.1 μg/mL), these compounds were not characterized by satisfactory scoring functions, which indicates a different target for their antimycobacterial activity. The work was financially supported by the Russian Science Foundation (grant 19-13-00231).

P-06.2-11
Search for potential mycobacterial MmpS5-MmpL5 efflux pump inhibitors

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Multidrug efflux pumps play a significant role in mediating drug resistance in mycobacteria by actively excreting antibiotics. The RND-type mycobacterial MmpS5-MmpL5 efflux system has been shown to provide drug resistance to a wide range of antimicrobials, such as bedaquiline, clofazimine, azoles, thiacetazone derivatives and imidazo[1,2-b][1,2,4,5]tetrazines. Thus, inhibition of MmpS5-MmpL5 efflux pump is an attractive strategy for overcoming mycobacterial drug resistance, which is a major challenge in treating tuberculosis. Virtual screening of around 8000 compounds (collected from Asinex database) was performed against the structure of MmpS5-MmpL5 system and 5 top-scoring compounds were selected for *in vitro* studies. We used a test-system involving 3 Mycobacterium smegmatis strains with different levels of mmpS5-mmpL5 operon expression and, thus, various susceptibility to antimicrobials subjected to MmpS5-MmpL5 efflux: mc2 155 (wild type), Δmmp5 (carrying a deletion in the mmpS5-mmpL5 operon, hypersensitive to imidazo-tetrazines and tryptanthrins) and atr9c (recombinant strain with a mutation in MSMEG_1380 gene, leading to overexpression of the mmpS5-mmpL5 operon, resistant to imidazo-tetrazines and tryptanthrins). We used the paper-disc method for screening active MmpS5-MmpL5 inhibitors: paper discs impregnated with an antimicrobial (an imidazo-tetrazine or tryptanthrin), the tested compounds, or the combination of the antimicrobial with the tested compound were laid on the bacterial agar layer, and the growth inhibition halos were measured. 2 out of 5 compounds turned out to be active MmpS5-MmpL5 inhibitors, increasing growth inhibition halos by 20–25% when combined with the antimicrobials on the M. smegmatis atr9c strain, while having little to no effect on the w.t. and Δmmp5 strains, confirming their specificity. This work is supported by the Russian Science Foundation grant 17-75-20060-P. *The authors marked with an asterisk equally contributed to the work.

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P-06.2-12
Discovery of novel antimycobacterial agents based on quinoxaline 1,4-dioxide scaffold

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Tuberculosis (TB) is one of the deadliest infectious diseases, caused by a single agent – *Mycobacterium tuberculosis*. The spread of multidrug and extensive drug resistant *M. tuberculosis* strains is a primary challenge in global TB control, urging the need for novel anti-TB drugs. Quinoxaline 1,4-dioxides have a wide range of biological activities and are known for their ability to undergo bioreductive activation under hypoxic conditions. This fact may be important for targeting non-replicating persister forms of *M. tuberculosis*, surviving therapy in hypoxic areas of the tuberculous granulomas' caseous core. We have developed a new series of water-soluble derivatives based on quinoxaline 1,4-dioxide scaffold. Screening on *Mycobacterium smegmatis* revealed that 7-amino-2-acylquinoxaline 1,4-dioxides were the most potent ones, with MIC values 2–4 μg/mL. We were able to obtain spontaneous *M. smegmatis* mutants resistant to 8-16×MIC for 2 compounds (LCTA-3163 and LCTA-3168) with a frequency from 1.4×10^{-8} to 7.8×10^{-8}. We have selected 6 spontaneous mutants for further analysis. These mutant strains showed increased resistance to the majority of the tested quinoxaline 1,4-dioxides, suggesting a common mechanism of resistance. Whole-genomic sequencing revealed a common mutation in MSMEG_4883 (AMP-dependent synthetase/ligase, homologous to *M. tuberculosis* fadD2) – deletion of 9 nucleotides – in all 6 strains. FadD2 is involved in fatty acids metabolism and its knock-out has been shown to lead to hypersensitivity to pyrazinamide in *M. tuberculosis* and to quinolones in *Haemophilus parasuis*. Thus, we show that quinoxaline 1,4-dioxides are perspective antimycobacterial agents, with the mutation in fadD2 being a possible clue to their mechanism of action/resistance. This work is supported by the Russian Science Foundation (grant 21-45-00018) and the National Natural Science Foundation of China (grant 82061138019).

P-06.2-13
Metabolic profiling of dormant *Mycolicibacterium smegmatis* cells reveals significant accumulation of tetramethyl coproporphyrin, which can be used for photodynamic inactivation of mycobacteria

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Under stress conditions, mycobacterial cells transit into a specific state characterized by low level of metabolic activity-dormancy. This state is directly linked to physiological drug resistance, complicating eradication of MDR-TB infection. In order to find new anti-TB strategies, the metabolic processes which may occur in dormancy as well as in reactivation should be characterized. In the current study we analyzed untargeted metabolomic profiles (LC-MS) of dormant and active *Mycolicibacterium smegmatis* (Msm) cells (a model microorganism mimicking physiological traits of MTB), on the global scale level. The metabolomic data were statistically evaluated (PCA, hierarchical clustering, differential analysis) and the succession of the metabolic pathway activation was analyzed. Comparison of the metabolic profiles revealed, the most abundant ion-chromatographic peak in dormancy was m/z 710.3329, which can be annotated as coproporphyrin III tetramethyl ester. Differential analysis reveals that the abundancy of this peak decreases significantly ($P < 0.01$, two-tailed t-test) to the point of metabolism activation. Porphyrins are well known photosensitizers in photodynamic therapy of cancer. Based on these findings, we checked the influence of light on dormant bacterial culture. Exposition of the dormant Msm cells at 532 nm up to 30 min results in appearance of the statistically-significant amounts (P = 3e-7– control vs illuminated cells, one-tailed t-test) of the damaged (dead) cells (assessed by the method of flow-cytometry). In conclusion, the metabolic profiling reveals significant changes of the specific markers of mycobacterial dormancy - tetramethyl esters of coproporphyrin III in transition from dormancy to active state, what pave a new way toward eradication of LTB / XDR-TB - photodynamic inactivation based on the accumulated endogenous photosensitizers. This study was supported by the Russian Science Foundation grant 19-15-00324.

P-06.2-14
Thorough unmasking of promiscuity in medicinal chemistry

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The identification of false positive hits is a crucial step in the early stages of drug discovery to avoid wasteful use of resources. Moreover, poor tool compounds in biological studies can generate false information about biochemical processes. The underlying promiscuous mechanisms of interaction with biological targets include aggregation, reactivity with protein residues, redox activity, interference of the assay system (PAINS), poor stability or solubility issues. There are well established methods for the detection of aggregators, and many interfering compounds can be filtered before screening, e.g., using PAINS filters (Baell JB et al. (2018) ACS Chem Bio 13(1), 36–44). We present guidelines for the detection of less obvious promiscuous mechanisms, namely redox activity and thiol reactivity. After a

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thorough literature review, selected assays were optimized and tested with 10 chemically distinct positive control compounds. Then we have further expanded our set of compounds that can be used as positive controls. Assay setups are quick and can be performed at low cost. They are orthogonal and capable of detecting multiple interference mechanisms, i.e. the horseradish peroxidase–phenol red assay detects H_2O_2 generation; the probe H_2DCFDA is sensitive to ROS; resazurin reacts with free radicals; and the Ellman's reagent detects covalent reactivity with thioles. Assay conditions have been optimized to improve robustness and sensitivity. A library of 99 chemical probes and bioactive compounds was used to demonstrate the capabilities of our assay cascade. We have identified new types of compounds that were not previously known to be redox active. Even well prepared, filtered and manually curated compound libraries can still contain nuisance compounds that can only be identified experimentally.

P-06.2-15
Application of the photodynamic effect of endogenous porphyrins for inactivation of dormant *Mycobacterium smegmatis*

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Mycobacterium tuberculosis (Mtb) is able to transit into a dormant state causing the latent state of tuberculosis (TB). Dormant mycobacteria acquire resistance to all known antibacterial drugs. We have established a model that mimics the state of the causative agent of TB in the latent form of the disease by reproducing a primary condition encountered by the pathogen in the host – acidification in macrophages. Previously published in: Shleeva MO et al. (2011) Tuberculosis (Edinb.) 91, 146–154. Similar approach was applied to induced morphologically altered dormant forms of rapidly growing relative to Mtb – *Mycobacterium smegmatis* (Msm). The obtained dormant cells of Msm were capable of long-term storage in acidified liquid cultures. The proteomic analysis of the obtained dormant Msm forms revealed a significant representation of porphyrin synthesis enzymes (porphybilinogen deaminase, delta-aminolevulinic acid dehydratase, uroporphyrinogen decarboxylase), in contrast to active mycobacteria. Indeed, dormant Msm cells were able to endogenously accumulate the red fluorescent pigment identified as coproporphyrin III and uroporphyrin III, as well as their methyl esters. Protoporphyrin IX was not found, which indicates a blockage of this site in the heme biosynthesis pathway in dormant mycobacteria. Accumulation of unbound porphyrins in dormant mycobacteria allows to apply photodynamic inactivation (PDI) for killing of these bacterial forms. The most visible effect on dormant cells illuminated for 5–60 min was observed at 395 and 575 nm, which correspond to porphyrin's absorption maximum. We did not observe PDI effect on active bacteria as these bacteria do not contain porphyrins in unbound forms in substantial amount. For the first time we have demonstrated the successful application of PDI for inactivation of dormant Msm cells due to significant accumulation of endogenous porphyrins. This work was supported by Russian Science Foundation grant 19-15-00324.

P-06.2-16
Modulating the activity of monoamine oxidases A and B with targeted covalent inhibitors

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The resurrection of covalent inhibitors led to several FDA approvals of covalent drugs that target catalytic or non-catalytic nucleophilic amino acid residues. Covalent inhibitors offer a number of advantages over non-covalent ligands such as high potency, prolonged duration of action and opportunity to inhibit intractable targets. Monoamine oxidases A and B (MAO-A and MAO-B) are validated targets in the therapy of neurological disorders such as Parkinson's disease and depression. Involvement of MAOs in numerous other disorders, such as cancer, heart failure and cardiomyopathy, was also demonstrated lately. MAO-A and MAO-B both contain cysteine residues in the active site or in close vicinity susceptible for covalent modification. Covalent fragment libraries containing diverse set of electrophilic fragments (i.e. warheads) were thus screened against human (h) MAO-A and hMAO-B. Several chemically diverse warheads inhibited hMAO-A in a time-dependent and irreversible manner. On the other hand, linear and predominantly hydrophobic fragments inhibited hMAO-B in a reversible manner with no indication of covalent interaction. Covalent modification of targeted Cys321 and Cys323 of hMAO-A was confirmed by LC-MS analysis of the native hMAO-A and subsequent to tryptic digestion (Petri L et al. (2021) ChemBioChem 22, 743–753; Scarpino A et al, (2021), J Comput Aided Mol Des 35, 223–244). Low-molecular weight analogues of active fragments were synthesized to explore the effects of substituents on the reactivity. Using computational approaches, active warheads were incorporated in the structures of known reversible hMAO-A inhibitors, namely toloxatone and harmine. The designed targeted covalent inhibitors (TCIs) were synthesized and evaluated in vitro on both MAO isoforms. Reactivity and selectivity profiles were investigated using model nucleophiles and enzymes. Further on, biological activity of TCIs was also studied in cell-based assays.

P-06.2-17
Search for the second generation of antifungal drugs among semisynthetic polyene antibiotics of the Amphotericin B group

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Although Amphotericin B (AmB) is the drug of choice for treating the most serious systemic fungal or protozoan infections, its application is limited by low solubility in aqueous media and serious side effects such as infusion-related reactions, hemolytic toxicity, and nephrotoxicity. Thus, it is essential to search for the polyene derivatives with better chemotherapeutic properties. Previous studies have demonstrated that the selectivity of AmB towards fungal membranes is linked to its self-association

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properties.[1] With an objective of obtaining AmB derivatives with lower self-aggregation and improved solubility, we synthesized and studied a series of amides of AmB bearing an additional basic group in the introduced residue. Based on the *in vitro* antifungal data, new potent AmD derivative (amphamide) was selected for further in-depth investigations and preclinical evaluation. The electrophysiological experiments of the selected candidate suggested that it had lower self-aggregation properties but higher pore-forming abilities in the model membrane compared to those of AmB under the same conditions. The enhanced selectivity of amphamide for the ERG-containing bilayers resulted in its lower toxicity compared to that of AmB. *In vivo* studies confirmed that amphamide had a much lower acute toxicity and higher antifungal efficiency compared to those of AmB. Thus, a novel AmB derivative, amphamide, with a considerably increased safety and better efficacy compared to those of AmB with a therapeutic index of almost 17-fold higher than that of AmB was discovered. Amphamide is the promising drug-candidate for the second generation of polyene antibiotics and is also perspective for in-depth preclinical and clinical evaluation. This work was partly supported by the Russian Foundation for Basic Research (grant № 20-04-00467). 1. Espada R. et al; Int. J. Pharmaceutics 2008, 361, 64–69.

P-06.2-18
New aspects of action of heliomycin on tumor cells

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Heliomycin (resistomycin), a polyketide antibiotic, has a broad spectrum of biological activity. Despite the fact that antitumor action was discovered over 20 years ago a precise mechanism of action on cancer cells remains to be unknown. Experimentally were demonstrated that heliomycin binds DNA ($K_b = 1.1\times10^4$ M^{-1}), inhibits of HDAC1 (IC_{50}~100 μM) and modulates apoptosis of cancer cells. Additionally, molecular docking studies predicted an ability of the antibiotic to interact with HER2 tyrosine kinase. We found that heliomycin effectively blocks proliferation of a number tumor cell lines including variants with multidrug resistance. Treatment of T24 cells with heliomycin do neither lead to reactive oxygen species nor induce apoptosis of cancer cells. Cell cycle distribution indicated that heliomycin induces arrest at G1 phase. Western Blot analysis revealed that antibiotic slightly enhances total p53 expression. On the other hand, a lack of significant changes in Bak, Bcl-2 and PARP cleavage further confirmed a non-apoptotic mechanism of death of T24 bladder cancer cells. Autophagy is a well-regulated cellular pathway that governs the adjustment of cytoplasmic organelles to maintain cellular homeostasis. Interestingly, we demonstrated that heliomycin triggers autophagy and growth inhibition in cancer cells by directly targeting a tumor-associated NADH oxidase (tNOX, ENOX2), supported by the cellular thermal shift assay (CETSA) and isothermal dose-response fingerprint curve ($ITDRF_{CETSA}$), a recent developed target identification procedure based on that their direct binding enhances the thermal stability of a target protein in intact cells. Thus, together with a high antiproliferative potency against MDR-positive cells and an original mechanism of killing tumor cells heliomycin can be considered as the interesting and valuable small molecule for future study and development of new anticancer agents. The study was funded by RFBR and MOST (project №19-53-52008).

P-06.2-19
Improving the selectivity of G-quadruplex ligands based on heteroarene-fused anthraquinones

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G-quadruplex (G4) structures of nucleic acids play an important role in metabolism of tumor cells, including maintaining stability genome, DNA replication and oncogene expression, etc. Numerous advances in the development of new small molecules targeting G-quadruplexes proved this strategy of anticancer therapy. Previously, we designed and synthesized a series of potent G4 ligands based on anthrafuran and anthrathiophene scaffolds and performed a preliminary SAR analysis. In this work we identified the structural features of ligands which improve interaction with a specific G4 over duplex or other G4s. Screening and study of binding was performed for G4-forming oligonucleotides MYC, KRAS, KIT, TERT, TQ24 and duplex ds26 using FID assay, fluorescent titration, FRET melting assay, CD spectra, etc. Modification of terminal amino groups of 4,11-side chains into guanidines magnifies binding to all tested G4 compared to duplex DNA. Introduction of an additional guanidinoalkylamino moiety to the 2 position of heterocycle gave an additional increase of both affinity (4–15 times) and selectivity (up to 75 times). A replacement of the third side arm from the position 2 to the position 3 reduces G4 stabilization. Bioisosteric modification of five-membered heterocyclic rings (furan, thiophene) into the six-membered pyridine bearing the third side chain at the position 3 led to selective binding only with TERT G4 over other oligonucleotides. Notably, all compounds exhibited high antiproliferative activity (up to submicromolar IC_{50}) against cancer cell lines. In summary, we found new important patterns of the relationship between the structure of antitumor heteroarene-fused anthraquinone derivatives and their ability to form tight complexes with G-quadruplexes and duplex of nucleic acids. This study was partially supported in part by the Grants Council of the President of the Russian Federation for State support to young scientists of Russia (grant MK-222.2021.1.3).

P-06.2-20
Light scattering method in homeopathic medicine

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Despite the widespread growth of homeopathic medicine around the world, with an inclusion in state pharmacopeiaof certain developed countries, such as France, Germany, Austria, Belgium, the main problem concerns the detection of the active component.

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Therefore, the purpose of the work is to study homeopathic preparations containing micro- and nano- concentrations of active substances by dynamic light scattering. For this study, particle size measurements of 3C dilution of the following homeopathic preparations aluminum oxidatum, silicidumacidum, barium carbonicum, Spongilla drug; 3D dilution of Spongilla drug and non-diluted solution of Traumel C and aflubins were analyzed on Nanofox (Sympathex, Germany). For the preparations Traumel C, 3C barium carbonicum, the nanoparticles counting rate of Nanophoxwas significantly lower, which led to a result with a 100% error. The analysis of the other 3C dilution and non-diluted preparations provided also negative results, even after changing the position of the cell to ensure optimal transmission of the laser beam. However, the positive results in the analysis of the 3D dilution of Spongilla drug show the possible application of dynamic light scattering method of photo-cross-correlation to study homeopathic preparations. Measurements during the shelf life (14 days) shows that the preparation retains its aggregative, sedimentation and flocculation stability, with an average particle size of 448 ± 18 nm at the beginning of the experiment and 442 ± 9 nm on the 14th day. Thus, we showed that dynamic light scattering method could be used to measure the particle size in homeopathic preparations. From our study, we also recommend studying a 3D dilution of the preparation, as analysis of 3C dilution and non-diluted preparations was ineffective. The publication has been prepared with the support of the «RUDN University Program 5-100.

P-06.2-21
Antimicrobial and antimycotic activities from multivitamin preparation of Urticae dioicae folia and Fructus Sorbi aucupariae

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Herbal medicinal preparations have long been used for the prevention and treatment of various diseases. One of the herbal medicinal preparations, multivitamin preparations of Urticae dioicae folia : fructus Sorbi aucupariae (1:1), is used in Russia. Vitamin preparations are recommended as they may increase immunity in colds, viral and other diseases. From literature reviews, Urticae dioicae folia and fructus Sorbi aucupariae contain various groups of biological compounds, such as phenolic compounds, with antimicrobial and antimycotic effects. The aim of our study was to study the pharmacological activity of a multivitamin preparation against bacteria and fungi and to establish the content of phenolic compounds in the preparation. Both aqueous and alcoholic extracts from a plant preparation were obtained and analysed. The study of the antimicrobial and antimycotic activity of a multivitamin preparation was carried out by agar diffusion. The results showed that both aqueous and alcoholic extracts of the multivitamin preparation were not active against *Escherichia coli* and *Staphylococcus aureus*, but active against *Candida albicans*, with a zone of inhibition of 16 mm for aqueous extracts and 25 mm for alcoholic. Regarding the content of biological compounds, the content of tannins was determined by permanganometric titration, and flavonoids - by a spectrophotometric method, based on flavonoid-aluminum chloride ($AlCl_3$) complexation. The content of tannins in percent in terms of tannin and absolutely dry raw materials in the multivitamin preparation was 2.88 ± 0.08%, and the flavonoid content was 0.40 ± 0.01% in terms of rutin and absolutely dry raw materials. The publication has been prepared with the support of the «RUDN University Program 5-100»

P-06.2-22
Metabolic biotransformation and *in vitro* safety profile of selected pan-phosphodiesterases (PDEs) inhibitors

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Phosphodiesterases (PDEs) are enzymes responsible in the cells for hydrolysis of phosphodiester bond in two second messengers': adenosine or guanosine 3',5'-cyclic monophosphate (cAMP or cGMP). An elevated intracellular level of cAMP and cGMP may lead to an inhibition of both immune and lung resident cells' proinflammatory response in various chronic lung diseases, including asthma and COPD. As it has also been shown that some PDE inhibitors can affect airway remodeling, there is now a growing interest in searching for new, effective PDE inhibitors. Our earlier studies revealed that selected, novel pan-PDE inhibitors from the group of 7,8-disubstitiuted-purine-2,6-dione represent both anti-inflammatory and anti-fibrotic properties in numerous cell lines of lung origin (1,2). Since it is known that organ toxicity and metabolic instability are one of the most important reasons for failure at various stages of drug development, here we decided to verify new compounds: 1) *in vitro* safety (hepato-, neuro-, cardio- and lung toxicity); 2) metabolic stability in rat liver microsomes (RLMs) and; 3) *in silico* predictions of metabolism direction. The obtained results indicate that all tested pan-PDE inhibitors are characterized by a good, *in vitro* safety profile. Simultaneously, they have been shown to possess good to moderate metabolic stability in RLMs, comparable to many known drugs. This was also confirmed by *in silico* liver biotransformation predictions. The obtained results allow us to conclude 7,8-disubstitiuted-purine-2,6-dione derivatives as a promising agents for use in lung diseases therapy. 1. Eur J Pharmacol. 2019 Dec 15;865:172779. https://doi.org/10.1016/j.ejphar.2019.172779. 2. Int J Mol Sci. 2020 Jun 3;21(11):4008. https://doi.org/10.3390/ijms21114008. Acknowledgements: This study was supported by the National Science Centre, Poland, funded grants No. UMO-2017/27/B/NZ7/01633

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P-06.2-23
In vitro and *in vivo* antibiofilm activity of a bacteriophage endolysin LysECD7

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Bacterial biofilms are recalcitrant biological targets for traditional antimicrobial therapy agents, especially in implantology, due to their complex and heterogeneous structure. Among the approaches to overcome this problem is the application of phage peptidoglycan hydrolases (endolysins) – novel antibacterial compounds with significant advantages including action on multidrug resistant bacterial strains. LysECD7 endolysin showed high effectiveness and activity against a wide spectrum of different species of Gram-negative bacteria. Here we investigate the antimicrobial activity of this enzyme against the preformed biofilms of *Acinetobacter baumannii*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* representatives *in vitro*. Incubation with up to 1 mg/mL of the LysECD7 totally reduced optical density of the biofilms stained with gentian violet. Also *in vivo* assessment of LysECD7 action on implant-associated bacterial biofilms was done using hermetical diffusion chambers filled with *Klebsiella pneumoniae* bacterial suspension and implanted into the abdominal cavity of the outbred rats. The treatment with 50 mkg/animal of LysECD7, amikacin as a positive control or control buffer (n = 9 in each group) started 4 days after the implantation, when strong biofilms were formed. The treatment was performed once a day for 7 days, then chambers were studied on the intensity of formed bacterial biofilms. On the 5th day of treatment the OD_{595} of the chamber membranes significantly decreased in endolysin treated group as well as in the positive control group comparing to the negative control, although this difference disappeared by the 8th day due to the biofilms weakening. CFU reduction from $1.1 \times 10^3 \pm 7.2$ in control group to 36.3 ± 29.2 and 2.4 ± 2.5 (5th day) and from $1.0 \times 10^3 \pm 128.1$ to 58.0 ± 37.0 and 18.5 ± 2.1 (8th day) in experimental and antibiotic groups, respectively, was detected. Thus, LysECD7 can be considered as a promising agent with strong antibiofilm activity. *The authors marked with an asterisk equally contributed to the work.

P-06.2-24
The prospective antibacterial conjugates of modified oligonucleotides

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The design of oligonucleotides able to specifically interact with bacterial RNA targets and block their functions is a very promising approach to the development of new generation of antibiotics. Bacterial RNase P can be used as both an instrument for the influence on bacterial RNA and a target for inhibition. In the first approach EGS oligonucleotides form the complex with the chosen sequence within mRNA and induce its cleavage by means of bacterial RNase P. In the second approach RNase P-inhibiting oligonucleotides bind with M1 RNA subunit of RNase P, provoke the disturbance of tRNA processing and hence suppress the bacterial growth. Our work is aimed to design of new conjugates of modified EGS oligonucleotides and inhibiting oligonucleotides interacting with bacterial RNase P. Conjugates of nuclease-resistant oligo(2'-O-methylribonucleotides) and phosphorylguanidine oligonucleotides guiding or inhibiting RNase P, with oligo(N-methylpyrrole) and with peptides were obtained. The cleavage of target RNAs corresponding to the mRNA fragment of the ftsZ and gyrA genes of *Acinetobacter baumannii* in the AUG codon region by RNase P in the presence of guiding modified and control unmodified oligonucleotides and their conjugates has been demonstrated. The penetration of conjugates of guiding and inhibiting modified oligonucleotides into *E. coli* and *A. baumannii*, as well as their ability to inhibit bacterial growth, were examined. The results obtained show the promise of using the studied conjugates of modified oligonucleotides guiding or inhibiting RNase P as antibacterial drugs. This research has been supported by the Russian Foundation for Basic Research (N 17-04-01892).

P-06.2-25
Synthesis, transformations and antitumor properties of new β-maleimide substituted meso-arylporphyrins

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Porphyrins and their analogues hold a great potential as photosensitizers for various biomedical applications including photodynamic therapy. A new panel of boronated porphyrins was prepared through the modification of maleimide group in porphyrins with S-nucleophiles. β-Amino-substituted derivatives for 5,10,15,20-tetraphenylporphyrin, 5,10,15,20-tetrakis(pentafluorophenyl)porphyrin and 5,10,15,20-tetrakis[4-(trifluoromethyl) phenyl]porphyrin were synthesized and used as key compounds for the introduction of maleimide substituent into the β-position of the above mentioned meso-arylporphyrins. New maleimide porphyrins were prepared via the reaction of β-amino group in porphyrins with maleic anhydride followed by the cyclization of maleic acid monoamides. The reactivity of porphyrin maleimides toward thiols was demonstrated using mercapto carboranes and cysteine, as a result corresponding succinimide thioethers were prepared selectively. New porphyrins formed stable complexes with albumin, a major drug carrier in the body. The flash photolysis showed the ability of new compounds to generate triplet states and reactive oxygen species upon photoactivation. New compounds had a low dark toxicity and their phototoxicity was tested for HCT116 cell line. The most promising compound, 2-{3-[(o-carboran-1'-yl)thio]pyrrolidine-2,5-dione-1-yl}-5,10,15,20-tertraphenylporphyrin, in low micromolar concentrations triggered rapid (within the minutes) generation of superoxide anion radicals and cell death via a necrotic mechanism. Thus, the maleimide- and succinimide-substituted porphyrins represent efficient

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compounds for an in-depth investigation as photosensitizers in cancer and other diseases. In particular, the maleimide-cysteine interaction can be fruitful for therapeutic targeting to proteins. This work was supported by Ministry of Science and Higher Education of the Russian Federation using the equipment of Center for molecular composition studies of INEOS RAS.

P-06.2-26
Inhibition of tumor cell growth by novel synthetic polyamine analogues

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Polyamines (PA) are essential for cell growth and proliferation. Tumor cells are known for their higher concentration of PA compared to normal cells, which contributes to the proliferation and invasion of tumor cells. Metabolism PA is an important target for anticancer therapy. The aim of the study was to identify the potential value of PA analogs among some azafluorene derivatives (AZD) for chemotherapy. The experiments were carried out on human breast cancer cell line SKBR3 and the basic mechanism of the effect of AZD on the cells was explored. The SKBR3 cells (Sigma-Aldrich), were grown in RPMI-1640 medium, containing 10% calf serum, 100 μg/mL streptomycin, and 100 U/mL penicillin in humid 5% CO2 at 37°C. The analysis of cell proliferation was carried out by MTT assay. The activity of polyamine oxidase (PAO), a key enzyme involved in the decomposition of PA and an important factor in the induction of cell apoptosis, was determined by the formation of hydrogen peroxide during the oxidation of cancer cells by PAO. It has been revealed that AZD can inhibit the growth of breast cancer cells of the SKBR-3 line, activating a key enzyme of PA catabolism, and has potential value for clinical therapy of human breast cancer. The synthesized analogues of AZD (2-chloro-N-(5H-indeno [1,2-b]pyridin-5-yl)acetamideandande and (5Z)-5-[nitro(phenyl) methylidene]-5H-indeno[1,2-b]pyridine are capable to inhibit the growth of breast cancer cells of the SKBR-3 cell line in different degrees and activate PAO. Moreover, (5Z)-5-[nitro(phenyl) methylidene]-5H-indeno[1,2-b]pyridine exceeds AZD in its antiproliferative activity with IC50 (6.83 mmol/L) , causing a stronger activation of PAO. Further search for effective analogues of PA, as well as search for combinations of PA analogues with other anticancer drugs should be recommended. «The work was done in the framework of the Russian Federation fundamental research program for the long-term period for 2021-2030»

P-06.2-27
Trichoderma is the producer of antitumor enzyme with L-amino acid oxidase activities

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Aim: The biosynthesis of L-lysine-α-oxidase enzyme by Trichoderma fungus has been studied at various cultivation methods and on the media with different carbon sources. In the work presented, various variants of medium and the possibility of wheat bran and hemicellulose substrate replacement with other carbon sources were studied in association with enzyme development, as well as the substrate specificity of Trichoderma during the use of this substrate depending on the culture and acidity medium age. Materials and Methods: The vegetative mycelium of mushrooms, grown during 8–10 days at 28°C on the Chapek medium, served as seed material. The study was carried out in a deep-seated manner using the laboratory shaker No. 357 ("Poland") and a surface method of cultivation. Results: The enzyme was synthesized actively only on the medium with wheat bran. Trichoderma strains, when they were grown on the medium with a hemicellulose substrate, exhibited a different spectrum of L-amino acid oxidase activities. With the age of the Trichoderma culture, the spectrum of aminoxidase activities expanded, but it remained individual for each strain and could serve as a marker for its characteristics. Conclusion: It can be seen from the presented results, that a specific amino acid degradation spectrum manifests itself for each strain of various representatives of Trichoderma - the producers of L-lysine-α-oxidase, which can serve as a biochemical characteristic of the strain. The publication has been prepared with the support of the "RUDN University Strategic Academic Leadership Program". Key words: Amino oxidase, Enzyme, L-lysine-α-oxidase biosynthesis, Trichoderma

P-06.2-28
Activators of polyamine oxidase as potential antitumor agents

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Our studies of polyamine metabolism in transplantable hepatomas 27, 22a, 60, 61, 46, 48 and during diethylnitrosamine-induced hepatocarcinogenesis were aimed to investigate the mechanisms of PA regulation in normal and regenerating liver. Results demonstrate the difference between PA synthesis and catabolism during the pathological proliferation compared to healthy tissues. Accumulation and increased concentrations of PAs observed during cancer growth process are associated mostly with their catabolism suppression but not with the acceleration of biosynthesis only as in regenerating liver. Decreased oxidative deamination of PAs in hepatocarcinogenesis is most likely to be the primary process, while the decarboxylation rate increase may be considered as the result of the transformed hepatocytes' dedifferentiation. Di- and polyamine oxidases (DAO and PAO), which regulate the PA levels, may have direct or indirect effects on the

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enhanced cell proliferation and tumor growth. Enzyme activity of PAO in the early stages of hepatic malignization can be down-regulated by higher concentrations of PAs and vitamin B6 deficiency. In the late phase of neoplastic transformation the DAO function may be genetically regulated by the termination of apoenzyme sinthesis (chronical regulation type). Elimination of DAO and PAO activity in tumor cells may be a defence against the toxic products of PA oxidation. Thus activators of PA catabolic enzymes migh thave the anticancer potency. Bis-(uracil) PA analogs have demonstrated not only inhibition of PA synthesis, but also activation of PA catabolism in acellular test systems of tumor and regenerating tissue. This is the probable reason for the proliferation suppression of cancer (CaOv) cells in culture. Our results indicate that stimulators of PA oxidation show potential antitumor activity and may be used for development of therapeutic agents. The study was supported by the "RUDN University Strategic Academic Leadership Program".

P-06.2-29
Quantitative evaluation of benzimidazole and azafluorene derivatives influence on polyamine metabolism as potential anticancer agents

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Substances inhibiting the oxidative deamination of polyamines are likely to manifest carcinogenic properties. And vice versa, chemical compounds activating the process of oxidative degradation of putrescine and polyamines may have antitumoral potential. We studied the effect of benzimidazole (1,2) and azafluorene (3–12) derivatives (polyamines structural analogs) on diamine oxidase (DAO) and polyamine oxidase activity (PAO) in model cell-free testing system made from rat regenerating liver tissue/ The compounds were the following: 7-aminopyrido[1,2-α]benzimidazole (1), 7-nitropyrido[1,2-α]benzimidazole (2), 1-amino-4-azafluorene (3), 1-amino-4-azafluorenole (4), 9-dicianomethylene-4-azafluorene (5), 9-[α-(β-hydroxiethyl)aminomethylene]-4-azafluorene (6), 9-[α-pyridilaminomethylene]-4-azafluorene (7), 1-amino-9-phenylamino-4-azafluorene (8), 1-amino-4-azafluorenone-9 (9), 1,4-diazoacetonaphtyleno[1.2-f]-fluorantene (10), 2-methoxycarbonyl-(β-benzoylethyl)aniline (11), 5-(2-methoxycarbonyle)-phenyle-α-furfurole (12). Benzimidazole derivatives (1, 2) inhibited polyamine synthesis as well as polyamine degradation. Azafluorene derivatives (3, 8, 9, 10 and 12) depressed ornithine decarboxylase activity (ODC) effectively in the test-system, but among the mentioned substanses the compound 10 only can activate diamine and polyamine oxidation. PAO activity was enhanced by the componds (4), (7), (9), and (12). DAO activity was depressed by (4) or without significant changes (7), (9), (12). Finally, azafluorene derivatives (4), (7), (9), (12), and to a large extent (10) have shown antiproliferative properties. They might be the object of future examining as cancerostatic agents. The publication has been prepared with the support of the "RUDN University Strategic Academic Leadership Program". Key word: oxidative deamination, polyamines, anticancer potential, diamine oxidase, polyamine oxidase, antiproliferative activity.

P-06.2-30
Prediction of the kinetics of photosensitizer accumulation in cells by the fluorescence contrast of healthy and pathological tissues

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The rate of accumulation of drugs in pathological tissues, photosensitizers (PS) for photodynamic therapy (PDT), may depend on several factors: the species composition of bacterial communities involved in the pathogenesis of wound infection, the degree of skin lesions, as well as the individual characteristics of the metabolic rate in the body. The efficiency of PDT is associated with irradiation at the time of maximum accumulation (Tmax) of PS. We studied the dynamics of the intensity of fluorescence of bacteriopurpurinimide (BPI) derivative on a model of infected wounds and healthy skin in BALB/c mice during the day after its intravenous administration at a dose of 20 mg/kg. The average value of the fluorescence contrast in relative units was 2.2 ± 0.2 in the group of mice with wounds infected with *P. aeruginosa*, and 2.0 ± 0.30 in the group of mice with wounds infected with *S. aureus*. Measurements were taken 30 minutes after administration of PS. The maximum fluorescence intensity of the BPI derivative in the wound was observed after 3 to 6 hours. Fluorescence contrast characterizing the selectivity of wound/healthy skin accumulation correlates with Tmax of PS. The correlation coefficient between fluorescence contrast (0.5 h) and Tmax was -0.81 in the group of mice with wounds infected with *P. aeruginosa*, -0.83 in the group of mice with wounds infected with *S. aureus* and -0.84 in the general group of mice. The obtained values of the correlation coefficient ranging from -0.9 to -0.7 indicate a strong negative inverse correlation between the considered values. It indicates the possibility of predicting the optimal exposure time according to the evaluation of fluorescence contrast after 0.5 hours after administration of PS. The revealed patterns indicate the feasibility of a personalized assessment of the pharmacokinetics of PSs in wound tissues in order to determine the optimal time of its accumulation in the wound and irradiation with maximum efficiency.

P-06.2-31
Fragment based design, synthesis and evaluation of novel O-GlcNAc transferase inhibitors

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Reversible glycosylation of nuclear and cytoplasmic proteins is an important regulatory mechanism across metazoans. Just one enzyme, O-linked N-acetylglucosamine transferase (OGT), is responsible for all nucleocytoplasmic glycosylation [1]. This modification can alter protein function by regulating cellular signalling and transcription pathways in response to altered nutrient availability and stress so there is a well-known need for potent, cell-permeable inhibitors to interrogate OGT function [1,2]. By using fragment-based drug design approach based on 3D OGT

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structure we designed and synthesized novel potential inhibitors that target the donor uridinediphosphate-O-b-N-acetylglucosamine (UDP-GlcNAc) OGT binding site. All the compounds composed of quinolone-4-carboxamide scaffold elongated with 4-(aminomethyl) benzoic acid (fragment F20) that target the donor UDP-GlcNAc binding pocket and has been previously published by Zhang H et al. [3] Fragment F20 has then been coupled with various amines by using coupling reagents to elongate the inhibitor towards O-GlcNAc binding pocket to improve compounds selectivity and potency. The inhibitory potency against OGT was measured by using UDP-Glo assay and recently published assay by Vocadlo et al. [3] The most potent inhibitors revealed were the compounds that enable formation of new hydrogen bonds and π-π interactions with amino acids residues in the binding site, with the same binding pattern as UDP fragment. The percentage of inhibition of the enzyme for most potent compounds were 61.25% and 46.81% at 240 μM concentration. Currently, the compounds are undergoing further testing to verify their effect on inselected cell lines. [1] Martin ES et al. (2018) J Am Chem Soc, 140: 13542–13545. [2] Zhang H et al. (2018) Med Chem Comm, 9 (5): 883–887. [3] Vocadlo D et al. (2020) GDCh

P-06.2-32
Bacterial signaling molecules in methicillin resistance expression

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Introduction: The spread and treatment of methicillin-resistant Staphylococcus aureus (MRSA) infections remains one of the most significant problems in the world medicine. The appearance of strains with reduced sensitivity to the first choice agents – vancomycin and teicoplanin – become more often during last years. Intensive development of antibiotic resistance in bacteria stimulate the search for new strategies to combat superinfection. One of such strategies could be the use of Gram-negative bacteria quorum sensing molecules (AHLs) as a new medicinal chemistry tool. The aim was to study the effect of AHLs on the antibiotic resistance of MRSA strains. Methods: Strains were isolated from skin and tissues wounds from hospital patients in MI Kyiv Regional Clinical Hospital. Bacteria were identified as *Staphylococcus aureus* by VITEK 2 compact 15 (France). The resistance to antibiotics was checked by disks diffusion method with disks certified in Ukraine (HiMedia, Oxoid, Liofilchem). The results were interpreted by EUCAST-2019. Two types – L and DL isomers – of C6 and C8–AHLs were used in the study. Results: In the presence of AHLs 3 strains (among 7) identified as MRSA have shown sensitivity to 2nd class of cephalosporines despite the fact that cassette chromosome mec was present in their genome. Inhibition zones were significantly increased (from 15 ± 1 to 22 ± 1 mm, from 13 ± 2 to 28 ± 1 mm and from 17 ± 1 to 29 ± 2 mm respectively) in the presence of AHLs (both L and DL isomers). Further cultivation of same strains without AHLs in media have shown the expression of methicillin resistance again. Perhaps, AHLs affect biochemical mechanisms of resistance expression. Conclusions: AHLs affect the expression of methicillin-resistance in staphylococci and could be used for combination therapy for MRSA-induced infections.

P-06.2-33
Visualisation of the urine metabolome composition by means of 3D fluorescence analysis is a tool for noninvasive detection of some gynecological diseases

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Native fluorescence is a phenomenon that characterizes the physiological state and can reflect the pathological state of the biological system, too. Vital functions of the cell depend on normal or pathological conditions. The response to these processes at the level of transcriptome and proteome are metabolites. Some metabolites are produced in excess amounts in cancerous tissues due to their rapid metabolism and they can be detected in body fluids, too. Many metabolites are native fluorophores. From this point of view, body fluid is defined as a multifluorescent metabolome and its composition can be monitored by a 3D fluorescence analysis. There are a number of studies using native fluorophores of tissues as tumor markers in fluorescence spectroscopy, but only a few of them focused on using body fluids to diagnose cancer are available. Urine is one of the biological fluids that could be obtained most simply and non-invasively. The urine fluorescent metabolome was defined as the concentration profile created by scanning of the synchronous excitation spectrum of urine diluted via geometric progression. The spectra were graphically processed into the fluorescent concentration matrix which provides diagnostically valuable information about quantitative and qualitative composition of urine. Concentration matrix represents visualisation of urine fluorescent metabolome. The philosophy of comparative profile analysis is applicated into the monitoring of urine composition. By comparing of the graphic record of the analysed urine with the defined metabolic profile standard, it is possible to immediately identify the abnormalities, even without a detailed knowledge of the composition. Fluorescent urine monitoring shows differences in the urine composition of healthy individuals and patients with different inflammatory diseases of the cervix or gynecological malignancy. Research supported by grants: VEGA 1/0620/19 and EU Structural Funds, Project ITMS 26220220143

P-06.2-34
New 4-aminoquinoline-based cholinesterase inhibitors

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Medical treatment of Alzheimer's disease (AD) and related dementias is mainly based on improving the level of the neurotransmitter acetylcholine in the brain by inhibiting the enzymes involved in acetylcholine breakdown, acetylcholinesterase and butyrylcholinesterase. Today, three out of four drugs in the pharmacotherapy of AD are acetylcholinesterase inhibitors that do not cure but only delay the progression of AD symptoms. 4-

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aminoquinoline represents a simplified tacrine structure and has been identified as the most promising structure for the development of new acetylcholinesterase inhibitors. We synthesised twenty derivatives of 4-aminoquinolines, differing in the substituent attached to the C(4)-amino-group, determined their ability to inhibit the action of acetylcholinesterase and butyrylcholinesterase and evaluated their capacity to pass the blood-brain-barrier. All of the tested compounds reversibly inhibited both acetylcholinesterase and butyrylcholinesterase in low micromolar range, forming noncovalent interactions within the active site of the enzymes with slight preference toward acetylcholinesterase. Generally, acetylcholinesterase showed the highest affinity toward compounds with a longer C(4)-amino aliphatic side chain, pointing to the importance of the existence of high lipophilic substituents. The replacement of a terminal amino-group by a hydroxy-group generally decreased the inhibition potency of compounds indicating the importance of a basic functional group. Furthermore, all of the tested compounds had the potential for passing the blood-brain-barrier. Our results suggest that the studied 4-aminoquinolines are a solid starting point for further structural refinement in terms of developing novel acetylcholinesterase inhibitors as centrally active drugs for use in the treatment of AD. Acknowledgment: this study was supported by the CSF (IP-2018-01-7683 and IP-2020-02-9343) and by the MoESTD.

P-06.2-35
Obtaining of recombinant antistasin-like proteins of leech *Hirudo medicinalis*

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Cardiovascular diseases are one of the main challenges for modern medicine, so the development of new drugs that affect hemostasis seems to be an extremely urgent task. Previously, we performed whole genome sequencing of the medicinal leech *Hirudo medicinalis* and carried out the genome annotation. In the bioinformatic analysis of the obtained data, a group of genes encoding homologues to the previously described anticoagulant antistasin was found. The structure of antistasin is characterized by the presence of two homologous domains, each of which contains 10 cysteine residues forming five intradomain disulfide bonds. Proteins with similar antistasin-like domains have been found in many multicellular animals. The aim of this work was to obtain recombinant antistasin-like proteins of *H. medicinalis* in eukaryotic and prokaryotic expression systems and to verify their effect on hemostasis. A total of 14 ORF of antistasin homologues were selected. DNA fragments encoding antistasin-like proteins were obtained by PCR using a cDNA library from salivary gland cells of a medicinal leech. The obtained DNA fragments were cloned into the plasmid vectors pETmin and pSlyD-TEV (under the control of T7 promoter) and pcDNA3.4 (under the control of CMV promoter). *E. coli* BL21(DE3) Gold cells were transformed with pETmin- and pSlyD-TEV-based vectors, and the human embryonic kidney cells (Expi293F) were transfected with pcDNA3.4-based vectors. Next, conditions were selected for culturing bacterial strains and human cell cultures providing the accumulation of recombinant antistasin-like proteins. Target polypeptides were purified by metal chelate chromatography and then dialyzed into PBS. A part of antistasin-like proteins was tested in blood tests, in which, using the obtained polypeptides, an increase of activated partial thromboplastin time was demonstrated in comparison with the control. This work was supported by the Russian Science Foundation (projects 17-75-20099 and 20-15-00270).

P-06.2-36
Structural studies of LysSi3, Gram-negative bacteria targeting endolysin with broad bactericidal activity

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Phage endolysins comprise a promising alternative capable to replace or supplement conventional antibacterial therapy used in medicine and veterinary in the face of increasing antimicrobial resistance. For endolysins targeting Gram-negative bacteria the specific activity against a broad host range has been shown, however, the molecular mechanisms specifying broad spectrum of action are obscure. The LysSi3 is a peptidoglycan hydrolyzing, lysozyme-like enzyme with predicted muramidase activity (GH24 family) and broad bactericidal activity against ESKAPE pathogens. It showed in vitro antibacterial effect for 75 out of the 120 investigated clinical isolates at concentration 100 μg/mL (5.4 μM), reducing the bacterial load up to 5 logs. X-ray crystallography and HDX-MS were applied to elucidate the mechanism of LysSi3 action. Site-directed mutagenesis was performed based on the structural data. Using the 3D structure of muramidase AcLys (PDB ID 6ET6) the LysSi3 catalytic triad (Glu15, Asp24 and Thr30) was predicted and mutated. Modifications of amino acids did not lead to changes in enzymatic activity against the *Acinetobacter baumannii* model strain. Thus, the existence of additional functional domains in the structure of LysSi3 can be proposed. Previously, a significant loss of LysSi3 activity at pH > 7, compared to mild acidic conditions has been shown. The addition of permeabilization agent (EDTA) restored its activity, indicating failure of LysSi3 to overcome the bacterial outer membrane at pH > 7. Analysis of HDX data revealed that C-terminal peptide of LysSi3, containing two α-helixes and β-strand, becomes less structured with increase of pH. We propose that this charged terminal peptide can play a particular role of membrane-translocating domain, allowing the enzyme to cross outer membrane of bacteria and reach the peptidoglycan substrate. The mechanism of enzymatic action and role of C-terminal peptide in broad bactericidal action of LysSi3 are under investigation.

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P-06.2-37
Revealing and resolving artifacts of FRET-melting assays

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FRET screening is a popular and promising method for studying intermolecular interactions. In the case of non-canonical DNA structures, such as G4s and i-motifs (IMs), this method allows to evaluate the effect of low molecular weight ligands on the thermal stability. This method is good for simple and quick initial analysis of the potential effects of a ligand, but has some limitations connected with different artifacts. For our work we use a wide panel of biologically significant targets, including G4s and IMs from the human genome and also from the genomes of viruses and bacteria. It includes the well-known G4s from oncogen promoters, such as c-myc, CKit, telomeric repeats, as well as bacteria, such as *M. tuberculosis*, Chlamydia, *N. gonorrhoeae*, Streptococcus, and viruses like HSV. As IM targets, we used complementary sequences from c-myc, CKit and telomeric repeats and from promoters of the DNMT1, EWS, SOX18, CL2, VEGF genes. We also included imperfect G4s and a classic hairpin as a control. The effects were tested using known G4/IM-binding ligands, such as berberine, Braco19, PhenDC3, etc. Based on data about of these compounds as an example, we investigated the influence of conditions on the stability of non-canonical structures and on the properties of ligands. We demonstrated the necessity of carefully choosing of conditions for more accurate prediction of possible effects. We also described the most common artifacts and give an explanation of their possible cause and offer and discuss methods of their resolving by changing conditions, titration or verification with another methods like CD spectra. This work was supported by RSF [20-15-00017].

P-06.2-38
Mass spectrometric method for analysis of cyclosporin A and isocyclosporin A

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Cyclosporins are a class of cyclic peptides containing β-hydroxy-N-methylamino acid. Cyclosporin A is the most common cyclosporin and is used in the clinic as an immunosuppressant. One of the most common impurities in cyclosporin A formulations is isocyclosporin A, a product of the N→O-acyl shift. During the study of cyclosporin A, we developed a new approach for distinction of cyclic peptides containing β-hydroxy-N-methylamino acids and isomeric depsipeptides (resulting from the N→O-acyl shift) from each other, in particular, cyclosporin A and isocyclosporin A. The technique is based on analysis of CID fragmentations of doubly protonated ions $[M+2H]^{2+}$. It allows to identify isomers of such cyclic peptides thanks to their characteristic N→O-acyl shift being suppressed by protonation of the β-hydroxyl group of the cyclic peptide. The method can be used for LCMS detection and identification of impurities in drugs based on cyclosporin A because it does not require chromatographic standards. Moreover, the N→O-acyl shift is common for other cyclic peptides containing β-hydroxy-N-methylamino acids. The discovered suppression of the rearrangement in doubly protonated molecules can be further used in structural studies of these compounds. This work was supported in part by Russian Foundation for Basic Research (project No. 20-33-70215).

P-06.2-39
Novel plant and peloid peptides inhibit fibrin monomer polymerization

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Peptides are broadly used as signal, transport, protective molecules in living organism. Due to its wide range of activity, peptides potentially can be used novel pharmacological agents. In this study, we discovered peptides from Boraginaceae family plants and Taraskul lake peloid which demonstrate anticoagulant activity. We have developed methods for the isolation of peptides from 0.1 M ammonium hydroxide plant and peloid extract using size-exclusion chromatography (Toyopearl HW-40, 0.02 M ammonium-acetate buffer) and cation-exchange chromatography (Sepharose CL-6B). Fractions were checked for peptide purity by gel-electrophoresis in gradient polyacrylamide gel (6–25% tris-tricine gel) and high pressure liquid chromatography (column 4.6X250, ReproSil C-18, 5 μm, gradient ranging from 5 to 40% acetonitrile in 0.1% trifluoroacetic acid/water). The molecular mass of peptides was estimated by size-exclusion chromatography (Sephadex G-25) and sodium dodecyl sulfate-polyacryamide gel electrophoresis (SDS-PAGE). To evaluate the effects of peptides (target purity of 99.5%) on coagulation parameters *in vitro*, we measured the influence of the peptide on the prothrombin time (PT), activated partial thromboplastin time (aPTT), thrombin time (TT) and test of fibrin monomer autopolymerization. In *in vitro* clotting time experiments (PT, aPTT, TT), peptides significantly prolonged blood clotting time in a dose-dependent manner. Peptide in concentration 5 mM more than four times increased time of fibrin monomer autopolymerization. We studied that peptides demonstrate the strong influence on the early stages of fibrin polymerization (the formation of oligomers and protofibrils), but practically did not affect the late stages of fibrin clot formation. Overall, this study sheds light on inhibition fibrin polymerization by novel peptides from Boraginaceae family plants and peliod, while putting forward these peptides as an new pharmacological agents.

P-06.2-40
Folate-containing liposomal drug formulations as potential anticancer agents

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To overcome the multiple drug resistance of cancer cells a development of anticancer agents with different mechanism of action is required. Lipophilic anticancer agents containing two functional domains: polyamine and glycerolipid promote apoptosis-dependent anticancer activity and significantly increase polyamine catabolism [1]. To reduce side effects of lipophilic anticancer

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agents, new liposomal drug formulations can be developed. The problem of tumor selectivity can be solved by introducing of targeting ligand into the liposomal drug formulations. Unlike normal tissues, aggressive or undifferentiated tumors overexpress folate receptors. Therefore, folic acid can be used as targeting ligand. Previously, we developed folate-containing liposomes for effective delivery of different types of nucleic acids into cancer cells [2,3]. The use of folate-containing liposomes is promising strategy for targeted delivery of the lipophilic anticancer agents into cancer cells by receptor-mediated endocytosis. In this work, we prepared a number of folate-containing liposomal drug formulations, studied their physicochemical parameters (size and zeta-potential) and biological activity. [1] K.A. Perevoshchikova et al. (2019) Mendeleev Commun 29, 616–618. [2] E. Shmendel et al. (2020) J Drug Deliv Sci Tec 57, 101609. [3] E. V Shmendel et al. (2019) Russ J Bioorg Chem+ 45, 719–725. This study was supported by the Russian Foundation for Basic Research (project no. 18-33-20192) and the Ministry of Science and Higher Education of the Russian Federation (project no. 0706-2020-0019).

P-06.2-41
Study of substrate specificity of bacteriocin modification enzymes

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Lantibiotics belong to one of the most numerous types of ribosomally synthesized antimicrobial peptides of bacterial origin (bacteriocins) that undergo significant post-translational modifications by specific modifying enzymes. A distinctive characteristic of this class of bacteriocins is the presence of intramolecular thioether bonds, which are formed between the amino acid residues of Cys and dehydrated residues of Ser or Thr. Lantibiotics attract attention due to their high activity against many clinically relevant gram-positive and some Gram-negative bacteria, including drug resistant pathogens such as methicillin resistant *Staphylococcus aureus* (MRSA) and vancomycin resistant Enterococci (VRE), as well as due to their low toxicity for humans. Interest in lantibiotics is associated with the potential for their use in medicine, livestock and food industry as new antibiotics and preservatives. Lantibiotic biosynthetic machinery can be extensively used in biotechnology. Study of these enzymes will provide an information necessary for the production of recombinant analogs of natural lantibiotics and their mutant variants, as well as other biologically active lanthipeptides. In our work, we developed a heterologous *Escherichia coli*-based system for coexpression of the two-component lantibiotic lichenicidin genes lchA1 and lchA2 with its modifying enzymes lchM1 and lchM2. Also, we evaluated these enzymes specificity by coexpression of a number of non-natural substrates with lchM1 and lchM2 genes. The aim of this study was to expand our understanding of mechanisms of bacterial antimicrobial peptide biosynthesis that may find medical application in the future. The reported study was funded by RFBR, project number 20-015-00369.

P-06.2-42
Study of biodiversity of β-hairpin antimicrobial peptides from marine Polychaeta

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Antimicrobial peptides (AMPs) are essential components of innate immunity system of multicellular organisms. Polychaetes is the class of annelid worms living mainly in marine environment. As these marine organisms do not exhibit adaptive immune response, the presence of AMPs is extremely important for them. Earlier, we have established that the BRICHOS domain (about 100 amino acids residues) is used by polychaetes similarly to the cathelin-like universal system of AMP biosynthesis in vertebrates. A panel of recombinant analogs of natural peptides from *Capitella teleta*, *Abarenicola pacifica*, *Urechis unicinctus* and *Arenicola marina* were obtained with the use of heterologous *E. coli* BL21(DE3) expression system. All investigated peptides were active against Gram-positive and Gram-negative bacteria including multidrug-resistant strains. Most of these peptides were shown to be non-toxic against normal mammalian cells at concentrations up to 64 μM. A putative mechanism of antibacterial action of these peptides was investigated, and also antibiofilm activity of the peptide from *Capitella teleta* was studied. The discovered AMPs may be considered as scaffolds for the creation of antimicrobial agents. This work was supported by the Russian Foundation for Basic Research (RFBR project No. 18-54-80026).

P-06.2-43
Anticancer activity of lipopolyamines

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Polyamines (PA) are small organic cations that are essential for normal cell growth and development in eukaryotes. Increased intracellular PA content are associated with malignant transformation mainly through an up-regulation of the PA biosynthesis and PA transport system. Terminally alkyl substituted natural and synthetic PA as well as drug-polyamine conjugates exhibited significant antitumor effect. Recently, we developed non-symmetric lipophilic polyamines (LPAs) and showed, that LPAs induced cell death at concentration from 8.50 ± 0.70 to 1.28 ± 0.24 μM [1]. Here we report synthesis of symmetric LPAs contained alkyl diglycerides with various hydrocarbon chain lengths. MTT-assay showed that a decrease of the alkyl length led to an increase of LPA cytotoxicity for cancer cells. The leader norspermine-based LPAs with tetradecyl and decyl residues induced apoptosis in KB-3-1 cells. Lipophilic norspermine with decyl substituent inhibited migration of B16 cells and promoted cell adhesion. This study was supported by the Ministry of Science and Higher Education of the Russian Federation (project no. 0706-2020-0019). [1] Perevoshchikova KA et al. (2019) Mendeleev Commun., 29, 616–618.

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P-06.2-44
Development of highly selective non-azole inhibitors of *Candida albicans* sterol 14α-demethylase

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Fungal infections are increasing dramatically, leading to high morbidity and mortality in immunocompromised patients. Azole drugs are the first line treatment for fungal infections, with the main target being sterol 14α-demethylase (CYP51), a cytochrome P450 enzyme crucial for the sterol biosynthetic pathway. However, resistance to available azole-based antifungal drugs is growing which raises the need to develop structurally distinct and selective fungal CYP51 inhibitors. In this work, we have investigated for the first time the binding of pyridylethanol(phenylethyl) amines to any fungal CYP51. With the comparative study of their binding to *Candida albicans* and human CYP51, we were able to reveal moieties critical for selectivity and potency and develop highly selective derivatives towards *C. albicans* CYP51 with significantly increased inhibitory activity. Nuclear magnetic resonance and molecular modelling methods were used to reveal the unique binding mode of this new chemical class of fungal CYP51 inhibitors. The low molecular weight of the lead derivatives and the in-depth studies on their binding provide directions for focused lead optimization that could eventually furnish structurally novel antifungals with clinical candidate potential.

P-06.2-45
Anti-HIV-1 activity and mode of action of a viral-derived and proteolysis-resistant peptide CXCR4 antagonist

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We report the anti-HIV-1 peptide pepRF1, a human serum-resistant peptide derived from the Dengue virus capsid protein. In vitro, pepRF1 shows a 50% inhibitory concentration (IC_{50}) of 1.5 nM with a potential therapeutic window higher than 53,000. This peptide is specific for CXCR4-tropic HIV-1 strains, preventing viral entry into target cells by binding to the viral co-receptor CXCR4. Upon binding, neither internalization nor intracellular Ca^{2+} influx are triggered, showing that pepRF1 is an antagonist of this chemokine receptor. pepRF1 is more effective than T20, the only peptide-based HIV-1 entry inhibitor approved by FDA for clinical use, and excels in inhibiting an HIV-1 strain resistant to T20 (HIV-1NL4.3 DIM) with an IC50 of 2.8 nM. Overall, our study led to the discovery of a peptide highly active against HIV-1, serum-stable, and with low toxicity, that acts as a CXCR4 antagonist. Potentially, pepRF1 can be used alone or in combination with other anti-HIV drugs to fight AIDS. Furthermore, one can also envisage its use as a novel therapeutic strategy for other CXCR4-related diseases.

P-06.2-46
Transcriptomic profiling of *Mycobacterium smegmatis* to reveal imidazo[1,2-b][1,2,4,5] tetrazine mechanism of action

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Tuberculosis (TB), caused by *Mycobacterium tuberculosis*, is currently the leading killer among the infectious diseases caused by one infectious agent. An important challenge for researchers today is the search for new anti-tuberculosis drugs and shorter chemotherapy regimens. We have previously investigated several imidazo[1,2-b][1,2,4,5]tetrazines as new potential anti-tuberculosis drugs. We have shown that mutations, leading to overexpression of mmpS5-mmpL5 efflux pump genes are the main mechanism of resistance to these compounds. However, the mechanism of action of imidazo-tetrazines still remains unknown. We sequenced total RNA (RNAseq) from *M. smegmatis* strains subjected to different concentrations of an imidazo-tetrazine (¼×MIC, ½×MIC, MIC), to reveal the transcriptomic response of the bacterial cells, which could shed light on its mechanism of action. We identified a large number of differentially expressed genes (at least 2-fold, FDR ≤ 0.05) in all three transcriptome profiles, as compared to the compound-free control: 344 up-regulated and 684 down-regulated genes. Filtering only the genes that had a dose-dependent expression fold-change revealed two operons involved in iron absorption to be up-regulated: mbtA-G (mycobactin biogenesis gene) gene cluster and ESX-3 operon. The mbt gene cluster is responsible for the synthesis of siderophores, the most important iron chelating compounds. ESX-3 is a type VII secretion system responsible for the efflux of siderophores. Both mbt and ESX-3 operons' expression is controlled by IdeR and HupB, which regulate the iron uptake in *M. smegmatis*. We suggest that imidazo-tetrazines interfere with iron metabolism, leading to the inability of the cell to absorb iron, and subsequently to cell death. This work is supported by the Russian Science Foundation (grant 17-75-20060-P).

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P-06.2-47
Long exposure of bacteria to ampicillin induces changes in sensitivity to other antibiotics

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Ampicillin is a broad-spectrum beta-lactam antibiotic used to prevent and treat a number of bacterial infections such as respiratory and urinary tract infections, meningitis, salmonellosis and endocarditis. It is one of the oldest and most used antibiotic worldwide and is unfortunately sometimes used for self-medication. The present study aimed to assess the changes in susceptibility to other antibiotics induced by recurrent exposure of bacteria to ampicillin. The bacteria used included 4 uropathogens namely *E. coli* (UPEC), *S. aureus*, *E. faecalis*, *St. agalactiae* and 2 standard strains namely *S. aureus* ATCC 6538 and *E. coli* M17. The minimum inhibitory concentrations (MIC) of Ampicillin were determined using the microplate mircodilution method and bacteria were exposed to increasing concentrations of ampicillin (from MIC/2 to MIC) prepared in the brain heart infusion broth during 8 days. The sensitivity of bacteria to antibiotics was assessed using the Kirby Bauer disc diffusion method and the antibiotics used were: Ciprofloxacin, Tetracycline, Ceftriaxone, Imipenem, Nitrofurantoin, Ceftazidime, cefazoline+clavulanate and Trimethoprim. The inhibition diameters (untreated bacteria = D0 and treated = D) were interpreted referred to the Clinical & Laboratory Standards Institute and the variation rates were calculated by:(D-D0)*100 /D0. The results showed that a variation in sensitivity to at least one antibiotic was observed in all the strains tested. The highest variations were observed on tetracycline, ceftazidime and cefazoline+clavulanate in *S. aureus* ATCC 6538, *E. coli* M17 and *St. agalactiae* with variation rate ranging from -68,3% to -45.8%. The uropathogen *S. aureus* became resistant to tetracycline (-50%) while variations observed in *E. faecalis* and UPEC were not highly significant ($P > 0.05$). In conclusion, long exposure to ampicillin can induce a decrease in sensitivity of bacteria to antibiotics and should only be used under prescription while avoiding prolonged use.

P-06.2-48
The activity of prospective HSP90 inhibitors in breast cancer cells

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The development of new classes of drugs for cancer therapy is an actual direction of investigation requiring the collaboration of medicine, biology and chemistry. We synthesized a novel class of inhibitors of the molecular chaperone, HSP90. This protein is involved in a proper folding of different proteins in cancer cells including oncogenes. So, it is extremely important to discover new drugs that can disrupt protein folding in cancer cells, which consequently will lead to changes in its metabolism and finally to cell death. Nowadays, several HSP90 inhibitors are used in cancer therapy, but they have some serious disadvantages like complicated synthesis, low efficiency *in vivo*, and various side effects. Test compounds were selected by virtual screening their binding affinity to N-domain of HSP90 and synthesized starting from 3-(phenyl)-6,7-dihydrobenzo[d or c]isoxazol-4(5H)-ones by an oximation of them followed by acylation with various carboxylic acids. All breast cancer cell lines were obtained from the ATCC. We performed screening over than 30 compounds in breast cancer cell lines. We selected the most active drugs and studied their cytotoxicity and effect on different cell signaling cascades. We showed that the inhibitors changed HSP90 intracellular targets levels. It was confirmed by decrease of Akt, phospho-Akt, Erk1/2 and pNF-κB. We observed the depression of Snail, a key regulator of epithelial-mesenchymal transition. This process initiates metastasis and invasion of tumor cells. Moreover, we noticed that leaders of HSP90 inhibitors had high cytotoxic activity. Cell cycle analysis showed the raised number of apoptotic cells simultaneously without any changes in other phases of the cycle. A novel class of HSP90 inhibitors could be considered as candidates for further chemical optimization into lead compounds and a preclinical investigation for breast cancer treatment. The reported study was funded by RFBR (project 19-54-04001) and BRFFR (project X19PM-013). *The authors marked with an asterisk equally contributed to the work.

P-06.2-49
The properties investigation of new antimicrobial peptides of *Hirudo medicinalis* metagenome

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The search and study of the new of antimicrobial peptides (AMPs), promising therapeutic agents, is an urgent task. Based on the metagenomic analysis of the microbiota of the medicinal leech *Hirudo medicinalis*, peptides were identified, the antimicrobial activity of which was confirmed against a number of pathogenic bacteria. The cytotoxicity of AMPs was investigated by viability evaluation of McCoy cells treated with peptides at a concentration equal to 4× MIC. Cytotoxic assays of the AMPs demonstrated that two peptides (pept_1545, pept_148) of five did not cause cell death after 24 and 48 h treatment. Other peptides exhibited high or moderate cytotoxic effects. The secondary structure predictions of AMPs were accomplished via the I–TASSER–MR server. According to the predictions, the only peptide pept_1303 is unstructured, whereas other peptides adopt α–helical conformation. To determine the secondary structure of AMPs we used CD spectroscopy. CD spectra of peptides were obtained in a buffer (100 mM KCl, 1 mM HEPES, 0.2 mM EDTA) and in a buffer with the addition of 1-palmitoyl-2-oleoyl-glycero-3-phosphocholine liposomes, which mimics the cell membrane. The analysis of CD spectra was carried out using the BeStSel web server. According to the results, only peptide pept_352 adopts an α–helical conformation in the buffer and in the presence of POPC liposomes. The other peptides are

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unstructured in different conditions. The examination of the AMPs' properties demonstrated that the nontoxic peptide pept_1545 is a promising candidate for drug development. The research was supported by the Russian Science Foundation (project № 20–15–00270).

P-06.2-50
Effect of three-day pretreatment of male rats with a thieno[2,3-d]pyrimidine-based allosteric agonist on the steroidogenic effects of human chorionic gonadotropin

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For the treatment of androgen deficiency in men, luteinizing hormone (LH) and human chorionic gonadotropin (hCG) are often used, but their use in pharmacological, sufficiently high doses leads to decreased sensitivity of the testes to endogenous gonadotropins and the drug withdrawal syndrome. LH/hCG receptor agonists, which bind to the allosteric site located in the transmembrane domain of the receptor, are an alternative to gonadotropins. Among them, thienopyrimidine derivatives, including 5-amino-N-tert-butyl-2-(methylsulfanyl)-4-(3-(nicotinamido)phenyl) thieno[2,3-d]pyrimidine-6-carboxamide (TP03) developed by us are of great interest. The aim of the work was to test the hypothesis that pretreatment of male rats with TP03 can enhance the stimulating effect of low-dose hCG on testosterone (T) levels and testicular steroidogenesis. It was shown that in animals pretreated for 3 days with TP03 (15 mg/kg/day, i.p.), the steroidogenic effect of hCG (10 IU/rat, s.c.) was significantly enhanced compared with intact rats. This indicates the enhancement of the hCG-induced increase in blood T levels and the testicular expression of the Creb1 and Sf1 genes encoding the CREB and SF1 transcription factors, and the Star and Cyp11a1 genes encoding the StAR protein and cytochrome P450scc involved in T synthesis. When using the half-maximum dose of hCG (20 IU/rat), the potentiating effect of TP03 pretreatment was significantly weaker and was detected only during the first two hours after hCG administration. Thus, the pretreatment of male rats with TP03 increases the stimulatory effect of low-dose hCG (10 IU/rat) on testicular steroidogenesis, which is due to the synergism of the action of orthosteric (hCG) and allosteric (TP03) agonists. Such pretreatment may be a promising approach to reduce pharmacological doses of gonadotropins, thus preventing the side effects of high-dose hCG and LH in the clinic. This work was supported by the Russian Science Foundation (No. 19-75-20122).

P-06.2-51
Anti-cancer properties of compounds from leaves of *Pergularia tomentosa*

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Pergularia tomentosa L. is a milkweed tropical plant, belonging to the sub-family Asclepiadaceae of the Apocynaceae. Different parts of *P. tomentosa*, including the aerial parts and roots, are used in traditional medicine for the treatment of several diseases as, for example, bronchitis, constipation and skin diseases. Among bioactive compounds isolated from *P. tomentosa*, some cardenolides present good antiproliferative properties. We have focused our attention on five cardenolides recently isolated from aerial parts of the plant and investigated their anti-proliferative properties on a human hepatocarcinoma cell line (i.e. HepG2 cell line). Compounds were: calactin (C21), calotropin (C20), 12β-hydroxycalactin (C14, a calactin derivative), 12β,6′-dihydroxycalotropin and C7, 3-O-β-glucopyranosiylcalactin (C4 and C7, respectively, both calotropin derivatives). We monitored cell viability by an MTT assay, then studied the occurrence of apoptosis by different approaches (caspase-3 and TUNEL assays) and evaluated the ability of these compounds to induce autophagy, by analysing two markers of the autophagic process (LC3 and p62). We found that all cardenolides had cytotoxic effects, with IC50 ranging from 0.127 to 6.285 mM. We verified that all compounds were able to induce apoptosis and autophagy, being C21 the most active one in all assays performed. In cells treated with C21, we also observed a slight reduction of cell migration and a partial block of cell cycle into S-phase. Our findings suggest that selected cardenolides from *P. tomentosa*, and in particular C21, possess potential desirable properties to be further investigated as anti-cancer agents. *The authors marked with an asterisk equally contributed to the work.

P-06.2-52
Effects of cholesterol on internalization Puumala and Dobrava hantavirus

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Hantaviruses are dangerous human pathogens and cause diseases that are often fatal. There is information about the important role of cellular cholesterol in the life cycle of the Andes hantavirus. Dysfunction of the regulatory sterol complex (SREBP-2, SCAP, S1P and S2P) leads to a decrease in the infectivity of the virus. By manipulating cholesterol levels, we have demonstrated that the penetration of the Puumala and Dobrava hantaviruses into the cell is also sensitive to changes in cellular cholesterol levels. The use of drugs that lower the level of cellular cholesterol by blocking NADP•H-dependent reductase HMG-CoA, statins (simvastatin, mevastatin) and PF-429242 dihydrochloride as an inhibitor of the sterol regulatory element binding protein (SREBP), affect the infectivity of Puumala and Dobrava viruses. The effect manifests itself during pharmacological treatment of host cells before infection is dose-dependent and unequal in relation to different types of hantaviruses. A decrease in the

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infectivity of Puumala and Dobrava/Belgrade viruses was observed at concentrations of 0.6–10 μM for mevastatin / simvastatin and PF-429242 in the range of 15–100 μM. The maximum (up to 80%) decrease in infectivity is at a concentration of 3–5 μM statins and 50–60 μM PF-429242. Neither PF-429242 nor statins had any significant effect on the infection of Vero E6 cells with the Hantaan virus. The results of the study suggest an important role of cholesterol in the life cycle of Puumala and Dobrava/Belgrade viruses, its necessity for penetration into the host cell, and, possibly, for their replication, and also indicate certain differences in the virus-cell interactions of different hantaviruses.The study of the pathways of internalization and intracellular traffic of hantaviruses provides an understanding of the molecular mechanisms of hantavirus infection at the subcellular level and helps to expand the theoretical basis for the development of targeted drugs.

P-06.2-53
New generation molecules as modulators of the mitochondrial permeability transition and potential therapeutic agents

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Neurodegenerative, cardiovascular and other diseases, associated with mitochondrial dysfunctions due to mitochondrial permeability transition pore (mPTP) opening, could greatly benefit from therapies which counteract the mitochondrial defect. The F_1F_O-ATPase, the well-known mitochondrial enzyme complex which forms ATP, is considered as the main candidate responsible for the mPTP formation when Ca^{2+} concentration suddenly increases in the mitochondria. The Ca^{2+}-activated F_1F_O-ATPase, being recently found as consistent with the signal propagation pathway that triggers mPTP opening [1], has raised interest as potential drug target. As potential drugs, small triazole molecules are particularly promising since they modulate the mPTP [2,3] and show antiviral and antitumor properties, by interacting with proteins [3]. Selected 1,2,3-triazole-1,5-disubstituted derivatives (TZs) were tested on the F_1F_O-ATPase activated by the natural cofactor Mg^{2+} or by Ca^{2+} in swine heart mitochondria. Their effects on mitochondrial respiration and energy metabolism as well as the possible cytotoxicity were also evaluated on porcine aorta endothelial cells. The TZs did not affect the Mg^{2+}-activated F_1F_O-ATPase while they inhibited the Ca^{2+}-activated enzyme activity. Inhibition kinetic analysis on the purified F_1 domain of the F_1F_O-ATPase allowed to localize the binding site of the TZs on the hydrophilic portion F_1. Interestingly, the tested TZs did not alter the mitochondrial respiration and cell metabolism and allowed to rule out any citotoxicity. The results shoulder the possible use of TZs as therapeutic agents to counteract mPTP-related diseases at the molecular level by hampering mPTP opening. [1] Algieri C et al. (2019) Ann N Y Acad Sci 1457, 142–157. [2] Stocco A et al. (2021) Pharmacol Res 105421. [3] Algieri V et al. (2021) Ann N Y Acad Sci 1485, 43–55.

P-06.2-54
Comparative analysis of polyphenolic biologically active compounds isolated from brown marine algae and green endemic algae of lake Baikal

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The isolation of biologically active compounds from natural sources is one of the most actual problems in biopharmaceuticals. Polyphenolic compounds are considered to be one of the promising agents in the treatment of various human diseases. This study aimed to compare the polyphenolic composition of extracts from the brown marine algae *Ecklonia cava* and the endemic freshwater green algae Draparnaldioides sp. Lake Baikal is a UNESCO World Heritage site. This unique freshwater ecosystem is characterized by an exceptional environment, such as high oxygen content in the entire water column. Thus, the studies of algae adaptation mechanisms are engaging in the focus of biopharmaceutical value. Brown algae *E. cava* inhabits the oceans near Japan and Korea, and it is a well-known source of natural products with biological activity. Polyphenolic extracts of these algae are used to treat various diseases in Asia. A comparative analysis of the polyphenolic composition of secondary metabolites between endemic green algae of Lake Baikal and marine brown algae has not been carried out before. Sampled algae were homogenized with ethyl acetate, shaken and centrifuged. The upper phase was separated and dried in vials for chromatography at 70° C to dry mass. Obtained extracts were dissolved in methanol before analysis. Samples were analyzed on Shimadzu LCMS-8060 Triple Quad and Agilent 6470 Triple Quadrupole LC/MS. Comparative analysis of algae extracts showed a high similarity of the qualitative polyphenolic content. We identified 2D-Bromotetraphlorethol C, Eckol, Trifuhalol, 4'-Bromeckol, and Eckstolonol in extracts of both studied species. Thus, polyphenols' ability in Baikal green algae can determine the development of endemic algae in pharmaceutical biotechnology, and biomedicine. This study was partially supported by foundations, including the main support Russian Foundation for Basic Research (18-29-05051) and Grant of President of Russian Federation (MK-1245.2021.1.4).

P-06.2-55
The effect of amino acid substitutions on the properties of food allergen Len c 3

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Lentil is an important component of the diet but lentil allergy occurs in many countries especially in the Mediterranean area. Previously we isolated the lipid transfer protein from lentil seeds which was characterized as the allergen Len c 3 cross-reacting with the major peach allergen Pru p 3. In this work, we investigated the influence of replacement for alanine of two conserved for plant LTPs amino acids, Arg45 and Tyr80, on the structure and biochemical properties of Len c 3. Mutant analogs of Len c 3 (R45A, Y80A, and R45A/Y80A) were obtained by protein

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expression in *E. coli*. Computer modeling demonstrated that amino acid substitutions affected the change in the size and shape of the hydrophobic cavity of Len c 3 as well as the structure of possible B- and T-cell epitopes. Simulation of gastrointestinal digestion of Len c 3 and its mutant analogs *in vitro* was performed. Len c 3 was not sensitive to gastric digestion but was cleaved efficiently during subsequent duodenal digestion. The replacement of Arg45 and Tyr80 led to a decrease in the rate of Len c 3 degradation, which was especially pronounced in the case of R45A/Y80A. Gastrointestinal digestion of all proteins was accompanied by the formation of a large fragment with a molecular weight of about 8 kDa. Cross-reactivity of the mutant analogs of Len c 3 was investigated by ELISA using polyclonal rabbit anti-Len c 3 antibodies. All mutant analogs interacted with rabbit antibodies with the same efficiency. Comparative study of IgE-binding capacity of Len c 3, R45A, Y80A, and R45A/Y80A was also performed by ELISA using sera of allergic patients. Substitution of only arginine 45, slightly reduced the ability of Len c 3 to bind IgE from the sera of allergic patients. But IgE reactivity of the double mutant analogue R45A/Y80A was significantly reduced. We showed that Arg45 and Tyr80 are important amino acid residues in the structure of food allergen Len c 3. Substitution of these amino acid residues leads to structural changes of Len c 3 and affects its allergenic potential. The introduced mutations increase the resistance of allergen to gastrointestinal digestion, but reduce the ability of Len c 3 to bind antibodies from sera of allergic patients. The work was supported by the Russian Science Foundation (project no. 20-45-05002).

P-06.2-56
Development of polymer complexes of chitosan, gelatin, alginate and albumin in bioactive layer design of caspase-3 biosensor

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Biosensors are a device based on the principle of the formation of a signal proportional to the amount of analyte on the transmitter surface as a result of the interaction of the substance to be analyzed with the biocomponent in the bioactive layer and the transmission of this signal. Charge transfer on the bioactive layer between electrode and biocomponent should be fast. This transfer is optimized by polymers used with biomolecules on the electrode surface. Apoptosis is a programmed cell death process. However, impaired apoptosis can cause diseases such as cancers, neurological and autoimmune diseases.In addition to caspase-3 is an important and reliable biomarker for the diagnosis and prognosis of apoptosis-related diseases, it is seen as a potential target molecule in treatment. The aim of this study is to develop polymers that will contribute to the optimization of bioactive layers for caspase-3 biosensor. For this purpose, polymers consisting of chitosan, alginate, gelatin and albumin were combined. Surface analyzes of the complexes of 4 types of polymers, namely gelatin-chitosan, alginate-chitosan, gelatin-alginate and albumin-gelatin, with caspase-3 antibody and crosslinking agents (glutaraldehyde and EDC/NHS) were performed with FTIR spectra and SEM images. FTIR spectra gave specific identifying bands to functional groups of polymers such as carbonyl-amino groups and amide bonds. Apart from these bands, specific peaks were also identified as a result of the interaction with the caspase-3 antibody. As a result of SEM analysis; It has been observed that the gelatin-albumin complex is porous, recessed and slightly fibrous 3D structures, the chitosan-gelatin complex is in the form of inhomogeneous round shaped particles, the alginate-gelatin complex is formed in the form of porous small lattices and the chitosan-alginate complex forms a heterogeneous blurry appearance. The most suitable polymer complex will also be supported by future electrochemical studies.

P-06.2-57
New borylated derivatives of amino acid esters

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Neutron capture therapy (NCT) is a promising method for the non-invasive treatment of malignant tumours. The creation of modern preparations for ^{10}B-NCT is based on boron cluster anions $[B_nH_n]^{2-}$ (n = 10, 12) due to their low toxicity and high boron content in the molecule. The primary efforts to create these drugs are focused on obtaining conjugates of boron clusters with influential transport groups. This work presents methods for synthesising conjugates of nitrile derivatives of closo-borate anions $[B_{10}H_9NCCH_3]^-$ and $[B_{12}H_{11}NCCH_3]^-$ with amino acid esters. This approach makes it possible to obtain target derivatives in quantitative yields under mild conditions. For the obtained derivatives based on ethyl ester of glycine Na $[B_{10}H_9NHC(CH_3)NHCH_2COOC_2H_5]$ and Na$[B_{12}H_{11}NHC(CH_3)NHCH_2COOC_2H_5]$ cytotoxicity was studied on NKE cell lines (IC50 2.4, 2.3 mM respectively) and Hep2 (IC50 3.0, 3.1 mM respectively). The obtained values indicate the low cytotoxicity of the obtained compounds and further study their properties as new potential agents for BNCT. This work was funded by RFBR, project numbers 20-33-70217 and 20-33-90119. *The authors marked with an asterisk equally contributed to the work.

P-06.2-58
Synthesis of conjugates of substituted porphyrins with 4′-(4-methylphenyl)-2,2′:6′2′-terpyridine for diagnosis and cancer therapy

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One of the most promising approaches for the cancer treatment is the development of diagnostic methods. Theranostics is the dual-purpose agents for earlier detection of a tumor, its visualization and subsequent therapy. Photodynamic therapy allows combining the capabilities of diagnosis and therapy of malignant tumors. This work is devoted to the design and synthesis of conjugates based on terpyridine derivatives and meso-arylporphyrins. Terpyridine molecule possess chelating properties allowing to incorporate large radius metal ions (for example, Gd(III)) into the nitrogen-bearing cavity for use in magnetic resonance imaging (MRI) contrast agents for tumor imaging. Meso-arylporphyrins are widely used for PDT. In this study, several photosensitizers (PS) were synthesized, and their toxicity studied on cells. The main strategy for A3B and ABAB type porphyrins was monopyrrole condensation reaction by the Lindsey method (A3B porphyrins) and the McDonald method from dipyrromethanes (ABAB porphyrins).

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Metal complexes (Zn(II), Pd(II)) of amino- and carboxy-substituted porphyrins were obtained, which were subsequently used to create conjugates with terpyridine derivatives. Commercially available 4'-(4-methylphenyl)-2,2':6'2"-terpyridine was modified to obtain functionally active derivatives chelating gadolinium ions. Dark toxicity of conjugates was carried out using MTT test on NKE cells (human kidney epithelium). The data obtained indicate a rather low dark toxicity of the obtained compounds (from 0.17 mM to 14 μM), which allows in the future to use rather large doses of PS for effective PDT. This work was supported by the Russian Foundation for Basic Research, project No 20-33-70218.

P-06.2-59
Gausemycins: meshanistic studies and preliminary results

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Gausemycins A and B, recently discovered antibiotics (Tyurin AP et al. (2020) ChemRxiv preprint) possess several unique structural features indicating that they constitute a new class of antibiotics. The compouns show high activity against some gram-positive bacteria. Cytological profiling using confocal laser microscopy indicates the similarity in action between gausemycins and daptomycin - well known antibacterial used in medicine. Like daptomycin and related lipopeptides, the studied antibiotics could form pores in bacterial cell memranes. It was supported by electochemical measurements using model lipid bilayers. Despite this the concentration dependence of ion current, response on two-valent cations addition and antibacterial activity spectrum were not identical with typical calcium-dependent lipopeptides. The UDP-N-AcMur-PP accumulation assay indicates that gausemycin B has no effect on cell wall biosynthesis precursor pool in contrast to vancomycin. These data point out the novel mechanism of antimicrobial action for gausemycin-type lipopeptides. Discovery of these antibiotics is promising for development of novel antibacterial drugs, both natural and semi-synthetic. The research was supported in part by the Russian Science Foundation (project no. 20-15-00361).

P-06.2-60
RDD-19 and RDD-142, two novel darunavir analogues, induce apoptosis, ER stress and autophagy in hepatic cancer cells

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Given the impelling need for innovative cancer treatment approaches to overcome novel mutations and chemotherapy resistance, a more rapid and not very expensive way seems to be the repositioning of already approved drugs as anticancer agents [Subeha MR et al. (2020) Cancers 12, 3427]. There are several pre-clinical and clinical evidences suggesting that HIV-1 protease inhibitors (HIV-PIs), in addition to the antiretroviral properties, possess pleiotropic pharmacological actions, including anticancer effects [Maksimovic-Ivanic D et al. (2016) Int J Cancer 140, 1713–1726]. Possible use of some derivatives of hydroxyethylamine, the central nucleus of the HIV-1 protease inhibitor Darunavir, as molecules with pro-apoptotic activity on tumor cells, has been demonstrated [Facchinetti V et al. (2015) Med Chem Res 24, 533–542], and two new analogous molecules, indicated as RDD -19 and RDD-142, have been synthesized. The two molecules showed a greater and dose-dependent cytotoxicity towards the hepatic tumor cell line HepG2 compared to the non-pathological hepatic cell line IHH, also higher than that shown by treating the cells with Darunavir. Therefore, several assays were performed in order to clarify the molecular mechanisms of their cytotoxic effect. Both molecules caused two types of cell death, a caspase-dependent apoptosis, ascertained by a series of biochemical and morphological assays, and a caspase-independent death that was characterized by induction of endoplasmic reticulum stress and autophagy. The strong increase of ubiquitinated proteins inside the cell suggested that the target of these molecules was the proteasome and in silico molecular docking experiments make this hypothesis plausible. Finally, treating the cells with the two analogs lowered p-AKT levels, interfering with cell survival and proliferation. These findings demonstrate that RDD-19 and RDD-142, two novel Darunavir analogues, are potent anticancer agents with pleiotropic effects.

P-06.2-61
Study of molecular bases of the mechanism of action of antifungal macrolide antibiotics

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Fungal infections affect more than one billion people and cause more than 11.5 million life-threatening infections and more than 1.5 million deaths per year. One of the most promising classes to search for a new generation of antifungal antibiotics is the antifungal polyene macrolides of amphotericin B group as they have a wide range of antifungal activity, and clinically significant drug resistance is rare enough. However, the use of amphotericin B is limited by its low solubility in aquatic environments and by serious side effects such as hemolytic toxicity and nephrotoxicity.

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The mechanism of action of antifungal polyenes has not yet been finally established. The key unexplained questions are the necessity of formation of pores in the membrane for antifungal activity, the influence of self-aggregation of antibiotics on toxicity in respect to host cells, ways to increase the selectivity of binding with ergosterol, factors affecting the ability to act on fungi in biofilms and etc. We have performed a comprehensive comparative analysis of the structure – antifungal activity relationship for the series of semisynthetic polyene antibiotics of the AmB group, including nystatin, natamycin and AmB. Chemical modification aimed to separation of antifungal and canal-forming activity and reduction of the ability of antibiotics to self-aggregate. Some of the derivatives were tested to determine whether they form pores in lipid bilayers and to compare their channel characteristics, also their ability to bind with ergosterol was evaluated by the quantum chemical method. The obtained data obtained allowed to establish some structure-activity relationships for a given class of antifungal drugs and suggested ways to increase the efficiency and selectivity of antibiotics, including those aimed at reducing toxicity and actions on resistant forms of pathogens. This work was supported by the Russian Science Foundation project number № 21-74-20102.

P-06.2-62
Investigating the surface of the parasite's drug target thioredoxin glutathione reductase by X-ray fragment screening

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The FAD/NAD-linked reductases are crucial but difficult targets of several drug discovery projects for the treatment of many human diseases. Thioredoxin glutathione reductase (TGR), a thioredoxin reductase-like enzyme, is a selenocysteine (Sec)-containing enzyme and a major drug target for the cure of schistosomiasis, a parasitosis affecting more than 200 million people. TGR is a chimeric enzyme that reduces the oxidized forms of both glutathione and thioredoxin, taking advantage of the mobile Sec-containing C-terminal arm. Currently, praziquantel is the only drug used to treat schistosomiasis, but such massive administration causes the onset of less sensitive strains, making the search of new drugs urgent. 97 small molecule fragments selected from a high throughput screen were probed by X-ray crystallography on TGR from *S. mansoni*; by means of a multi crystal data analysis, functional studies, and molecular dynamics, we discovered four new secondary sites (Site 1–4) exploitable in drug-design [Previously published in: Silvestri et al. (2018), ACS Chem Biol 13 (8), 2190–2202; Silvestri et al. (2020), Free Radic Biol Med, 147, 200–211]. Molecules bound to Site 1–2 interferes with NADPH binding and reduction, while 2-naphtholmethylamino compounds bound in Site 3 are transformed by this secondary site into covalent modifiers, which in turn, attack the nucleophilic centres of SmTGR, inhibiting the enzymatic activity. The fourth one is found between two symmetry related SmTGR subunits of the crystal lattice. Surprisingly, one compound bound to this latter site stabilizes, through allosteric effects, the crucial redox active C-terminus of SmTGR, making it finally visible at high resolution. The results further promote fragments as small molecule tools shedding light on functional aspects of the target protein, as the possibility to allosterically stabilize some relevant conformation of the target and providing fundamental chemical information exploitable in drug discovery.

P-06.2-63
EuPSIC: the database of eukaryotic small molecule translation inhibitors

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Translation machinery is an attractive target for the treatment of various human diseases. Many small molecule inhibitors that specifically affect protein synthesis in eukaryotic cells are potent anticancer, antiviral, anti-inflammatory, antifungal or antiparasitic drugs. However, despite their medical significance, there is no single resource combining data on eukaryotic translation inhibitors. Thus, we have created a manually curated, continuously updated database, EuPSIC (for Eukaryotic Protein Synthesis Inhibiting Compounds), containing information about small molecules targeting eukaryotic ribosomes, translation factors, aminoacyl-tRNA synthetases, and signaling pathways associated with translation. Compounds are classified according to their mechanisms of action, targets, binding sites, and organism specificity. EuPSIC is linked to the ChEMBL, PubChem, and CMap databases, provided with appropriate filters and an interactive diagram. It can be used as a handbook of translation inhibitors and as a data source for *in silico* approaches. The database is available at http://eupsic.belozersky.msu.ru and currently contains information on 429 inhibitors. This work was supported by the Interdisciplinary Scientific and Educational School of Moscow University «Molecular Technologies of the Living Systems and Synthetic Biology» and by the Russian Foundation for Basic Research (grant no. 19-34-51047 to S.E.D.)

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Pharmacogenomics and biomarkers

P-06.3-01
Potential platelet's miRNA biomarkers of acute coronary syndromes

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MicroRNA microarray technology provides a high-throughput tool for the analysis of thousands of miRNAs in a single experiment, allowing to identify an altered expression and distinguish signatures associated with diseases. Modifications of gene expression are a frequent phenomenon marking the existing health disorder. The discovery of those changes helps in the early diagnosis and prognosis of patients and allows to understand the pathophysiological mechanisms of disease. Due to high resistance to harmful conditions, and remarkable stability in biological fluids, miRNAs are considered as ideal potential biomarkers. The general aim of our work was to find the miRNA signatures that could help to define human predisposition to the Acute Coronary Syndromes (ACS). For this purpose, we performed for the first time a screening and comparative analysis of platelet's miRNome between ACS diagnosed patients and healthy donors. Data obtained from microarrays showed altered expression of 172 platelet miRNAs which are associated with more than 2,000 mRNA molecules. Out of 172 miRNAs, we selected 7 miRNAs to be validated by Real-Time PCR based on molecular interaction, function, and signaling pathways. Altered expression was confirmed for miR-30e-3p, miR-148a-3p, miR-28-5p, miR-33a-5p, miR-454-3p, miR-139-5p, and miR-15b-5p, associated with genes linked with platelet activation (*AKT3, RASGRP1, PLCB1*), adhesion process (*VEGFA, PIK3R1, PDGFRA*), fibrinolysis (*PLAU*) and structure of platelets (*ITGA2, ITGB3*). Our results provide a new insight into the molecular mechanism of platelet disorders in ACS and indicate the direction for our further studies whose primary goal is to look for molecular markers of human predisposition to ACS.

P-06.3-02
Altered plasma s-Tie2, G-CSF and HGF concentrations observed in patients with endometrial cancer

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Endometrial cancer (EC) is the most frequent gynecological malignancy in more developed countries. Identification of novel biomarkers for EC would be invaluable for preoperative stratification of patients as low/high-risk, detection of primary and recurrent disease and thus enabling personalized therapeutic approaches. Our objective was to identify novel biomarkers for EC. Methods Women underwent surgical treatment at the University Medical Centre Ljubljana (38 patients with endometrioid EC and 38 control patients with prolapsed uterus or myoma). Plasma samples were measured using commercially available kits that enables determination of 37 different angiogenic factors with the Luminex xMAP multiplexing technology. Analysis of experimental data was carried out using 5–parameter weighted logistic regression modelling on the xPonent 4.2. Software. Results 38 patients (65.9 $\pm$ 8.2 years) with endometrioid EC had mean body mass index (BMI) of 31.8 $\pm$ 6.1 kg/m^2. Lymphovascular invasion (LVI) was present in five patients while deep myometrial invasion (DMI) was present in 12 patients. The control goup included 38 patients (66.8 $\pm$ 8.3 years) with mean BMI of 27.6 $\pm$ 3.9 kg/m^2. Plasma levels of sTie-2 (soluble angiopoietin-2) and G-CSF (granulocyte-colony stimulating factor) showed significantly decreased levels in patients with EC compared to the control group ($P < 0.05$). On the contrary, HGF (hepatocyte growth factor) was increased in patients with EC when compared to controls ($P < 0.05$). Tenascin C and angiostatin showed decreased levels in patients with EC and present LVI or DMV, respectively, while osteopontin levels were trending toward an increase in patients with EC and present DMI. Based on different grade of endometriod EC neuropilin showed significant difference between grade 1 and 3. Conclusion Results of our pilot study indicate that plasma levels of different angiogenic factors might have a diagnostic and/or prognostic value in endometrioid EC.

P-06.3-03
Metabolomic and proteomic approaches for discovery of diagnostic and prognostic biomarkers for endometrial carcinoma – BioEndoCar

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Endometrial carcinoma (EC) is the most frequent gynecological malignancy in the developed world. Currently there are no valid non-invasive diagnostic or prognostic methods available, and thus diagnosis and treatment of EC patients is guided by

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histopathological and surgical findings. The lack of non-invasive biomarkers of EC is addressed in the clinical study titled "Biomarkers for Diagnosis and Prognosis of Endometrial Carcinoma" (NCT03553589). Within the prospective observational case – control study patient recruitment takes place at six medical centers (University Medical Centre Ljubljana, Slovenia; University Medical Centre Maribor, Slovenia; Maastricht University, The Netherlands; Lublin Medical University, Poland; University Hospital Brno, Czech Republic and University of Genoa, Italy). Plasma samples from women with diagnosed EC and controls are currently examined using non-targeted and targeted metabolomics and targeted proteomics approaches. Combined blood metabolome (>850 metabolites), proteome (>900 proteins), clinical and epidemiological data will be analyzed in order to construct diagnostic/prognostic algorithms for early diagnosis of EC and to identify patients with low/high risk for cancer progression and recurrence. BioEndoCar project has defined inclusion/exclusion criteria and a strict standard operating procedure for sample collection, processing and storage that is followed in all medical centers. Since the beginning of the project we recruited more than 440 patients. Targeted proteomics and targeted and non-targeted metabolomics analysis are currently in progress and we are awaiting the results. Within the project we expect to find different metabolic and protein profiles in patients with early stages of EC as compared to controls and in patients with poor prognosis and high risk of disease progression and recurrence as compared to those with favorable prognosis. Funded by ERA-NET Transcan2 and MIZS.

P-06.3-04
Diagnosis of patients with erythrocytosis of unknown etiology

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Erythrocytosis is an increased production of red blood cells due to several acquired or congenital factors. Variants located in nine genes, that regulate oxygen-sensing and hemoglobin oxygen affinity, have been already associated with familial erythrocytosis. Despite full screening of those variants, 70% of patients remain undiagnosed. Our aim was to extend the genetic testing of selected patients with idiopathic erythrocytosis, using targeted NGS of 39 genes associated with erythropoiesis and iron metabolism. We also explored additional disease-driven mechanisms through a comprehensive review of the literature and several in silico tools, including Reactome, String, UniProt, GeneCards, and Human Protein Atlas. Targeted NGS revealed the pathogenic heterozygous variant c.1609G>A in the EPAS1 gene in one patient. Genetic screening of other patients uncovered several rare VUS potentially involved in the disease development and a high frequency of variants in the HFE gene, which is associated with iron balance impairment. Possibly causative variants need further functional assessment to explore a connection with erythrocytosis development. In silico we identified new mechanisms in HIF-EPO-EPOR pathway, containing genes involved in epigenetic modifications, post-translational modifications, nuclear transport, transcriptional regulation, and signal transduction, that could be correlated with this rare hematological disorder. With targeted NGS we improved Slovenian diagnostic testing for erythrocytosis and we were able to identify the genetic cause in one patient. However, for further investigation of disease etiology in patients with yet unexplained erythrocytosis, NGS analysis that includes new in silico identified genes or broader approaches, like WES and WGS, is suggested. *The authors marked with an asterisk equally contributed to the work.

P-06.3-05
DPYD gentoyping as a promising tool for preventing fluoropyrimidine toxicity – case series

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Fluoropyrimidines (FPs) are the most frequently prescribed anticancer drugs for variety of cancers, especially for breast, colorectal and gastric cancer. FPs are inactivated in the liver by dihydropyrimidine dehydrogenase enzyme (DPD). As DPD is encoded by DPYD gene, variations within this gene can result in DPD deficiency. DPD deficiency occurs in approximately 5% of population and leads to significant increase in risk of severe and potentially lethal toxicity when a patient is treated with standard FP doses. Although preemptive DPYD genotyping presents valuable tool in identifying DPD deficient patients at risk for serious, potentially fatal toxicity, it has not yet been implemented in standard clinical care. Our goal was to explore the importance of preemptive DPYD testing and upfront dose adjustment in DPD deficient patients, to avoid FP toxicity and lower the cost of patient's care. Six patients that experienced gastrointestinal and/or hematological events grade 3 or higher (CTCAE v4) leading to 16–34 days long hospital admissions, were referred to DPYD testing. DPYD genotyping of four clinically most relevant SNPs (DPYD*2A, DPYD*13, DPYD 2846A>T and DPYD 1236G>A/Hap3) was performed on DNA isolated from whole blood using competitive allele specific PCR. DPYD genotyping confirmed DPD deficiency in 3 patients (50%), while in the other 3 patients DPD deficiency was not confirmed. Our results indicate that in DPD deficient patients serious events such as febrile neutropenia, thrombocytopenia, mucositis, diarrhea, vomiting and hand-foot syndrome could be avoided if they were treated according to pharmacogenomic recommendations, advising to reduce FP dose or to avoid treatment with FPs. Since additional hospitalization could also be avoided, this approach would be more cost effective. In addition, rarer variants in DPYD or other genes involved in FP metabolic pathways should be further investigated as a cause of FP toxicity.

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P-06.3-06
The role of catecholamines, melatonin, serotonin, and nitric oxide in the response of tissue microhemodynamics to low-intensity electromagnetic radiation of extremely high frequency

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The hypothesis of the study based on the fact, that the physiological mechanisms of the positive effect of low-intensity electromagnetic radiation of extremely high frequency (EMI EHF) on tissue microhemodynamics are associated with an increase in the level of catecholamines, melatonin, serotonin, and nitric oxide in blood serum. The aim of the study was to determine the dynamics of the content of catecholamines, melatonin, serotonin, and nitric oxide in the blood serum of rats after 10-fold EMI EHF. On the 10th day of the experiment, after a 10-fold session of EHF exposure (wave length 7.1 mm, power flux density from 4 to 10 mW/cm^2), experimental animals were slaughtered by decapitation on a guillotine. After that, the blood collected in vacuum tubes with a separation gel for serum. We used double centrifugation for 10 minutes at 1300 g at 25°C to obtain blood serum for research. Determination of the level of biogenic amines (epinephrine, norepinephrine, serotonin), melatonin, and nitric oxide was performed using enzyme immunoassay on an microplate photometer "Anthos 2010" (Biochrom, UK). We used five kits of reagents in our research (Cloud-Clone Corp., USA): ELISA Kit for 5-Hydroxytryptamine (5-HT), ELISA Kit for Epinephrine (EPI), ELISA Kit for Noradrenaline (NE), ELISA Kit for Melatonin (MT), Nitric Oxide (NO) Assay Kit (microplate format). After 10-fold EHF exposure, an increase in the concentration of epinephrine by 10.6% and norepinephrine by 8.0% found in the blood serum at the trend level. The concentration of serotonin in the blood serum of rats after 10-fold EHF exposure increased relative to the values in the control group by 12.9 % ($P \leq 0.05$), the concentration of melatonin – by 19.7% ($P < 0.05$), and the concentration of nitric oxide – by 27 % ($P \leq 0.01$). Thus, the hypothesis about the participation of melatonin, serotonin, and nitric oxide in the development of a positive effect on the microcirculation of 10-fold EHF exposure confirmed.

P-06.3-07
Opportunities of telomeres use as biomarkers in toxicology studies

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In toxicological studies of low-toxic objects as new sources of food, dietary supplements, some pharmaceuticals, a special attention is focused on the use of specific biomarkers that allow to identify the minimum negative impact. These biomarkers include indicators of the adaptive and protective systems such as the 1st and 2nd phases of xenobiotics metabolism, lysosome enzymes activity, antioxidant system enzymes and lipid peroxidation processes. The development of agro- and pharmaceutical industry leads to the appearance of new factors of chemical and biological nature that come into contact with humans. Hence the constant improvement of safety assessment approaches are the crucial point to the widespread adoption of novel technologies. The search for new biomarkers is one way to improve these approaches. Progressive research of recent years in the field of aging and cells proliferative activity suggests that a very promising and sensitive to many destructive factors biomarker may be a complex of telomere DNA, proteins and telomerase which protects a cell genome from degradation and participates in the regulation of proteins transcription in the near-telomere region. Possibility of this biomarker use should be confirmed in a complex of model experiments that aimed at (1) determining the background levels of telomere DNA, proteins and telomerase in the tissues and organs of laboratory animals, (2) research of external factors (alimentary, toxic, etc.) influence on these indicators, (3) choice of the most informative biomarkers (concentration of protein of telomere complex, relative length of hematopoietic cells telomeres, etc.) and methods of research (ELISA, PCR or flow cytometry method). This work was supported by the Ministry of Science and Higher Education of the Russian Federation (research project No. 0529-2019-0056).

P-06.3-08
Allelic polymorphism of SLC6A3 (DAT1), COMT and SLC6A4 (5HTT) genes as genetic risk factors for suicidal behavior

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According to WHO, more than 800 000 people die because of suicide each year. The evidence of the influence of genetic factors on suicidal behavior is provided by family, twins, and adoption studies. We investigated 11 polymorphisms of the 9 genes in patients who attempted suicide at least two times (n = 100) and the control group (n = 154): SLC6A3 (DAT1) 40 bp VNTR, DRD2 rs1800497, DRD4 120 bp in/del and 48 bp VNTR, COMT rs4680, SLC6A4 5-HTTLPR and rs25531, HTR1A rs6295, HTR2A rs6311, HTR1B rs6296, BDNF rs6264. The genotyping was carried out using locus-specific PCR and treatment with specific restriction endonucleases. Logistic regression, negative binomial regression, and odds ratios with the corresponding 95% confidence intervals were applied to analyze the data. We have first found an association between SLC6A3 (DAT1) 40 bp VNTR locus and suicidal behavior. This association was significant in the co-dominant ($P = 0.006$), dominant ($P = 0.001$), over-dominant ($P = 0.004$) and log-additive ($P = 0.004$) models, LL genotype played a protective role (OR = 0.48, 0.29–0.82). Difference in the genotypes distribution of COMT rs4680 was significant in the co-dominant ($P = 0.04$), dominant ($P = 0.013$) and log-additive ($P = 0.02$) models, AA genotype was associated with low risk of suicide (OR = 0.49, 0.26–0.91). We took into account the influence of two polymorphic loci (5-HTTLPR and rs25531) of the SLC6A4 gene on its expression and grouped alleles according to their expression levels: high (L_A) and low (S, L_G). Locus SLC6A4 5-HTTLPR+rs25531 was significant only in the recessive model ($P = 0.024$) and also affected the severity of symptoms of depression ($P = 0.044$) and personal anxiety ($P = 0.029$). Our results suggest that allelic variants of SLC6A3, COMT, and SLC6A4 genes might be considered as risk factors for suicidal attempts.

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P-06.3-09
A systematic review and meta-analysis reveal potential genetic risk factors for non-syndromic orofacial clefts in Caucasian populations of European ancestry

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Up to now, the roles of different genetic variants in the aetiology of non-syndromic orofacial clefts (nsOFC) have been inspected in numerous studies. Nevertheless, the obtained results are often inconsistent, particularly when studies are performed on populations of different races or geographic origins. Hence, we conducted this study to systematically review accessible literature and discover potential genetic markers for nsOFC, while focussing only on studies covering Caucasians of European descent. We identified eligible articles by searching two databases, Ovid Embase and Ovid Medline. Our search was limited to population-based case-control studies, from which we later extracted all the genetic markers that have been studied in relation to nsOFC. In instances, when the same marker was examined in more than one study, we conducted a meta-analysis using four different genetic models (the allelic, dominant, recessive, and overdominant). Furthermore, a stratified analysis was conducted to test association with different nsOFC subtypes. Effect estimates were expressed as pooled odds ratio (OR) with 95% confidence interval (CI) and *P*-values of 0.05 or less were regarded statistically significant. Altogether 84 studies met our inclusion criteria and were included in a systematic review. More than 700 genetic markers, distributed all over the human genome were extracted. 52 studies (out of 84) were included in a subsequent meta-analysis, in which 49 genetic markers in 32 genes/loci were analysed. We recorded statistically significant results for 22 genetic markers in 15 genes/loci (8q24, ABCA4, AXIN2, DVL2, FOXE1, GREM1, GRHL3, IRF6, MTHFD1, NOG, RARA, TGFA, VAX1, WNT3A, WNT5A). Our findings suggest that markers in these genes/loci have a huge potential as biomarkers of the disorder in Caucasian populations of European ancestry. We performed the first complete systematic review and meta-analysis of all genetic variants studied in relation to nsOFC in Caucasians.

P-06.3-10
Analysis of mitochondrial DNA mutations in patients with epilepsy

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Mitochondrial dysfunction and oxidative stress are defined as factors that play a crucial role in the pathogenesis of a number of neurological diseases, including epilepsy. Epilepsy is the main phenotypic symptom of some syndromic mitochondrial disorders such as Lee syndrome, Alpers-Huttenloher syndrome, MELAS and MERRF. The aim of this study was to identify mitochondrial DNA mutations associated with the development of epilepsy in patients with burdened family histories and clinical symptoms of mitochondrial syndromes. The whole mitochondrial genome sequencing on Illumina MiSeq platform was performed for 5 epilepsy patients and 12 conditionally healthy individuals, which corresponds to the case cohort by age, sex and ethnicity. To verify the epilepsy type, the electroencephalography, magnetic resonance imaging of the brain and biochemical blood analysis were evaluated. In comparison with reference mtDNA nucleotide sequence, a total of 183 variations were found in the control group and 115 in epilepsy patients. We analyzed each variation that could be associated with the disease, and studied statistically significant variants. An analysis of functional significance indicates that 13 mutations may be associated with the development of epilepsy, 7 of which (MT-HV2 A183G, MT-RNR2 A1736G, MT-ND1 G3697A, MT-tRNA Ala T5628C, MT-ND3 G10310A, MT-ND4L T10609C, and one analogous variant of MT-ATP6 C8794T, which, according to the MITOMAP database, was associated with the development of epilepsy) were detected only in the experimental group. It should be noted that 2 mutations (MT-CYB C14854T and MT-ND3 C10095A) and 1 deletion at the MT-DLOOP regulatory locus (16180_16181del) were found for the first time in patients with epilepsy and were not previously annotated in the literature. To further assess the contribution of mitochondrial DNA dysfunctions to the development of epilepsy, studies involving more patients and proper analysis of mitochondrial DNA mutations are needed.

P-06.3-11
Genetic variability in pathways associated with Alzheimer's disease: a comprehensive review

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Introduction: Alzheimer's disease (AD) is the most common neurodegenerative brain disease affecting the cerebral cortex and hippocampus. Accumulation of insoluble amyloid-β (Aβ) plaques in extracellular spaces, as well as in the walls of blood vessels, and aggregation of the microtubule protein tau in neurofibrillary tangles in neurons are important hallmarks of the disease. Late-onset AD (LOAD) cases comprise the vast majority of all AD patients (>90%), with the typical age of onset 65 years or more. Although LOAD is not as strongly linked to familial background

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as early-onset AD, complex genetic and environmental interactions have been associated with risk for AD even late in life. Our aim was to find genetic factors associated with AD risk based on published data and determine underlying molecular pathways to enable identification of potential biomarkers of disease susceptibility and disease prognosis. Methods and results: We performed a literature synthesis of genome wide association studies (GWAS) and their meta-analyses that investigated AD susceptibility. 109 loci involved in different cellular pathways were associated with AD risk and 26 additional loci were associated with biomarker levels in CSF or brain tissue abnormalities. Functional polymorphisms in identified genes were grouped into six pathophysiological pathways: APP and tau processing, vesicle mediated transport, endocytosis and cytoskeleton; lipid metabolism; energy metabolism; inflammation; mechanisms involved in transcription, translation, post-transcriptional and post-translational modification; signaling and transport activity. Conclusion: GWAS and meta-analyses enabled us to identify key pathways of AD pathogenesis. Our comprehensive review revealed functional SNPs that could play a role in AD progression and could serve as novel genetic biomarkers of AD. These SNPs should be further investigated in studies of AD susceptibility, disease progression and early diagnostics.

P-06.3-12
Catalase and paraoxonase 1 polymorphisms are associated with late adverse events of radiotherapy in breast cancer

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Introduction: In early breast cancer patients, treatment with radiotherapy enables good long-term local control and survival. However, some patients experience adverse events that can affect treatment protocol and quality of life. As radiation leads to increased production of reactive oxygen species and oxidative stress, antioxidant enzymes and their genetic variability can contribute to the interindividual variability in occurrence of adverse events. Our aim was therefore to evaluate the association of polymorphisms in antioxidant genes with late adverse events of breast cancer radiotherapy. Materials and Methods: Our study included 101 HER2-positive early breast cancer patients treated with adjuvant radiotherapy. We genotyped all patients for six common polymorphisms CAT rs1001179, GSTP1 rs1138272, GSTP1 rs1695, SOD2 rs4880, PON1 rs854560 and PON1 rs662 using competitive allele-specific PCR. Association of polymorphisms and haplotypes with adverse events was evaluated using logistic regression. Results: In total, 33 (32.7%) patients reported grade ≥2 late adverse events according to LENT-SOMA criteria, while 12 (11.9%) patients experienced grade 2 skin toxicity according to CTC criteria. Late adverse events grade ≥2 were significantly more common in patients with at least one polymorphic paraoxonase 1 (PON1) rs854560 allele (OR = 3.03; 95% CI = 1.07–8.53, $P = 0.036$) and carriers of at least one polymorphic catalase (CAT) rs1001179 allele (OR = 2.71; 95% CI = 1.00–7.33, $P = 0.049$) after adjustment for clinical variables. Compared to the most common reference PON1 TA haplotype, carriers of AA haplotype experienced less late adverse events, but the difference did not reach statistical significance (OR = 0.46; 95% CI = 0.21–1.03, $P = 0.059$). Conclusions: Genetic variability of antioxidant genes was associated with more pronounced adverse events of radiotherapy and could contribute to the personalization of radiotherapy in breast cancer patients.

P-06.3-13
Analysis of polymorphisms of DNA repair genes in people exposed to residues of unutilized outdated pesticides of Almaty Region

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Environmental pollution by persistent organic pollutants (POPs) is one of the global environmental problems, which is closely related to health issues, as POPs adversely affect the human body, having a genotoxic, immunotoxic and carcinogenic effects, creating a real threat to the health of present and future generations. It is known that significant, constant and uniform pesticide contamination of agricultural areas significantly increases the frequency of chromosome aberrations (CA). There is evidence that mutations in DNA repair genes are directly proportional to the number of CA in lymphocytes. Our studies are aimed at analyzing the association between the number of chromosomal abnormalities and the polymorphism of DNA repair genes for pesticide toxicity. We examined 172 people living in close proximity to the centers of localization of unused pesticide reserves in rural areas of the Almaty Region (Kazakhstan). Based on our data on cytogenetic analysis, we divided people into a control group with a spontaneous level of CA (0–2% aberrations) and an experimental group – people with a CA level above 2%. DNA excision repair genes were selected as candidate genes XRCC1 Arg399Gln, XRCC1 Arg194Trp, XRCC3 Thr241Met, XPD Lys751Gln. The polymorphisms were examined by PCR-RFLP. Analysis of the distribution of alleles and genotypes of polymorphisms of the XRCC1Arg399Gln, XRCC3 Thr241Met, XPD Lys751Gln genes did not reveal statistically significant differences ($P > 0.05$). An exception is the XRCC1 gene polymorphism Arg194Trp. For this polymorphism, an increasing negative effect of the 194Trp allele was found in people with an increased level of CA. According to the dominant model of inheritance, people with a CA level above 2% show a 4-fold increase in the homozygous mutant allele (194Trp) genotype (OR = 4.06, $P = 0.02$), which indicates violations of the repair system in residents exposed to pesticides. The work was supported by Scientific Program: No. BR05236379.

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P-06.3-14
Human and horse milk extracellular vesicles: proteins and peptides

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Extracellular vesicles are secreted in milk by breast cells. Exosomes are vesicles with a diameter of 40–100 nm and contain CD9, CD63, and CD81 tetraspanins on the surface. Horse milk is a unique source of exosomes since is less allergenic and not prion-prone comparing to bovine milk and can be obtained in larger amounts than human milk. Different nucleic acids (plasmid DNA, microRNA) and drugs can be delivered in cells with milk vesicles. According to the literature data, milk exosomes present hundreds and thousands of proteins, mRNA, and microRNA molecules. Our recent findings show that the results on the protein and nucleic content in milk exosomes are significantly overestimated. Isolation of exosomes with an additional step of gel-filtration allows us to decrease the number of proteins, that co-isolate with vesicles. Here we show the content of proteins and nucleic acids in human and horse milk exosomes before and after gel-filtration of sediment obtained after ultracentrifugation. According to these data, human and horse milk exosomes may contain only a few major proteins with well-known biological functions and just several dozens of different microRNA. Our data confirm that horse milk exosomes can be used for the delivery of biologically active molecules to cells in vitro since their protein and nucleic acid content is highly similar to exosomes obtained from human milk. The study was funded by the Russian Scientific Foundation (research project 18-74-10055 to S. Sedykh).

P-06.3-15
Angiogenic factors and miRNA as diagnostic markers

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Endometriosis is an estrogen dependent gynaecology condition and almost 7–10% of women in reproductive age are affected by this pathological condition that can complicate pregnancy and cause the infertility. A novel research data considers a combination of pro/angiogenic factors that play an important role in angiogenesis during endometriosis and endometrial cancer development. The aim of presented work was detection of expression changes of selected miRNA molecules, of mRNA and proteins levels of specific pro/anti angiogenic factors of patients with different stages of endometriosis (n = 22) and endometrial cancer of corpus uteri (n = 13) compared with the control group (n = 63). Expression of individual genes was detected by qRT-PCR and the results were compared with the control group. The enzyme-linked immunosorbent assay were used for detection of proteins level. Expression levels of specific miRNA molecules were different, and depend on the differential disease of the patients. The mRNA level for sEng was about 273% higher in patients with-frozen pelvis and about 126% higher in patients with peritoneal endometriosis than in controls. We analysed significantly the hights level of Endoglin protein of patients with endometriosis. Similarly, ROC analysis showed that Endoline protein level could distinguish patients with endometriosis versus patients with endometrial carcinoma of the corpus uteri with a sensitivity of 92% and 67% of AUC specificities of 0.801 (0.625–0.977): $P = 0.01$. The Notch 3 protein could differentiate uterine endometrial cancer from a control group with 61% sensitivity and 75% AUC specificity of 0.792 (0.634–0.951) $P = 0.01$. Study of the specific markers expresion is highly current topic, can help understand of the mechanisms and can lead to the development of new diagnostic and therapeutic applications in monitoring and treatment of patients with endometriosis. This work was supported by grant project VEGA 1/0620/19

P-06.3-16
Cooperative interaction of dimeric enzyme prostaglandin H synthase with substrate – arachidonic acid

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Prostaglandins are regulators of inflammation, immune response and many other physiological processes. Synthesis of prostaglandins is a result of cyclooxygenase oxidation of arachidonic acid (AA), which is catalyzed by homodimeric enzyme prostaglandin H synthase (PGHS). PGHS is irreversibly inactivated during catalyzed reaction and inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs). There was some evidence of cooperative interactions between enzyme subunits, but cooperativity of AA binding to the active sites was not investigated sufficiently. In this study, we used the PGHS-1 isoform purified from sheep vesicular glands. The cyclooxygenase reaction was detected amperometrically from the consumption of dissolved molecular oxygen. Obtained data were analyzed using Origin and MATLAB. It was shown that the dependence of cyclooxygenase oxidation rate in the wide range of AA concentrations is not described by Michaelis-Menten equation. However, it can be described by model taking into account the negative cooperativity of AA binding to PGHS subunits (the enzyme affinity to the first AA molecule is 14 μM, and the affinity to the second AA molecule is 1 mM). The investigations of integral kinetics of cyclooxygenase reaction demonstrated that total enzyme turnover number (from the beginning of the reaction until complete inactivation) decreases with the increase of AA concentration. The experimental dependences were described by cooperative model, and the values of kinetic constants were found. Obtained results should be taken into account during the investigation of prostaglandin synthesis and pharmacological effects of NSAIDs *in vivo*. The reported study was funded by RFBR according to the research project № 19-04-01150a. It was carried out using the equipment purchased via the Moscow State University Development Program and the equipment of the shared research facilities of HPC computing resources at Lomonosov Moscow State University.

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P-06.3-17
Correlations between the total antioxidant activity and mineral components of pig blood serum

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The study of the physiological and biochemical (including antioxidant) status of the organism of productive animals is an important for evaluation of animal health. Using various methods, it is possible to estimate the concentration of various activated oxygen metabolites, the content of antioxidants in tissues, etc. The amperometric detection method (used by us) gives a general estimation of the total amount of water-soluble antioxidants (TAWSA); however, the relationship of TAWSA with specific biochemical parameters and antioxidant defense factors has been relatively little studied. Our task is assessing the TAWSA values and the content of some macronutrients in the blood serum of fattening pigs (from 65 to 101 days), as well as their relationship. The following values of TAWSA (mg/L); Ca (mM/L); Mg (mM/L) were obtained: 9.58 ± 1.28; 2.68 ± 0.10; 1.30 ± 0.08 (65 days of feeding), 7.68 ± 0.31; 2.48 ± 0.04; 1.15 ± 0.02 (72 days), 15.19 ± 0.60; 2.35 ± 0.07; 1.14 ± 0.05 (84 days), 14.66 ± 0.52; 2.78 ± 0.05; 0.93 ± 0.02 (89 days), 19.63 ± 1.61; 2.61 ± 0.08; 0.95 ± 0.02 (91 days); 19.16 ± 0.92; 2.37 ± 0.13; 0.97 ± 0.02 (100 days); 16.48 ± 1.48; 2.60 ± 0.07; 0.78 ± 0.11 (101 days), respectively. The correlation coefficients of TAWSA with the blood levels of calcium and magnesium were calculated: 0.326 and -0.643 (65 days of fattening), -0.069 and 0.335 (72 days), 0.436 and -0.062 (84 days), 0.204 and -0.299 (89 days), -0.976 and 0.768 (91 days), -0.599 and -0.319 (100 days), -0.987 and 0.998 (101 days). The study of the correlations between biochemical parameters of blood and indicators of the state of the antioxidant system of the fattening pigs was carried out for the first time. These data will contribute to the clarification of the mechanisms of the antioxidant activity in pig tissues and serve as a theoretical basis for the development of practical methods of pig fattening. The study was supported by a grant from the Russian Science Foundation (project RSF 20-16-00032). *The authors marked with an asterisk equally contributed to the work.

P-06.3-18
Possible effects of black cohosh extracts on estrogen levels in postmenopausal women

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Menopause is associated with the onset of major health problems and vasomotor symptoms occurring in 80% of peri and postmenopausal women. As a substitute to hormone replacement therapy (HRT), phytochemicals are increasingly offered to alleviate symptoms associated with menopause. For example, black cohosh root extracts (Remifemin®, Klymadinon®) are widely available. The aim of our pilot study is thus to evaluate systemic effects of black cohosh extracts by determining concentrations of estrogens and estrogen metabolites in blood samples from postmenopausal women before and after treatment. The collected serum samples will be analyzed for levels of follicle-stimulating hormone to confirm menopause and for concentration of 27 steroids mainly estrogens and catechol estrogens, but also adrenal precursors and androgens using validated methods. Gas-chromatography coupled to mass spectrometry (MS) will be used to quantify levels of DHEA, androsterone, androstenedione, testosterone, dihydrotestosterone, estrone and estradiol, while liquid chromatography tandem MS will be used for conjugated steroids, sulfates and glucuronides and for measuring catechol estrogens. Blood samples were taken before and after 2–3 month treatment with Remifemin® following a strict standard operating procedure for sample collection, processing and storage. All relevant clinical and life style data and information about menopausal symptoms of the enrolled women were gathered using validated questionnaires. To the best of our knowledge this is the first study that will examine the potential systemic effects of black cohosh extracts on the concentrations of steroid precursors, estrogens and estrogen metabolites in blood samples of postmenopausal women. We expect that the results of our study may reveal preventive or promoting effects of these extracts for development of hormone dependent diseases. The project is funded by the Slovenian Research Agency grant J3-8212 to T.L.R.

P-06.3-19
Efficiency evaluation of an anticancer recombinant targeted toxin based on DARPin and truncated exotoxin A

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Targeted therapy is a rapidly developing approach in cancer treatment. Here we focus on characterization and efficiency evaluation of targeted toxin DARPin-PE40, designed for treatment of HER2-overexpressing tumors. HER2 overexpression is common for various carcinomas and is associated with poor prognosis and low treatment outcome. DARPin-PE40 is comprised of non-immunoglobulin scaffold protein DARPin_9-29 which is targeted to HER2 and a fragment of exotoxin A of Pseudomonas aeruginosa. DARPin-PE40 binding to HER2 is followed by its internalization into the cell and protein synthesis arrest via exotoxin A activity, namely irreversible inactivation of eEF2. DARPin-PE40 coding plasmid vector was designed and then used for DARPin-PE40 expression in E. coli BL21(DE3) strain followed by purification by HPLC. DARPin-PE40 is shown to enter cells in vitro and undergo retrograde trafficking to EPR similarly to wild-type exotoxin A. Specific toxicity of DARPin-PE40 is manifested at picomole concentrations in monolayer culture in vitro and is determined by induction of apoptosis. In 3D models in vitro (tumor spheriods) DARPin-PE40 efficiency is reduced, which might be due to its limited penetration (~50 um) into the depth of the spheroid. Antitumor efficacy of DARPin-PE40 in vivo was evaluated in immunodefficient tumor-bearing mice and manifested as a pronounced tumor growth inhibition at doses of 25, 50 and 80 ug per mouse with TGI values of 48%, 66% and 94% respectively by the end of the experiment. No signs of acute toxicity were registered. Thus DARPin-PE40 can be considered as an effective antitumor agent and its further study and improvement is relevant. This work was supported by

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P-06.3-20
Sample preparation for metal analysis from serum using ICP MS

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The analysis of metal ion content in human biological fluids provides ample information, which can be used for evaluation of general health status as well as for predicting and preventing diseases. Inductively coupled plasma mass spectrometry (ICP MS) enables quantitative and fast analysis of many elements in a single run and is a method of choice. However, multielement analysis of human blood is still very challenging, due to highly variable concentration levels of different metal ions and a lack of unified sample preparation protocol. We compared several acidic and alkaline sample digestion methods proposed in the literature by using ClinCheck serum trace element samples (ClinCheck Serum Control, L2, Recipe, Germany) and Agilent 7800 ICP MS instrument coupled with SPS4 autosampler. After careful analysis, we elaborated a reliable acid- and heat-based method for metal content analysis from blood serum. The stability of the acid-digested samples and the intra-day variability of the results was confirmed. The method is applicable to human serum samples. The advantages of our method is low intra-day variability and the possibility to analyze abundant and trace metals in the same run.

P-06.3-21
Biomarkers in sport medicine: prevention of infections, inflammations, injuries and cardiovascular diseases

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Moderate physical activity is considered protective factor against cardiovascular disorders, muscle injuries, dysregulation of immune system, infections and oxidative stress. In this scenario, our study aimed to examine the responses of 15 basketball players during training, competition and recovery, considering the immune system; examining: (a) mucosal immunity [alpha and beta-defensins (HNP1 and HBD-1)], (b) muscle metabolism, such as cortisol and testosterone, (c) oxidative stress, by glutathione (GSH and GSSG) and γ-glutamyl-transpeptidase (GGT). First, we measured the levels of HNP1 and HBD-1 in the serum of athletes by ELISA assay; showing that the levels of both defensins increased in proportion to the intensification of physical activity; most likely the increase of these antimicrobial peptides consists of a sort of self-defense mechanism that is activated in the athletes, protecting them own body from any external attacks caused by pathogenic microorganisms. Then, we evaluated cortisol and testosterone through Pearson's regression. These hormones increase between 0 and 6 months of physical activity. These data show a perfect balance between anabolic and catabolic muscle metabolism. Finally, we dosed the GSH and GSSG (reduced and oxidized glutathione) in the serum of athletes, correlating their concentration with the main enzyme GGT involved in the metabolism of GSH. In this case, we showed that the levels of GGT in the serum of athletes are normal, underlining that the liver function of athletes is not compromised and that the metabolism of GSH works perfectly despite intense physical activity. The increase in GSH in athletes' serum and the simultaneous reduction in GSSG helps athletes fight the build-up of ROS (oxygen free radicals) . In conclusion, we shed light on the role of human defensins, hormons and GSH as self-defense molecules during a continuous stressor such as long-term exercise, and recognize them as potential markers of athlete's diseases.

P-06.3-22
Variation in the gamma-glutamyltransferase 6 gene: relation with body mass index and risk of type 2 diabetes

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Glutathione (GSH) is a key regulator of the redox homeostasis. Gamma-glutamyltransferase 6 (GGT6) is one of the enzymes of glutathione metabolism that provides cysteine for intracellular de novo synthesis of GSH. The aim of this work was to study the associations of polymorphic variants rs11657054 (A>G) and rs2100986 (T>C) in the GGT6 gene with parameters of glucose and redox homeostasis of blood plasma and the risk of T2D in residents of Central Russia. The study included 1562 patients with T2D (584 men and 978 women, mean age 61.1 ± 9.7 years) and 1575 healthy volunteers (590 men and 985 women, mean age 60.5 ± 6.3 years). Genotyping of the GGT6 gene polymorphisms was performed by matrix-assisted laser desorption / ionization method on a MassArray Analyzer 4 genomic time-of-flight mass spectrometer (Agena Bioscience). Analysis of the parameters of redox homeostasis revealed an increase in the content of hydrogen peroxide and a decrease in the level of total glutathione in the plasma of patients with T2D compared with the control group ($P < 0.05$). Correlation analysis revealed that the level of plasma hydrogen peroxide in patients is directly proportional to the concentration of their fasting blood glucose (rs = 0.54, $P < 0.05$) and inversely proportional to the level of plasma glutathione (rs = -0.41, $P < 0.05$). We have also established for the first time the associations of genotypes A/G-G/G rs11657054 (OR1.19, 95CI 1.01–1.40, $P = 0.035$) and genotypes T/C-C/C rs2100986 GGT6 (OR1.22, 95CI 1.03–1.44, $P = 0.019$) with an increased risk of T2D exclusively in patients with body mass index >25 kg/m^2. Our study in the Russian population revealed the association of SNPs rs11657054 and rs2100986 in the introns of the GGT6 gene with an increased risk of T2D, thereby demonstrating the potential involvement of the GGT6 gene in the pathogenesis of the disease. The study was supported by Russian Science Foundation (№20-15-00227). *The authors marked with an asterisk equally contributed to the work.

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P-06.3-23
The method of quantitative determination of circulating microRNA in blood of athletes in doping control

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MiRNAs are promising markers of peptide abuse in sports. The development of a method for the quantitative determination of circulating miRNA in the blood of athletes will significantly increase the effectiveness of doping control. Blood cells are one of the sources of circulating miRNAs. Changes in the cell formula or hemolysis significantly change the level of circulating miRNAs. Using reverse transcription and QX100 ™ DropletDigital ™ PCR System, Bio-Rad, we have shown an increase in the expression of circulating miR-144 after a single injection of erythropoiesis stimulator Mircera® from the Prohibited List of WADA. Blood samples were taken from 5 volunteers before the use of Mircera® (control), 24 hours after the use of Mircera® and then every 7 days until the 28th day. MiRNAs were isolated from 2 mL of blood plasma. Reverse transcription and PCR were performed using Applied Biosystems kits, including TaqMan MicroRNA Assay hsa-miR-144-3p. miR-144 showed a gradual 5–12-fold increase in expression by the 28th day after Mircera® injection compared with the control sample (FDR < 5%; $P < 0.0005$) in all volunteers. For comparison, hematological biomarkers (measured on a SysmexXN-1000, Germany), such as the percentage of reticulocytes, showed a peak at 6–8 days and a return to baseline 16 days after injection, and the hemoglobin concentration was practically not changed. These data also suggest that circulating miR-144 may be more sensitive indirect biomarker than current hematologic parameters. Considering the fact that miR-144 is an important agent of erythropoiesis in various organisms, these results suggest the possibility of using miR-144 as a sensitive and informative biomarker for detecting abuse of Mircera® and, presumably, other erythropoiesis stimulants. We were able to detect a change in the expression of miR-144 28 days after the injection of Mircera®. And it is likely that the detection window for this marker can be longer.
*The authors marked with an asterisk equally contributed to the work.

P-06.3-24
The contribution of Fanconi anemia genes mutations in breast cancer development

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A defect of the DNA repair system underlies genomic instability in breast cancer (BC) patients. While the BRCA1/2 are well described BC susceptibility genes, involved in DNA damage recognition and repair (DDR) pathways, their molecular and functional interactions remain insufficiently explored. In this case, the study of rare genetic diseases may bring new findings of biomarkers for predicting the development and sensitivity of tumors. Fanconi Anemia (FA) is caused by the pathogenic germline mutations in several genes known as FA genes which encode proteins involved in DDR. Recent studies revealed, that FA proteins interact with BRCA1/2 to maintain resistance to DNA crosslinks. Thus the aberrations in these genes may indicate sensitivity to inhibitors of DNA repair or chemotherapy with DNA crosslinking agents. We performed targeted NGS in a cohort of 630 BC patients using the Illumina MiSeq and implemented a targeted panel, which included 34 genes related to hereditary cancers. For effective large-scale bioinformatic analysis, we developed a cloud platform, which provides automated annotation and interpretation for targeted sequencing panels. 39 (6,1%) patients had heterozygous mutations in FA genes. Among these variants 30,5% were pathogenic, and 69,5% had uncertain significance. All variants had strong susceptibility with BC, the combined odds ratio of having a mutation was 27,3 (95% CI 1.25–4.64; $P = 0.002$) Six patients carried complementary pathogenic mutations in BRCA1/2, XRCC2, or MLH3 genes. The most frequent mutation was c.712_714del in FANCL (26 cases). This deletion was located in the UBC-RWD domain, which mediates interaction with FANCI and FANCD2. FANCI gene contained two other frequent mutations c.3721G>A and c.3853C>T (13 and 6 patients respectively). Both missense mutations are presented in the Solenoid 4 domain. Such molecular aberration in FANCD2/FANCI disrupts the ubiquitination of this complex and deactivates the downstream FA molecules.

P-06.3-25
Antibody microarrays for detection of peritoneal endometriosis biomarker candidates

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Endometriosis is a complex inflammatory gynecological disease that affects around 190 million women worldwide. Since symptoms of endometriosis are highly heterogeneous, patients are often misdiagnosed. Earlier diagnosis and treatment of patients could be achieved with discovery of non-invasive diagnostic biomarker for endometriosis. Compared to other proteomics-based methods, antibody microarrays enable simultaneous and fast measurement of large number of proteins from different sample types. The purpose of this study was to discover novel plasma biomarker candidates for peritoneal endometriosis using antibody microarray platform. The plasma samples were collected from 24 patients characterized with the absence (controls, n = 12) or presence of peritoneal endometriosis (cases, n = 12). Patients were further divided according to their menstrual cycle phase into secretory and proliferative group. Collected plasma samples were labelled with two fluorescent dyes and anaylsed in a dual-colour approach on eight scioDiscover antibody microarrays (Sciomics®) targeting 1360 different proteins with more than 1830 antibodies. Proteins were defined as differential between sample groups for log-fold changes (logFC) >0.5 and adjusted P value < 0.01. In secretory phase group, 14 proteins were found to be upregulated (5 of them with logFC >1.5) and 3 were downregulated in cases compared to the controls. In proliferative phase group, only 3 proteins were downregulated (logFC < -1.2) in cases versus the controls. A non-metric multidimensional

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scaling and hierarchical cluster analysis revealed a clear separation between cases and controls in both group of patients. String analysis exhibited involvement of differential proteins in biological processes like apoptosis, inflammation and cell adhesion. Using antibody microarray platform, we were able to identify several potential biomarker candidates for peritoneal endometriosis and these should be validated in a larger number of patients.

P-06.3-26
Identification of novel glioblastoma biomarkers for non-invasive liquid biopsy

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Glioblastoma is the most common and lethal form of brain tumor. Glioblastoma is usually diagnosed with imaging (CT, MRI), complemented with histopathological examination of biopsy sample and determination of molecular biomarkers. However, sampling of tumor tissue does not capture the entire tumor heterogeneity and requires invasive surgery. In addition, biomarkers for early disease diagnosis and treatment follow-up are still unavailable. Therefore, we aim to determine expression changes of circulating blood biomarkers from plasma of glioblastoma patients compared with healthy individuals. Selected biomarkers ALYREF, DPYSL2, FREM2, TUFM, TRIM28, VIM, CRMP1, SPRY1 and NAP1L1 were identified as differentially expressed in high grade compared to lower grade gliomas and reference non-tumor brain samples, previously published in Jovcevska et al. Oncotarget. 2017 44141-58. In this study, we will examine their utility as non-invasive liquid biomarkers. Firstly, we will isolate cfRNA and proteins from exosomes of 50 glioma patients and 50 healthy individuals. Next, we will proceed with determination and validation of the selected biomarkers at gene expression and protein levels. Gene expression levels will be determined with digital droplet PCR due to the high sensitivity of the method which is crucial for biological samples where the amounts of the RNA of interest are limited. At protein level, we will study the expression of proteins with western blot. We will also evaluate their potential role as biomarkers for treatment follow-up by monitoring their gene and protein expression levels in blood samples from 8 glioblastoma patients undergoing standard treatment. At last, to obtain broader panel of putative liquid biomarkers, we will create a mRNA-lncRNA-miRNA coexpression network. With this research we aim to provide accessible biomarkers for non-invasive liquid biopsy that can be used for early glioblastoma detection and, potentially, successful treatment monitoring.

P-06.3-27
Biochemical parameters of bone tissue in violation of carbohydrate metabolism

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Implantation is a modern replacement for traditional dentures. However, implantation is unsafe for patients with impaired carbohydrate metabolism since hyperglycemia increases the risk of rejection and complications. In this regard, it is important to use convenient and affordable methods of clinical screening for patients before implantation in order to identify risk factors. 50 men (average age 58 years) were examined before dental implantation. The main group was patients with type 2 diabetes, the control group was conditionally healthy. Patients were interviewed to identify risk factors. The laboratory examination included a biochemical blood test, indicators of bone metabolism (alkaline phosphatase, calcitonin, parathyroid hormone, osteocalcin and vitamin D). According to the results of the survey, patients with diabetes mellitus were more often to have a tendency to bleed when removing teeth, bleeding gums, the presence of bone operations, rashes on the oral mucosa and inflammation in the maxillofacial region. The main cause of tooth loss in both groups was dental caries. Fasting blood glucose and glycated hemoglobin were 4.6 mmol / l and 5.3 % in the control group and 9.2 mmol / l and 8.4% in the main group. The results of laboratory analysis showed that there was a statistically significant increase ($P < 0.05$) in alkaline phosphatase by 26%, calcitonin by 255%, parathyroid hormone by 36% and a decrease ($P < 0.05$) in vitamin D by 23 % in the group with type 2 diabetes mellitus. The osteocalcin parameters did not undergo significant changes. The data confirms the link between type 2 diabetes and diabetic bone disease. It can be used further to develop clinical screening for patients before implantation in order to identify risk factors. The studies were performed in accordance with the research plan for 2019–2021 of the Federal Research Center of Biological Systems and Agrotechnologies RAS No. 0526-2019-0001.

P-06.3-28
Potential new biomarkers for assessing the effects of manganese on the body

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The prenatal period is critical for the development of the nervous system. Despite the fact that manganese is an essential element, excessive exposure to this element can lead to negative postnatal consequences. The study examined the prenatal effect of manganese sulfate on the concentration of serum arginase I (ARG1) and manganese superoxide dismutase II (SOD2) in two generations. For this purpose, non-pregnant Wistar rats received a basic diet with the addition of $MnSO_4$ at a dose of 1433 mg/ kg/day for 28 days before gestation and during gestation (20–26 days). The assay was performed using the Rat ARG1 (Arginase I) ELISA Kit (Elabscience, USA) and Rat SOD2 (Superoxide

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Dismutase 2, Mitochondrial) ELISA Kit (Elabscience, USA) in accordance with the instructions. The obtained data were processed using Statistica 10.0 software (StatSoft, USA). According to the results of the study, the levels of ARG1 and SOD2 in females were statistically significant higher relative to the control group by 145% and 111%, respectively. The ARG1 level of the first generation of animals was statistically significant higher than the control values by 14%, but lower than in females rats exposed to direct oral exposure to metal. The ARG1 level of the second generation of animals was close to the control values. The level of SOD2 in animals of the first and second generation did not change. Thus, the study demonstrated that prenatal exposure to manganese sulfate can affect the concentration of arginase in the blood serum not only in the mother's body, but also in the generation. The concentration of this protein in the blood serum may serve as a screening biomarker for the neurotoxicity of manganese and the assessment of the risk of exposure to this metal on the body. The studies were performed in accordance with the research plan for 2019-2021 of the Federal Research Center of Biological Systems and Agrotechnologies RAS No. 0526-2019-0001.

P-06.3-29
Holistic metabolomic laboratory-developed test for the diagnosis of Parkinson's disease

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Metabolomics studies have demonstrated the possibility of using the metabolite sets for the successful diagnosis of many diseases and consequent development of new methods for precision medicine. A laboratory-developed test (LDT) is a type of *in vitro* diagnostic test that is developed and used within a single laboratory and usually is based on measuring of a wide range of substances. To date, numerous LDTs have been developed for the diagnosis of various diseases and can be considered as the most evident solution for implementation of metabolomics into the clinic. The holistic metabolomic LDT integrating the currently available data on human metabolic pathways, changes in the concentrations of metabolites, and modern data processing algorithms for analysis of panoramically measured blood composition data was developed. In this study, the LDT using the high-resolution mass spectrometry with data processing by in-house software was applied for diagnosing of early-stage Parkinson's disease (PD). The early laboratory diagnosis of PD is currently unavailable and urgently needed for effective therapy. Using the LDT for blood plasma samples, the diagnosis of PD based on diagnostic score reached an accuracy of 73%. The diagnosis was based on the detection of overrepresented metabolite sets such as disease-associated, pathway-associated, associated with abnormal concentrations of metabolites, and location-based. Mainly the overrepresented metabolite sets were relevant to PD and other neurodegenerative diseases. The developed LDT demonstrates the current capabilities of metabolomics for diagnostic and precision medicine. This study was supported by the Russian Foundation for Basic Research (grant № 19-29-01125 "A metabolomic analysis of biochemical disorders in the prodromal stage of Parkinson's disease for a systematic assessment of the risk of disease and personalization of pharmacotherapy"). Previously published in: Lokhov PG et al. (2021) Metabolites 11(1), 14.

P-06.3-30
Fabry nephropathy: a search for urinary biomarkers

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Fabry nephropathy has an important impact on morbidity and mortality in Fabry disease (FD). Current biomarkers are associated with late signs of kidney damage, but they do not predict Fabry nephropathy progression. Urinary extracellular vesicles (uEVs) are secreted by cells lining the urinary tract and have not been studied in FD so far. Our aim was to evaluate the association of uEVs and their cargo as possible early biomarkers of Fabry nephropathy. Small uEVs were isolated by size exclusion chromatography from two urine samples per FD patient (n = 21) obtained 5 years apart. We used nanoparticle tracking analysis to determine uEVs size and concentration. We analysed the expression of seven uEVs miRNAs using miRCURY LNA miRNA PCR Assays, two of which served for normalisation. uEVs concentration, size, and expression of miR-200a-3p, miR-29a-3p, miR-30b-5p, miR-23a-3p, and miR-34a-5p did not differ significantly between patients with and without Fabry nephropathy at last follow-up. However, expression of uEVs miR-200a-3p and miR-29a-3p differed significantly between chronological samples ($P = 0.013$ and $P = 0.011$, respectively). These differences were no longer significant among patients without Fabry nephropathy. However, when analysing only patients with Fabry nephropathy, the concentration of EVs was significantly different ($P = 0.015$) in addition to the above miRNAs ($P = 0.021$ and $P = 0.028$, respectively). In patients with Fabry nephropathy, uEVs concentration decreased, while the relative expression of miR-200a-3p and miR-29a-3p increased in the 5-year period. uEVs miR-200a-3p and miR-29a-3p may represent candidate biomarkers of renal function in FD. Further studies are needed to confirm this association. *The authors marked with an asterisk equally contributed to the work.

P-06.3-31
Influence of the missense Ile337Met mutation on the functioning of the cardiac Kv7.1 channel

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More than 80 drugs may cause drug-induced LQTS. All new therapeutic substances should be tested for their ability to Influence cardiac repolarization. Even asymptomatic individuals carrying functional genetic variants might be at risk of the life-threatening ventricular events taking common and otherwise efficient medication. We performed a functional study of the rare missense mutation c.1011C>G (p.Ile337Met) in the KCNQ1 gene identified in a patient with asymptomatic QT interval

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prolongation. We artificially inserted this substitution into the plasmid encoding Kv7.1 channel and expressed the mutant gene in CHO-K1 cells. We used a comprehensive approach involving the study of the integral IKs current using the standard whole-cell patch-clamp. The peak tail current transferred by mutant Kv7 channel was significantly (one-way ANOVA, $P < 0.05$) smaller in amplitude after depolarization to potentials from 20 to 60 mV, than the control current. The activation curve was shifted to more positive potentials in comparison to control current. The kinetics of tail current deactivation was much slower when the current was transferred by mutant channels. Thus, the mutation c.1011C>G leads to slowing of K+ current kinetics and right shift of activation curve, which in turn might decrease the extent of IKs current transferred by mutant Kv7.1 channels at the same values of membrane potential in cardiac myocytes. The major consequence of that changes in IKs is slowing of action potential repolarization reflected as QT prolongation in ECG. We assume that rare c.1011C>G variant is responsible for the mild QTc prolongation. Carriers of this variant should be aware about possible side effect of all drugs listed at www.qtdrugs.org source, and require appropriate genetic counseling and cardiological follow-up. The work was supported by RFBR, project 20-54-15004. *The authors marked with an asterisk equally contributed to the work.

P-06.3-32
Comparison of RNA yield and purity from small cell numbers following TRI reagent, Direct-zol™ RNA Microprep and NucleoSpin® RNA XS extraction

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RNA isolation providing high yield and purity is crucial when a limited number of sorted cells is used as a starting material for downstream RNAseq analysis. Here, we have used small numbers (1E4, 5x1E4 and 1E5) of peripheral blood mononuclear cells (PBMCs) to compare average RNA yield and purity following Direct-zol RNA Microprep (DZ), NucleoSpin RNA XS (NS) and TRI Reagent (TR) extraction procedure. Three healthy volunteers were recruited for blood collection and PBMC isolation, using discontinuous Lymphoprep centrifugation. Countess II cell counter was used for number and viability assessments prior to cell aliquot preparation. RNA quantification was performed by DeNovix DS-11 Series with the use of Qubit™ RNA HS Assay Kit for fluorometry. The A260/280 absorbance ratio, Lin's correlation coefficient (r) and Bland-Altman (BA) statistics were applied to estimate sample purity and concordance between spectrophotometric (SF) and fluorometric (F) RNA measurements. Compared to the average F RNA values obtained across all TR sample groups (2.4 ± 1.8 ng/μL), higher average concentration was observed in both DZ (4.9 ± 3.9 ng/μL, $P < 0.02$, Wilcoxon signed-rank test) and NS (4.8 ± 3.2 ng/μL, $P < 0.01$) RNA samples. Judging by the A260/280 ratios, NS (1.9 ± 0.3), but not DZ RNA (1.4 ± 0.3) samples were of higher purity compared to their TR counterparts (1.5 ± 0.1, $P < 0.01$). Alternative SF and F quantification values were concordant in DZ (r = 0.98), but not NS (r = 0.04) or TR (r = 0.02) samples, with a considerable BA differences [pair diff, mean(bias)] noticed across DZ (-3.2, pbias = 0.18), NS (-10.9, pbias = 0.012) and particularly TR (BA = -433 ng/μL, pbias = 0.009) cohorts. Our data demonstrate ability of all tested protocols for successful RNA isolation from as low as 1E4 cells, with higher average yield and purity achieved via DZ and NS protocols. TR, however, seems to be less effective and less reliable in samples with small cell numbers, particularly when combined with SF RNA quantification.

P-06.3-33
Treatment with the PCSK9 inhibitor Evolocumab improves vascular oxidative stress and arterial stiffness of hypercholesterolemic subjects with high cardiovascular risk

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Cardiovascular diseases (CVD) are still the leading causes of hospitalization and death worldwide. High levels of low-density lipoprotein cholesterol (LDL-C) and excessive production of reactive oxygen species (ROS) play a key role in the vascular functional and structural damage underlying all major causes of CVD. To reduce ciculating LDL-C level, the new lipid-lowering drug Evolocumab, able to inhibit the protein convertase subtilisin/kexin type 9 (PCSK9), has been recently introduced into clinical practice. However, the direct effect of this drug on vascular function, such as ROS generation derived from circulating leukocytes and arterial stiffness (AS), has not been so far reported. In our study, we present data from fifteen male subjects with high or very high CV risk who experienced 2-month treatment with Evolocumab (140 mg every 2 weeks). The AS was investigated by measurement of carotid-femoral pulse wave velocity (cfPWV) and augmentation index (AI). Oxidative stress was measured by an effect-based chemiluminescent bioassay for the detection of intracellular H_2O_2 production, based on the use of an adamantylidene-1,2-dioxetane probe (previously published in: Calabria D et al. (2020) Analytical Biochemistry, 600: 113760), in freshly isolated peripheral blood mononuclear cells (PBMCs). The expression of inflammatory and antioxidant genes in PBMCs were determined by qReal Time-PCR. After 2 months of Evolocumab treatment, we observed an improvement in blood pressure (BP)-adjusted cfPWV (P-value = 0.044), which was significantly associated with a decrease of H_2O_2 production (P-value = 0.007) and an increase in the antioxidant heme- oxygenase 1 (HO-1) gene expression (P-value = 0.009) in $PBMC_S$. Our data support the view of systemic oxidative stress involvement in hypercholesterolemic subjects and give further rationale for using Evolocumab to reduce CVD risk independently of its lipid-lowering effect. *The authors marked with an asterisk equally contributed to the work.

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P-06.3-34
Molecular biomarkers of cardiovascular risk: any role for the gut derivative trimethylamine N-oxide?

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Recent studies indicate a potential role of trimethylamine N-oxide (TMAO), the oxidative form of trimethylamine (TMA), in the development of cardiovascular disease (CVD). Since TMA is produced by the gut microbiota from dietary precursors (e.g., carnitine and choline), this association has a direct impact on dietary advice aimed at preventing CVD. Despite TMAO appearing as a promising biomarker, contrasting evidence on the association between TMA metabolism and CVD has emerged and a clear mechanistic explanation of this association is still missing. Together with classical CVD predictors, another promising molecular biomarker is the measurement of circulating mitochondrial DNA copy number (mtDNAcn). MtDNAcn changes have been proposed as an early biomarker of damage and mitochondrial dysfunction. Since mtDNAcn changes have been associated with both intrinsic and extrinsic factors, it has been proposed as a potential biomarker for complex multifactorial diseases. With the aim to identify biomarkers predictive of CVD and clarify the impact of TMA and TMAO on health, we investigated TMA, TMAO and mtDNAcn in a population of 389 coronary artery disease (CAD) patients and 151 healthy controls, in association with established risk factors for CVD (gender, age, hypertension, smoking, diabetes, glomerular filtration rate (GFR)). MtDNAcn was significantly lower in CAD patients and in hypertensive subjects; it correlates with GFR and TMA, but not with TMAO. A biomarker including mtDNAcn, gender, and hypertension (but neither TMA nor TMAO) emerged as a good predictor of CAD. Our findings support the usage of mtDNAcn as a plastic biomarker to monitor the exposure to risk factors and the efficacy of preventive interventions for a personalized CAD risk reduction. On the other hand, the contribution of TMAO in the prediction of CAD risk in our population is not confirmed. *The authors marked with an asterisk equally contributed to the work.

P-06.3-35
Liquid biopsy in colorectal cancer

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According to WHO, colorectal cancer (CRC) is currently the third most frequently diagnosed type of cancer worldwide and a major public health concern. Despite the efforts and the outcomes reached in the development of therapeutic strategies, the prognosis of CRC still depends on the disease stage at diagnosis. The molecular characterization of CRC tumors is crucial to guide treatment decisions and leads to significant impact on disease outcome. However, tumors are highly heterogeneous and sampling in its entirety is challenging and also tumors are highly susceptible to genotype alterations, making clinical decisions based on historical biopsy data out of date. In this view, liquid biopsy is a promising approach that could help clinicians screen for disease, stratify patients to the best treatment, and monitor treatment response and resistance mechanisms in the tumor in a regular and minimally invasive manner. For this, liquid biopsy was developed as an easily accessible and minimal invasive way to trace the molecular makeup of a patient's tumor allowing the detection and analysis of different tumor derived circulating markers such as cell-free nucleic acids (cfNA), circulating tumor cells (CTCs) and extracellular vesicles (EVs) in the bloodstream. The major advantage of this approach is its ability to trace and monitor the molecular profile of the patient's tumor and to predict personalized treatment in real time. In this context, we have recruited in our study patients with CRC in advanced stages and after obtaining their written consent we have harvested venous peripheral blood before tumor resection surgery. We have extracted cfNA from their blood and performed NGS analysis to detect specific mutations. Finally, we have compared the results obtained with the NGS profile resulted from tumor tissue analysis. This work was supported by a grant of the Romanian Ministry of Education and Research, CNCS - UEFISCDI, project PN-III-P2-2.1-PTE-2019-0577, within PNCDI III *The authors marked with an asterisk equally contributed to the work.

P-06.3-36
Determination of salivary levels of procollagen type I N-terminal propeptide gene expressions in different age groups by real time PCR

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Procollagen type I N-terminal propeptide (P1NP), a bone formation marker is derived from collagen type I. PINP is considered the most sensitive marker of bone formation and it is particularly useful for monitoring bone formation therapies and antiresorptive therapies. For PINP two automated method have been recently accepted for the routine use of serum samples. The aim of this study was to investigate the expression of P1NP in various age groups of saliva samples that is considered as an easy and repetable invasive sampling method. Saliva samples were collected in saliva RNA collection and preservation devices which provide high analytical performance. After saliva RNA isolation, cDNA synthesis, quantitation was performed using qPCR. In order to determine the optimum working range of primer, melting curve analyze was performed and the PCR method was validated accordingly. cDNA concentrations of 300–700 ng/μL and OD 260/280 of all samples were in the range of 1.8 ± 0.1. The age range of samples were between 19–23 years and 50–86 years old female and male subjects. ACTB for PINP were designed and the amount was determined by qPCR. Cq values of ACTB primer encoding the amino terminal propeptide, depending on the temperature values, were

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calculated. According to the data obtained, Cq values of ACTB primer in the temperature range of 48–63°C were seen in the range of 24–26. All values about that range suitable but the optimum temperature used in quantitative PCR reaction and gene expression analysis was 54.6°C. When compared to the GAPDH gene, ACTB gene expression was found in the saliva samples and average expression rate was 0.99. PINP was obtained from saliva regardless of age. The emission of the parameter was considered to be significant and it can be suggested to be a biomarker detected in saliva. These data may have an impact in the diagnosis and treatment follow-up of diseases associated with bone metabolism. *The authors marked with an asterisk equally contributed to the work.

Stem cells and regenerative medicine

P-06.4-01
Poly(lactic-co-glycolic) acid-based scaffolds for tissue engineering

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Nowadays, stem-cell-based technologies provide perspective solutions for the effective regeneration of injured tissues and organs. Advanced tissue engineering strategies are based on ex vivo cultivation of multipotent stem cells on a biodegradable three-dimensional (3D) scaffold and subsequent implantation of the formed tissue-engineering construct in damaged tissue or organ. Synthetic polymers have several advantages when applying as materials for stem cells carriers. These polymers can be synthesized using reproducible quality control procedure and are presented in various forms with expected physical-chemical, volumetric and surface characteristics what makes it possible to adapt their mechanical properties and biodegradation kinetics according to the requirements of tissue engineering. In this study a novel low-temperature antisolvent 3D-printing technique is presented which enables to form desired computer-aided porous structures in direct contact with cell cultures to achieve a homogeneous distribution of cells within the scaffolds. Poly(lactic-co-glycolic) acid (PLGA) was used as a row material. 3D printed PLGA scaffolds with the netlike inner structure were seeded with adipose-derived stem cells (ADSC) for our further in vitro investigation. Metabolic activity of cells was studied using MTT test. Cell adhesion has been analyzed by fluorometric microscopy of PKH-26 labeled stem cells. The samples did not demonstrate any cytotoxic effect on ADSC cultures. It was shown that PLGA scaffolds fabricated by our original antisolvent 3D-printing technique are conducive to cell growth, migration and proliferation. Thus, developed PLGA scaffolds with ADSC seeded on them have potential applications in tissue engineering and regenerative medicine. This work was supported by the Ministry of Science and Higher Education within the State assignment FSRC «Crystallography and Photonics» RAS in part of «3D-printing of PLGA scaffolds» and RSMG in part of "cellular experiments".

P-06.4-02
Studying the dynamics of the development of the therapeutic effect of initial and preconditioned MSCs on a model of acute abdominal inflammation of mice

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The aim of this research was to compare the therapeutic effects of initial and preconditioned MSCs from human umbilical cord based on a model of acute inflammation in mice. We studied the dynamics of sterile inflammation in mice after intraperitoneal injection of a 3% solution of proteose peptone. The experiment showed that 20 min. after the introduction of peptone, the number of peritoneal macrophages increased compared with the control and after 24 hours the number of macrophages significantly increased compared with the control. Comparison of the effectiveness of transplantation of different doses of MSCs (from 5 $x10^3$ cells / mouse to 100 $x10^3$ cells / mouse) showed that the injection of MSCs 20 min. after proteose peptone leads to almost complete disappearance of inflammatory signs in mice. When transplanting high-doses of initial MSCs (25 - 100 $x10^3$ cells/-mouse) 24 hours after the injection of peptone that is in conditions of acute inflammation, the inflammatory signs almost completely disappeared within 1 hour after injection of MSCs. The transplantation of low doses of MSCs (5 - 10 $x10^3$ cells / mouse) led to a 2-fold decrease in the number of peritoneal macrophages compared to proteose peptone. High doses of MSCs reduced the phagocytic activity of peritoneal macrophages almost to control and low doses of MSCs reduced the phagocytic activity by 1.5 times in 30 min after transplantation MSCs. The injection of low doses of MSCs, which don't lead to the complete clearance of inflammation, allows comparing the therapeutic efficacy of initial MSCs and preconditioned MSCs. The expression of the IL-10 gene decreased after the injection of proteose peptone to mice, and increased after transplantation of the initial and preconditioned MSCs. The date showed that the use of low doses of MSCs more clearly demonstrates the difference between MSCs variants preconditioned by various factors. *The authors marked with an asterisk equally contributed to the work.

P-06.4-03
Embryonic lumenogenesis is controlled by selective mRNA decay triggered by LIN28A relocation

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The naïve pluripotent epiblast cells become polarized into a rosette-like structure, followed by irreversible transition into primed pluripotency during one of the fastest morphological switches termed lumenogenesis. This requires rapid decay of pluripotency-associated mRNAs, but the underlying mechanism

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remains unknown. Guided by machine learning and metabolic RNA sequencing, we identified RNA binding proteins (RBPs), especially LIN28A, as primary mRNA decay factors. To understand if RBP dynamics steer embryogenesis, we used mRNA-RBP interactome capture during naïve-rosette-epiblast-gastrulation progression. We identified a dramatic increase in LIN28A mRNA binding, driven by its nucleolus-to-cytoplasm translocation during the naïve-primed pluripotency transition. Cytoplasmic LIN28A binds to 3'UTRs of pluripotency-associated mRNAs to directly stimulate their decay, and thereby progression to lumenogenesis. Accordingly, forced nuclear retention of LIN28A impeded lumenogenesis, causing an unforeseen embryonic multiplication and impaired gastrulation. This reveals selective mRNA decay, driven by nucleo-cytoplasmic RBP translocation, as an intrinsic mechanism for cell identity switch that controls embryonic timing of lumenogenesis.

P-06.4-04
Myoblasts and MSCs influence to each other's proliferative and differentiation status in the *in vitro* model of the Facioscapulohumeral muscular dystrophy (FSHD)

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Studies of muscular dystrophies are mainly focused on the molecular genetic mechanisms of the development of pathology, while cellular interactions in the affected muscles have not been studied in detail. In this regard, the goal of this work was to investigate the cellular mechanisms of fibrosis in FSHD. In the FSHD development muscle tissue degenerates followed by fibrosis. Previously, we showed that FSHD myoblasts (MB) can stimulate MSCs migration due to the increased secretion of SDF1α. The further role of MSCs in the lesion focus remained unclear. In this work, we founded that the proliferation of MSCs increases when exposed to conditioned media from FSHD-MB compared with a healthy control. However, we did not found that factors secreted by myoblasts affect to the adipogenic differentiation of MSCs during their co-cultivation. Further analysis of extracellular matrix (ECM) proteins showed: 1) immunocytochemically: an increase in fibronectin under the influence of FSHD MB-conditioned medium compared with Norm MB-conditioned medium; 2) biochemically: an increased collagen synthesis by MSCs cultured in FSHD MB-conditioned medium under normal conditions. Under conditions of inflammation (in the presence of TNFα), an increase in the collagen secretion by MSCs cultured in conditioned medium, especially FSHD MB-conditioned medium took place. In addition, MSCs prevented the fusion of myoblasts. However, FSHD myoblasts were significantly more sensitive to the presence of MSCs. Thus, FSHD-MB can stimulate proliferation of MSCs in the lesion focus, stimulate ECM protein synthesis and collagen secretion by MSCs in inflammatory conditions, but do not effect to MSCs adipogenic differentiation. In turn MSCs prevent myoblasts differentiation. The work was conducted under the IDB RAS Government basic research program in 2021 № 0088-2021-0016.

P-06.4-05
Evaluation of mesenchymal stem cell biodistribution in experimental renal tuberculosis in rabbits

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Mesenchymal stem cells (MSCs) have been reported to possess therapeutic potential in treatment of various pathological conditions, including the active forms of tuberculosis (TB). However, for further clinical application of MSCs the analysis of cells biodistribution in vivo as well as assessment of their targeting potential is of high importance. In the current study, a highly sensitive method of nonlinear longitudinal response (NLR-M_2) to a weak ac magnetic field was employed to analyze the biodistribution of MSCs labeled by superparamagnetic iron oxide nanoparticles (SPIONs). Following overnight co-incubation of cells with SPIONs the latter incorporated into the cytoplasm (as shown by confocal and electron microscopies) and did not induce any cytotoxic effects (MTT test). *In vivo* assessment of MSCs distribution was performed in a preclinical model of renal TB in Chinchilla rabbits induced by intraparenchymal injection of Mycobacterium tuberculosis (Mtb) (strain H37Rv). Following intravenous injection of the SPIONs-labeled MSCs the infected animals were sacrificed on days 1, 3, and 7 for evaluation of cells retention in different organs and tissues. As shown by NLR-M2 measurements, labeled cells accumulated in the infected renal tissue with a high retention at day 3 (that was further proven by the subsequent histological analysis). Intriguingly, magnetic measurements also detected the presence of MSCs in the lung and paratracheal lymph nodes indicating the extrapulmonary dissemination of Mtb into these tissues. In conclusion, MSCs demonstrated a high targeting potential in vivo and could be employed in further studies for diagnosis of tuberculosis or delivery of therapeutic agents. The work was supported by a grant from the RFBR №19-58-45012 and the Ministry of Science and Higher Education within the State assignments № 0103-2019-0012. *The authors marked with an asterisk equally contributed to the work.

P-06.4-06
Development of a biotechnological method for producing analogues of bone morphogenetic protein-2 with collagen-binding domains

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About 20 million various injuries are recorded annually in Russia, 15% of which are associated with bone fractures. One of the most effective treatments is to fill bone defects with various biocomposites that accelerate bone growth. Bone morphogenetic proteins (BMPs) provide high regeneration and have

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osteoinductive properties, so BMPs are one of the key components for creating a new generation of osteoinductive materials. Among these promising proteins is BMP-2. The main function of BMP-2 is associated with the growth of bone and cartilage, as well as in the differentiation of mesenchymal cells into osteoblasts and myoblasts. At present, one of such promising approaches to the creation of new materials is the creation of bone grafts based on osteoinductive materials that carry immobilized specific growth factors on the surface and provide a longer lasting effect. Previously in Laboratory of biotechnology of IBCH RAS was obtained a strain producer, then was developed protocol for isolation and purification of recombinant BMP-2, and on a culture of a mesenchymal stem cells was confirmed identity obtained BMP-2 with pharmacopeia standarts. To obtain a growth factor immobilized on a demineralized bone matrix, a genetic engineering construct was developed and a producer strain of a chimeric protein was obtained, in which the BMP-2 molecule is connected at the N-terminus with the adhesin CollBD fragment from *Staphylococcus aureus* (SAu), which is responsible for collagen binding. The created producer strain provided a high level of production of the CollBD-BMP-2 hybrid protein. To obtain the biologically active CollBD-BMP-2 dimer, was developed efficient isolation and purification scheme of recombinant protein. At this stage, experiments are being conducted to study the biological activity of the hybrid protein in comparison with unmodified rhBMP-2 in cell culture and in animals for a comparative evaluation of bone tissue repair.

P-06.4-07
Senescence of progenitor cells mediates dysfunction of endometrial tissue

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One of the primary consequences of senescence phenomenon is stem and progenitor cell exhaustion what may mediate tissue dysfunction. Here we focused on the impact of endometrial stromal cells' (ESCs) senescence on functioning of endometrial tissue. ESCs are the main structural units composing endometrium. ESCs undergo waves of extensive proliferation and tissue-specific differentiation/decidualization what determines endometrial plasticity and receptivity to embryos. Initially, we compared primary ESCs lines obtained from various donors by the basal level of senescence and differentiation response. By assessing the plethora of parameters we revealed the negative correlation – the higher rate of basal senescence within the ESCs line the lower its ability to decidualize. Further by using in vitro model of ESCs premature senescence we discovered that senescent ESCs almost completely lose their ability to decidualize compared to young cells, at least in part due to the increased intracellular ROS levels and reduced expression of progesterone and estrogen receptors. Performed RNA-seq analysis allowed us to detect significant alterations in expression profiles of young and senescent ESCs before and after stimulation of decidualization. Finally, we checked whether senescent ESCs may affect the main physiological function of endometrial tissue – receptivity to invading embryos. To do so, we applied an *in vitro* implantation model using choriocarcinoma cells and decidualized ESCs. Notably, ESCs senescence impaired adhesion and invasion of modeled "blastosysts", thus negatively influencing implantation process. The present data is first to indicate dramatic consequences of senescent ESCs appearance within endometrial tissue. Unraveled outcomes may be further considered in terms of altered endometrial plasticity and sensitivity to invading embryo, thus contributing to the female infertility curing. This study was funded by the Russian Science Foundation (# 19-74-10038).

P-06.4-08
Hsp70 mediates viability of senescent endometrial stromal cells

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Endometrial stromal cells (ESCs) ensure endometrial plasticity and receptivity to embryos. Our previous investigations showed that ESCs undergo premature senescence in response to sublethal stresses, followed by proliferation stop and differentiation impairment, critical for normal endometrium functioning. Moreover, we established that senescence may be transmitted within the ESCs population via senescence associated secretory phenotype. Together with the other results of our research group these facts indicate that senescence of ESCs may mediate endometrium dysfunction. We suppose that selective removal of senescent cells is one of the possible approaches to avoid their negative influence on the properties of endometrial tissue. It is known that senescent cells are characterized by enhanced survival. Here we focused on the mechanisms of this phenomenon in senescent ESCs with the aim to find the potential molecular target for their elimination. Firstly, we checked, whether there is a difference in viability of young and senescent ESCs in response to various stresses. As expected, we observed that senescent ESCs are more stress resistant than the young ones. Secondly, we investigated the molecular basis underlying increased stress resistance of senescent cells. We managed to find the elevated basal and stress-induced levels of Hsp70 in senescent ESCs. Finally, we tested the effects of the inhibitor and activator of Hsp70, CL-43 and U-133, respectively, on the young and senescent ESCs viability and their reaction to stresses. We discovered that induction of the Hsp70 contributes to enhanced stress resistance of senescent cells, while inhibition of this protein decreases their survival. These results allow us to conclude that Hsp70 is responsible for viability of senescent ESCs and may be considered as potential target to develop new pharmacological compounds with senolytic activity. This study was funded by the Russian Science Foundation (# 19-74-10038).

P-06.4-09
Scar formation under an influence of tissue-engineered skin equivalent in non-healing skin wound model

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Non-healing wound often leads to cosmetic defects. The aim of our study was investigation of the effect of tissue engineered Biological skin equivalent (BSE) on scar development in a mouse

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model of an ischemic non-healing wound. BSE includes mouse MSCs and keratinocytes cultured on a matrix containing hyaluronic acid. The experiment was performed on Balb/c mice. Animal manipulations were carried out under general anesthesia with the approval of Bioethics Commission of the IDB RAS. The mice were divided into three groups: «open wound», «matrix» and «BSE». All mice were surgically developed on the back with an H-shaped skin flap with parameters 10 × 30 mm with a wound 5–7 mm in the middle. Than the animals from the «matrix» and «BSE» groups were transplanted with a matrix alone and BSE, respectively. Wounds were sanified during days 3–5 after surgery with a sterile 0.7% gentamicin solution in DPBS. Cell lines were obtained from the Cell culture collection of the Institute of developmental biology RAS. On the day 7 in the «control» group, the wound bed was located significantly below the edges of the wound, which may indicate a developing of cosmetic defect. The smoothing coefficient (SC) shows the ratio between thickness of the tissue remodeling area of the wound bed andthe dermis of wound edges. Morphometric and statistical analysis showed that the SC of mice from the «BSE» group was significantly higher than that from the «control» and «matrix» groups. This indicates that BSE transplantation promotes the regeneration of a non-healing wound without a cosmetic defect. Research funding. The work was performed under the IDB RAS Government basic research program 2020.

P-06.4-10
Vectors system for delivery of morphogens for heterotopic ossification mechanisms studying

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Novel methods of molecular biology, surgical manipulation in combination with intravital visualization and transgenic technologies have been played significant role in understanding cell mechanism underlying in processes of organogenesis and tissue development. Development of bone at heterotrophic ossification site in murine model for example, regeneration of phalange, could be studied precisely by means of exogenic gene expression using viral vectors. Lentiviruses, baculoviruses and poxviruses can provide high level of expression during limited segment of time. In this research we constructed vector for expression of firefly luciferase (fluc) based on vaccinia viruse, lentivirus and baculovirus. Precise quantitative evaluation of gene expression in vivo is mandatory for assessment of developmental process in tissues and organs. IVIS visualization system is used for detection of fluc expression after intermuscular injection. Lentivirus vector containing fluc gene under control of cytomegalovirus promoter has shown effective gene expression during 14–20 days. Vaccinia virus (LIVP strain) expressing fluc gene under control of 7.5 kilobase promoter support expression during 3–4 days. Baculovirus was produced using bac-to-bac system and has shown signal in coarse of 5–7 days. However, vaccinia virus expression system supports the highest level of fluc in muscle tissues. Depending on purpose of the task suitable system for morphogen expression can be chosen. All three models showed to be useful for modeling of heterotopic ossification mechanism.

P-06.4-11
Obtaining gene-activated osteoplastic material based on plasmids with the BMP2 gene

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The treatment of traumatic injuries and degenerative bone diseases is a topical problem. The development of new effective materials to ensure organotypic bone regeneration is required. The materials impregnated with plasmid with the genes of osteoinductive factors for transfection of resident cells can be an effective and safe tool providing bone regeneration. In this work, the pcDNA3-BMP2 and TaqRFP-N-BMP2 plasmids encoding the target BMP2 gene were obtained. TurboFect (TF) was used to deliver plasmids to mesenchymal stem cells (MSC). The vector pEGFP-C1 was used to choose transfection conditions for MSC. Transfection efficiency was assessed by fluorescence microscope and by flow cytometry by counting cells expressing EGFP after 24 h. Cytotoxicity of the transfection complexes was estimated by the MTT assay. MSC were stained with membrane dye PKH26 to study the migration of cells into collagen and chitosan-based materials. The development of osteogenic differentiation was evaluated by PCR-RT and staining with alizarin red. The highest transfection efficiency (about 15%) with maintaining high cell viability (more than 80%) was observed during incubation of MSC with polyplexes 2 μg/mL pEGFP-C1 + 4 μl/mL TF for 1 h. When cells were added to the collagen and chitosan-based material impregnated with transfection complexes, cells migrated into the material and were transfected with plasmids with the EGFP gene. Our results demonstrate the ability of material impregnated with plasmid to transfect MSC with high efficiency. The matrices impregnated with plasmids encoding the BMP2 gene increased the expression levels of BMP2, OCN and OPN and increased extracellular matrix mineralization in MSC. Thus, the osteoinducing effect of the developed collagen and chitosan-based material impregnated with plasmid with the BMP2 gene in vitro was shown. The research was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation for RCMG.

P-06.4-12
In vitro and *in vivo* biocompatibility analysis of new 3D scaffolds for neurotransplantation

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Nowadays, an approach for morphofunctional nervous tissue reconstruction after traumatic brain injury by using 3D constructs (scaffolds) seems promising. Application of scaffolds with given architectonics allows maintaining necessary anatomical structure and provide free transport of biological fluids in the damaged area. Moreover, integration of biologically active substances into 3D constructs helps accelerate regenerative processes and, subsequently, will lead to gradual replacement of lesion site by natural nervous tissue. The current study was aimed to access biocompatibility of scaffolds based on hyaluronic acid glycidyl methacrylate and neurotrophic factors (BDNF/GDNF) with

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nervous cells *in vitro* and *in vivo*. Scaffolds based on hyaluronic acid glycidyl methacrylate were fabricated by extrusion 3D printing technique. The obtained 3D constructs were then supplemented with recombinant human BDNF (248-BDB, R&D Systems) or GDNF (212-GD, R&D Systems) at concentration of 100 ng/scaffold. Primary neuronal cultures obtained from C57BL6 mice embryos (E18) were cultivated in the presence of scaffolds during 14 days. We observed an intensive growth of neuronal processes towards scaffold during first 7 days of cultures development. The most pronounced effect was shown for the "scaffold BDNF" group. Cell viability assessment revealed high biocompatibility of scaffolds with primary neuronal cultures; the number of viable cells in the "scaffold BDNF" and "scaffold GDNF" was comparable with the intact group values. In experiments in vivo, the scaffolds was implanted to the damaged area of C57BL6 mice after day 7 of traumatic brain injury. We showed that scaffolds supplemented with neurotrophic factors BDNF, GDNF contribute to reduce the severity of neurological deficits and preserve mnemonic and cognitive abilities in experimental animals. This study was supported by the Ministry of Science and Higher Education of the Russian Federation (project No. 0729-2020-0061).

P-06.4-13
Cytokine-induced mesenchymal stem cells type 1/2 polarization affects their phenotype and metabolism

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Adipose tissue-derived mesenchymal stromal cells (MSC) are widely used in regenerative medicine to restore the function of damaged tissues, but their efficacy is hampered by cell heterogeneity. One of the modern approaches to overcome this obstacle is the polarization of MSC subpopulations into a specific phenotype by cytokines and other factors that activates receptors and signal transducion pathways. We polarized MSC with factors affecting the inflammatory signaling and functional properties, followed by verification of their expression profile by proteomics and RT-PCR analysis. We identified a list of potential protein markers whose expression up-regulates after MSC treatment with anti-inflammatory factors and simultaneously decreases in inflammatory conditions. Among 14 proteins that we suggest may serve as polarization markers, three are associated with the autophagy SQSTM1, BAG2, and RAB1A. Their expression was higher in MSCs incubated with IL17a, LPS, and TNFa compared to MSCs incubated with IL4 and p (I:C). Therefore, we additionally evaluated autophagy in MSCs using autophagosomal fluorescent probes – monodansylcadaverine and CYTO-ID®. Monodansylcadaverine staining was more intensive in MSCs incubated with IL 17a and LPS that correlate with the SQSTM1 expression. CYTO-ID staining also were highest in MSCs treated with inflammatory cytokines and decreased in anti-inflammatory-treated MSC compared to intact MSCs. These data collectively suggest that MSC polarization could lead to phenotypic and metabolic alterations, that appears in particular, in an autophagy activation. Thus we conclude that MSC polarization via pro-inflammatory or anti-inflammatory pathways allows to obtain cell subpopulations that have a distinct phenotype [work was supported by RFBR grant 20-015-00405 and RSF grant 19-15-00384].

P-06.4-14
Collagen-based microenvironment mechanically similar to native extracellular matrix for skin engineering applications

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The physical properties of the extracellular matrix play an important role in the regulation of cellular functions, affecting gene expression and cell physiology. Stiffness of the native dermal extracellular matrix is much higher than the stiffness of the collagen matrix widely used in traditional cell cultures. To imitate cell behaviour in native human dermis we created high-density constructs from highly concentrated type I collagen (30 mg/mL), inhabited by dermal cells of human skin, which were subjected to long-term cultivation in 3D conditions. As a control, the same cells were cultivated in a traditional low-density 5 mg/mL collagen matrix. An increased concentration of collagen did not affect the spreading and survival of fibroblasts (over 90%), but it prevented the contraction of the matrix and promoted the formation of mechanically stable constructs. The mechanical properties of such constructs remained unchanged even after prolonged cultivation for four weeks. The cell proliferation inside the dense collagen matrix was reduced recapitulating the fibroblast behaviour observed in native human adult skin. Analysis of the specific protein marker expression revealed that the cells cultivated in the dense matrix demonstrate the same expression programs as those observed during cultivation in a traditional low-density matrix. The results of this study indicate the prospects of using dense matrices for the production of stable tissue-engineered cell structures mimicking native microinvironment, which may prove usefull in application in tissue engineering. The reported study was funded by RFBR according to the research project № 19-29-04060

P-06.4-15
Hippo pathway is involved in oocyte selection during prenatal oogenesis

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Ovarian reserve in mammals starts to form during the embryonic development. It is well-known that there are several waves of germ cell death, and the first one takes place before the birth, when primordial ovarian follicles start to form. The reasons of the oocyte failure and the mechanism of the oocytes' selection during embryonic development remain unknown. Hippo signaling pathway is an evolutionary conservative pathway that regulates several important processes during cell life: proliferation, differentiation and death. According to literature Hippo pathway is active at the postnatal stages of folliculogenesis and regulates the activation of primordial follicles and follicles' death.

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Summarizing these data we hypothesized that Hippo signaling can be involved in regulation of the oocyte selection during the first wave of oocyte death. We compared the mRNA expression levels of the main participants of the Hippo cascade in mammals (mst1/2, lats1/2, yap) between the developmental stages (E14,5 - 18,5) of *Mus musculus* embryos by qRT-PCR. It turned out that the expression level of the main kinases (mst1/2 and lats1/2) increased at the E16,5 stage, but decreased at 17,5. The ovarian cyst break-down takes place during these late stages of development, so the rise of the kinases' expression can be connected with this process. The immunocytochemical staining of the embryonic ovaries for LATS1/2 and YAP showed that these proteins were mainly localized in the somatic cells of the ovary. The cyst break-down occurs with the direct involvement of somatic (future follicular) cells, that are necessary for the primordial follicles' formation. So the condition of the somatic cells around the oocytes can be the factor of oocyte selection. Understanding of this process can help to find out the ways of ovarian reserve restoration and maintainance in the females of reproductive age. The work was funded by the Fund of President RF research project MK-378.2020.4. *The authors marked with an asterisk equally contributed to the work.

P-06.4-16
Proteomics investigation of microgravity conditioned human primary stem cells, in presence of SrHA nanoparticles

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Microgravity-induced osteoporosis is one of the main problems affecting astronauts during and after space traveling. Furthermore, osteoporosis and non-union fractures still represent worldwide problems. Nanotechnological applications are gaining field as remarkable solutions to improve bone healing, especially where conventional drug approaches fail. Our group developed and characterized calcium and strontium hydroxyapatite nanoparticles (nCa-HA and nSr-HA, respectively) 1 as a countermeasure for osteoporosis (NATO project). Nanoparticles efficacy was studied on human bone marrow-derived mesenchymal stem cells (hMSCs), both under Earth's gravity (G) and simulated microgravity (μG), using a Random Positioning Machine (RPM). The investigation was conducted in terms of gene expression, extracellular matrix protein composition and proteome characterization 2. Particularly, hMSCs proteome has been studied using a bottom-up and label-free approach. Samples have been in-gel digested and analyzed with EvosepOne online coupled to the mass spectrometer. Data Independent Acquisition (DIA) was conducted to obtain a high proteome coverage. Results were statistically process and significative difference in the analyzed proteomes were correlated to the gravity and the nanoparticles treatment variables. The enrichment of the differentially expressed proteins between G and μG highlighted the involvement of actin filament organization, ossification and angiogenesis pathways. Furthermore, pathways involving actin filament organization were found enriched also in the presence of nSr-HA, both in G and μG. Gene expression analysis showed up-regulation of the osteogenic markers in the presence of strontium. Although further analysis and validation of these data will be carried on, preliminary conclusions suggest nSr-HA are counteracting the effects of μG, promoting ossification in hMSCs.

P-06.4-17
Skeletal muscle as a source of mesenchymal stem cells for autologous cell therapies

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Advanced cell therapies employing autologous mesenchymal stem cells (MSCs) for joint regeneration have shown promising results in alleviating symptoms despite the lack of detailed understanding of the underlying mechanism of action. To date, the most well-recognized tissue sources of mesenchymal stem cells in regenerative medicine are autologous bone marrow and adipose tissue. The biological properties of MSCs from other sources such as skeletal muscle, however, are poorly understood. The aim of our study was to compare the skeletal muscle MSCs with well-recognized bone and bone marrow-derived MSCs in osteoarthritis patients. Paired samples of skeletal muscle and trabecular bone tissue were obtained from 21 patients with osteoarthritis. MSCs were isolated using collagenase digestion and isolated cells compared using ex vivo immunophenotyping and detailed in vitro analyses. These included the colony forming unit fibroblast assay, growth kinetics, senescence, multilineage potential, immunophenotyping (CD90, CD73 and CD105), and gene expression profiling. Freshly isolated MSCs from skeletal muscle showed improved viability over bone-derived MSCs, with similar mesenchymal fraction. Muscle-derived MSCs also showed superior clonogenicity, higher growth rates, lower doubling times, as well as superior osteogenic and myogenic properties compared to bone-derived MSCs. We also showed a positive correlation between CD271 expression in skeletal muscle MSCs and adipogenesis. Due to their superior properties skeletal muscle-derived MSCs represent a suitable candidate for autologous stem-cell therapies. Previously published in: Čamernik K et al. (2019) Stem Cell Res 38

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P-06.4-18
Emulsion-templated synthetic polypeptide-based scaffolds for tissue engineering

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Ultimate goal in preparing a scaffold for tissue engineering is to create a suitable micro-environment for optimal cell attachment, migration, growth, differentiation and function. Creating the macroporous scaffold of desired pore volume, size distribution and interconnectivity enables it to better meet the tissue-specific demands and provide for fast migration of cells in it and efficient exchange of nutrients and metabolites. By employing ring-opening polymerization (ROP) of N-carboxyanhydrides (NCAs) we prepared scaffolds composed of synthetic polypeptides known for their biodegradability, biocompatibility and cell adhesive properties. Combination of high internal phase emulsion (HIPE) templating and ROP of NCA leads to the three-dimensionally interconnected macroporous morphology typical of polyHIPE scaffolds as revealed by SEM. A good control over the polymerization initiation and propagation prevented HIPE's phase separation and foaming effect of CO_2 byproduct. The scaffolds were cross-linked using the NCA derivative of the natural di-amino acid, L-cystine. The cell viability tests revealed that the scaffolds were nontoxic for cells. They supported rapid cell proliferation and colonization of the surface cavities and deeper voids suggesting efficient cell migration as demonstrated by confocal microscopy and microscopy of scaffold cross-sections. The cell metabolic activity in scaffolds demonstrated their exponential growth. Our approach in preparation of polypeptide scaffolds offers a control over the chemical composition and morphology, giving countless possible post-polymerization modifications for scaffolds with tailored properties. *The authors marked with an asterisk equally contributed to the work.

P-06.4-19
Multiplex proteomics in the evaluation of hepatocyte transdifferentiation into pancreatic beta cells

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Diabetes mellitus shows increasing prevalence worldwide; loss of β-cell function and of β-cells occur in both type 1 and type 2 diabetes, with earlier onset of cellular loss in type 1 diabetes, also in late-stage type 2 diabetes. So far, only symptomatic therapies, to control the symptoms, are available. Novel therapies, aiming to generate insulin-producing cells are investigated, aiming to replace deficient β cells. Such approaches include differentiation of human stem cells or mature cell types into β cells. Characterization of mechanisms involved in transdifferentiation, identification of key factors in the process and their interactions are essential for such strategies. One approach is the transdifferentiation of hepatocytes, followed by implantation of transformed cells [1]. One important tool for detection and monitoring of protein profiles is represented by protein arrays, highly specific and with ability to detect endogenous proteins [2]. We used a Bio-Plex Pro Human Diabetes 10-Plex Assay kit to reveal modified protein signatures due to transdifferentiation. Biological material used in the experiments was represented by supernatants and lysates of 2D, 3D and bioreactor cultures of human hepatic cells induced to transdifferentiate into β islet cells [1]. Out of the molecules investigated, relevant modifications were found for visfatin, glucagon, and insulin. C-peptide could also be detected, at measurable levels, in all the variants. Thus, we can conclude that transdifferentiation of hepatocytes into beta cells could be a promising alternative for a cure of diabetes mellitus. Acknowledgement – Funding of research was provided by COP- E 2016 Contract no. 148/2016, ID: P_37_794, My SMIS No.: 106897 1. Meyvar-Levi I. et al - Stem Cell Res Ther. 2019 Feb 13;10(1):53. https://doi.org/10.1186/s13287-019-1157-5. 2. Popa ML, et al. - J Immunoassay Immunochem 2019 - PMID 30632882 *The authors marked with an asterisk equally contributed to the work.

P-06.4-20
Urokinase–type plasminogen activator receptor regulates pro-survival properties of cardiac progenitor cells

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Urokinase-type plasminogen activator receptor (uPAR) expression is elevates during inflammation and remodeling, which is an important step for the realization of cardiac repair/regeneration. uPAR binds with vitronectin and urokinase-type plasminogen activator, regulates proteolysis and also activates different intracellular signaling pathways in cardiac cells. Here, we hypothesize that uPAR may determine pro-survival/proliferative properties of resident cardiac progenitor cells (CPC). Myocardial infarction (MI) was induced by anterial descendant coronary artery ligation in mice. CPC were isolated from hearts (WT and uPAR–/– mice) by retrograde perfusion according to Langendorff method followed by immunomagnetic selection. Down-regulation of uPAR expression was performed with short hairpin RNA. uPAR expression level were analyzed by Western blot and RT-PCR methods. It was shown, that uPAR is located on the surface of most CPC and required for their mobilization in the myocardium after infarction *in vivo*. In wild-type mice, we observed a twofold increase number of CPC in damage zone on the 5th day after myocardial infarction in comparison with the uPAR–/– mice. uPAR down-regulation leads to inhibition of clonogenic activity and CPC proliferation properties. uPAR down-regulation increased apoptosis of CPC under serum starvation and cell death after H_2O_2-induced oxidative stress. Taken together, our

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findings suggest that uPAR promotes the survival/growth of CPC cultured *in vitro*. These results imply that the efficiency of CPC homing to the injury site as well as their survival after transplantation may be improved by modulating the activity of uPAR. This work was supported by RSF grant (#17-15-01368P).

P-06.4-21
Investigation of CD133 gene expression before and after *in vitro* rFSH treatment in Sertoli cells of OA and NOA patients

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Sertoli cells are somatic cells that have essential functions throughout spermatogenesis. CD133 is a member of the multi-span membrane protein family and has an important role in the production and differentiation of germ cells. Our previous studies demonstrated that testicular CD133 gene expression was significantly related with testicular histology and that FSH treatment might have positive influence over spermatogenesis via changing the expression levels of certain genes. In this study, our aim was to investigate the CD133 expression from Sertoli cells in obstructive azoospermia (OA) and nonobstructive azoospermia (NOA) patients and to analyze the effects of rFSH treatment on its expression. A total of 10 azoospermic men diagnosed as OA (n = 5; control group) and NOA (n = 5) were included in the study. Primary Sertoli cell cultures were prepared from the testicular tissue samples collected during the micro-TESE procedure. CD133 gene expressions were evaluated by real-time PCR analysis before and after rFSH supplementation into culture medium. In primary cultures of Sertoli cells, mean CD133 gene expression in NOA group was found to be 0.273 fold lower than that of OA patients ($P < 0.05$) and rFSH treatment caused 0.135 fold change in the NOA patients against to control but couldn't reach to statistically significant level. In conclusion, CD133 gene expression in Sertoli cells may be a causative factor responsible for impaired spermatogenesis and FSH treatment has no effect on the gene expression. We suggest that CD133 plays an important role in the maintenance of spermatogenesis and development of germ cells through Sertoli cells. Also, CD133 may be an important marker in the management of the infertile males with NOA. Keyword: CD133, Spermatogenesis, Male Infertility, Sertoli Cells, Nonobstructive azoospermia

P-06.4-22
The differences in proliferation and cytotoxicity of MC3T3-E1 cell line on tricalcium phosphate composites containing physically and chemically bonded diclofenac to polyhydroxyoctanoate (PHO) polymer

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This work presents research related to the possibilities of application of organic / inorganic macroporous scaffolds prepared for bone tissue engineering applications. Tricalcium phosphates were used as the matrix. These materials have excellent bioactive, osteoconductive and osteoinductive properties. Polymer blends of bacterially derived polyhydroxyalkanoate containing physically and chemically bounded diclofenac were used as the coating of composites. The use of nonsteroidal anti-inflammatory drugs as medicaments in osteoarthritis after surgery is a very common practice. Studies related to the determination of direct and indirect cytotoxicity as well as the rate of proliferation in a mouse pre-osteoblast cell line model (MC3T3-E1) were performed. The indirect cytotoxicity studies showed no cytotoxicity effect of the supernatant collected after incubation of the composites in the culture medium. In contrast, direct proliferation studies showed that the materials containing chemically bound diclofenac did not show a cytotoxic effect after 7 days of cultivation, unlike the same sponges containing physically bound diclofenac. In addition, cell nuclei were imaged using DAPi dye, which allowed the confirmation of cell migration on three-dimensional scaffolds. Cell morphology was imaged using a scanning electron microscope (SEM) [1,2]. [1] Skibiński et al., Ceramics International 2020, 47(3), 3876-3883. [2] Haraźna et al., International Journal of Molecular Sciences 2020, 21(24), 9452. Research funded by the NCRDs projects, no. LIDER/27/0090/L-7/15/NCBR/2016 and TEG2/407507/1/NCBR/2019. KH also acknowledges the support of InterDokMed project no. POWR.03.02.00-00-I013/16 as PROM project no PPI/PRO/2018/1/00006/U/001.

P-06.4-23
Cultured endometrial cells as a model for screening of new progesterone analogues

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Drugs based on female sex steroid hormones are widely applied in obstetrics and gynecology. However, hormonal therapy is not always successful. Latest achievements in cell biology can be useful in the development of new compounds and their personalized

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use. The aim of this work was to explore the possibility of using human endometrial mesenchymal stem cells (eMSC) as a model to identify new promising progesterone analogues. eMSC were obtained from endometrial biopsys of 12 donors (healthy and with gynecological pathologies). All ethics approvals and written informed consents have been obtained. Immunophenotype, karyotype, expression of progesterone and estrogen receptors of eMSC have been determined. Decidual differentiation of eMSC was reached by culturing in media containing estradiol and progesterone or its novel analogues. Secretion of prolactin and IGFBP-1 was used as marker of decidualization and was measured by ELISA. eMSC derived from endometrial tissue of gynecologically healthy donors showed high ability for decidual differentiation. The response (increase of IGFBP-1 and prolactin secretion) to highly active progesterone analogues was more considerable than to progesterone itself. eMSC obtained from patients with gynecological pathology did not respond to hormonal exposure to both progesterone and its analogues. Despite similar immunophenotypic characteristics of eMSC from different donors, it is more preferably to use eMSC derived from gynecologically healthy donors as a cell model for screening of new progesterone analogues. Unusual response to hormone-induced decidual differentiation of eMSC from donors with endometriosis and hyperplastic processes supports the evidence of disruption of decidualization processes in the endometrium of patients with such pathologies. The work was supported by RFBR according to the research project № 18-015-00449 and RAS Presidium Program № 18.

P-06.4-24
Stem cell therapy for the regeneration of an adult BALB/c mouse hippocampus after neonatal stress

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Early life stress increases the risk for mental illness and diminished cognitive function due to reduced neurogenesis, impaired memory and learning. In our study, consequences of neonatal maternal separation were tested in mice with focus on behavioral alterations and hippocampal function. Moreover, an impact of cell therapy on brain regeneration after stress was examined. BALB/c mouse pups were exposed to early life stress by maternal separation from birth to day 21, while control pups were kept undisturbed with their mothers. Around day 75, 300.000 stem cells in DPBS were injected into the CA1 hippocampal region (in left and right hemisphere) using stereotaxic technique. Stem cells were isolated from abdominal adipose tissue of an adult BALB/c mice. Control mice were injected with DPBS buffer without cells. The effect of stress on brain and the ability of stem cell therapy to induce hippocampal regeneration were tested with different behavioral tests and also on tissue and molecular level. Sucrose preference drinking test was assessed at the age of 90–100 days to determine anhedonic behavior in stressed and control mice, followed by behavioral tests: elevated plus maze, open field test, sociability test and light/dark box to explore anxiety-like behavior, locomotor activity, exploration activity and social behavior and preferences. Body weight was monitored every 10 days. Mice were euthanized on day 130 and their hippocampi were isolated and weighed. RNA was extracted from the hippocampi and used for gene expression analyses to determine mRNA expression of glucocorticoid receptor Nr3c1.

P-06.4-25
The effect of transplantation of olfactory ensheathing cells from olfactory mucosa on the size of post-traumatic spinal cord cysts

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Introduction. Approximately 30% of patients with spinal cord injury (SCI) suffer postraumatic cavitation that impede the conduction of nerve impulses and regeneration of nerve tissue. Surgical removal of cysts and drug therapy is ineffective because it does not promote regeneration of the spinal cord. A promising direction in the treatment SCI is the study of the effectiveness of cell transplantationof olfactory ensheathing cells from olfactory mucosa. The aim of this workwas to study the effect of transplantation of olfactory ensheathing cells from olfactory mucosa on the size of post-traumatic spinal cord cysts. Materials and methods. We obtained olfactory mucosa of 70 rats. The tissue was treated with dispase and then mechanically divided into olfactory epithelium and lamina propria. Olfactory ensheathing cells were isolated from lamina propria according to the protocol developed by us (Voronova AD et al., 2018). Posttraumatic cysts were simulated at the TH4 level (Zhang Cet al., 2015). The presence of cysts was confirmed by MRI 4 weeks after SCI. We provided transplantation of 7.5×10^5 and 1.5×10^6 olfactory ensheathing cells into the cyst cavity. The control group was injected with a medium without cells. Results. It was shown that transplantation of olfactory ensheathing cells contributed to significant decrease in the volume of cysts and to the complete disappearance of cysts in some animals. Discussion. It is known that transplanted olfactory ensheathing cells can migrate through the glial scar and thus promote axonal regeneration. In addition, these cells secrete matrix metalloproteinases which contribute to the degradation of the extracellular matrix of the cyst capsule and thus contributing to a decrease in its size. Currently, further research is needed to study the mechanisms of spinal cord regeneration during olfactory ensheathing cells transplantation in cyst. This work was supported by the Russian Science Foundation (Grant No. 17-15-01133). *The authors marked with an asterisk equally contributed to the work.

P-06.4-26
Determining the mechanism and possibilities of brain damage regeneration using Mesenchymal Stem Cells (MSCs) after hypoxic-ischemic injury

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The majority of neonatal seizures are related to common diagnoses, including hypoxic-ischemic encephalopathy (HIE) and intraventricular hemorrhage. HIE is brain disturbance caused by e.g reduction of oxygen in the neonatal blood combined with reduced cerebral blood circulation. Clinical therapies allows for reducing the degree of brain damage (hypothermia) or alleviating

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complications (drug treatment), but there is still no effective clinical treatment that enable the regeneration of damaged brain tissue. Hence, it is an urgent problem to develop a novel therapy for HIE, that would make the reversal of injury possible. Cell-based therapy has attracted much attention because of not only its regenerative property but also its long therapeutic time window. The study determine how intracerebroventricular administration of Mesenchymal Stem Cells (MSCs) activate brain regeneration using animal model of HIE. Experiments were provided on NOD-SCID mice strain with induced HIE. MSCs were administrated into mice cerebro-spinal fluid via intracerebroventricular stereotaxic surgery. To determine influence of MSCs on damaged brain was provided battery of behavioral (cognitive and motoric) tests. Establishement of immunity stage of the brain was conducted due to measurement of choosen pro-inflammatory factors. Additionally, the distribution of given MSCs was tracked by survey of bioluminescence signal. Our data report MSCs cell therapy as effective in preclinical studies in terms of cognitive and motoric amelioration, as well as improvement of immune stage of damaged brain tissue. We have also proven feasibility, safety of the procedure and long viability of transplanted MSCs in cerebro-spinal fluid. Easy obtainability of MSCs with no ethical problem in cell collection, no tumourigenicity, low immunogenicity and easy preparation are seem to be crutial when translating to the clinic. Project was supported by National Scientific Center in Poland 2018/31/B/NZ3/01879.

P-06.4-27
Effectiveness of olfactory mucosa cells transplantation into experimental post-traumatic spinal cord cysts

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Introduction. After spinal cord injury (SCI) as many as 30% of affected patients suffer from post-traumatic deformation (cysts) that inhibits neural conduction and nerve tissue regeneration. Traditional surgical and therapeutic methods, unfortunately, remain ineffective. A promising area in SCI therapy is cell transplantation of cells from olfactory mucosa. The aim of this work is to investigate the effectiveness of olfactory mucosa cells transplantation into rat spinal cord cysts. Materials and methods. Using our protocol olfactory ensheathing cells were obtained by isolating from lamina propria of olfactory mucosa. Neural stem/progenitor cells (NSPCs) were obtained from epithelium of olfactory mucose. Post-traumatic cysts were modeled at the thoracic vertebrae level (TH4). After four weeks with a SCI the cysts were confirmed by MRI. Different cell combinations were transplanted into the area of the cysts: 7.5×10^5 and 1.5×10^6 olfactory ensheathing cells, 2×10^5 NSPCs, combinatory preparation (7.5×10^5 olfactory ensheathing cells + 2×10^5 NSPCs). Cultural medium without cells and astrosytes were transplanted as a control. Results. After cell transplantation, improving of hindlimb motility of rats was observed using weekly BBB scoring tests. It was demonstrated that transplantation of olfactory ensheathing cells and combined cell preparation almost equally helped to improve the motor activity of the hind limbs of rats. Conclusions. For the first time, we have shown that the effectiveness of olfactory mucosa cells in post-traumatic spinal cord cysts. Olfactory mucosa cells are highly promising in personalized medicine. They can be obtained from patients with spinal cord cysts and after cultivation transplanted into the same patient. This work was supported by the Russian Science Foundation (Grant No. 17-15-01133). *The authors marked with an asterisk equally contributed to the work.

P-06.4-28
Protein acetylation: the role in ischemic tolerance

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The balance between the processes of acetylation and deacetylation of proteins manages the functioning of a variety of enzymes and signaling pathways, determining both the ischemic tolerance and regeneration of tissue. The process of acetylation is determined by the metabolic status of the cell, depending on the acetyl-CoA from glycolysis, beta-oxidation of fatty acids, and catabolism of amino acids, and is regulated by deacetylases or acetyltransferase. A decrease in the levels of protein acetylation is associated with an increase in tissue tolerance to damage, primarily due to the activation of the autophagy process. Agents that reduce the levels of acetyl-CoA represent a promising approach for the treatment of ischemic pathologies. One of the most effective ways to reduce acetyl-CoA in the cell and to activate autophagy is dietary restriction. We have shown that dietary restriction for 4 weeks had a protective effect against kidney ischemic injury. Interestingly, in ischemia, as well as in aging, we observed an increase in the level of acetylated proteins in the kidney cells. The correlation of acetylation and ischemic tolerance was demonstrated in experiments with ischemic preconditioning of the kidney. We have shown that autophagy removes non-functional biological structures, including mitochondria, and deterioration of the effectiveness of mitochondria quality control causes the manifestation of the pathological phenotype and a decrease in ischemic tolerance, including with aging. Thus, reducing protein acetylation and activating autophagy may be a successful approach in the treatment of ischemic pathologies by increasing tissue tolerance to damage, and slowing down cellular aging. This strategy can be implemented through dietary restriction, activation of metabolic switching, for example, with a ketogenic diet, and the use of deacetylases activators and acetyltransferases inhibitors. The study was supported by Russian science foundation (grant #21-75-30009).

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P-06.4-29
Identification of proteins potentially responsible for antifibrotic activity of human mesenchymal stromal cells

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Multipotent mesenchymal stromal cells (MSCs) are key contributors to tissue renewal and repair after injuries, with many effects mediated by secreted factors where proteins are responsible for a notable part of biological effects. Recently we showed that MSC secretome fractions containing extracellular vesicles (EV) and soluble proteins (SP) prevented TGFβ-induced fibroblast to myofibroblasts transition (published in: Basalova NA et al. (2020) Cells, 9(5):1272), although non-fractioned MSC conditioned media (CM) demonstrated much less antifibrotic activity. For these effects, we aimed to investigate potential antifibrotic protein agents and interfering proteins based on their content in different fractions of MSC secretome. Immortalized human MSC (hTERT-ASC, ASC52telo, ATCC) conditioned media was collected for 48 h at 80% confluency. For CM fraction, media was concentrated on 10 kDa filter; for EV and SF fractions, media was concentrated on 1000 kDa filter (EV) and the filtrate was then concentrated on 10 kDa filter (SF). Antifibrotic activity of each fraction was tested in the model mentioned above. Tryptic peptides were obtained as described in (Nowacki FC et al (2015) JEV, 4:1), separated with HPLC, and analyzed using Q Exactive HF-X mass spectrometer. Analysis for protein abundance was performed with MaxQuant software. Results obtained were verified by western blotting assay for each fraction. Eight proteins potentially mediating EV and SF antifibrotic effects were identified, such as TIMP-1, PIP, C1 inhibitor, and Gal-3BP. A list of proteins depleted during fraction isolation was also made. These proteins are suggested to impede antifibrotic effects in CM (e.g., IGFBP-6, TIMP-2, HDGF) and SF (e.g., TFPI, protein SET, SPARC). After spotting potential antifibrotic and profibrotic proteins, their mechanism of action affecting the TGFβ signaling pathway was suggested. However, their contribution to MSC antifibrotic effects needs to be validated in further research.

P-06.4-30
PDGF-PDGFR network modulates myogenic progenitor fate, migration, and proliferation

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Platelet-derived growth factors (PDGFs) bind PDGFRα and PDGFRβ to regulate embryonic development, wound healing, and tissue repair. PDGF signaling pathway also participates in wound healing and tissue repair but exacerbated PDGF signaling can lead to fibrosis development in several tissues and organs. However, the role of PDGF signaling in regulating muscle development and regeneration remains elusive, and the cellular and molecular responses of myogenic progenitors are understudied. This study explores the contribution of PDGF signaling in regulating myogenic cell fate, proliferation, and myogenesis. Here, using single-cell and bulk transcriptomic analyses, we show that satellite cells and myoblasts express PDGF ligands and PDGFRα and PDGFRβ, albeit differentially. Myogenic commitment and differentiation reduced PDGFRα and PDGFRβ expression. Furthermore, PDGF-AB and PDGF-BB activated two pro-chemotactic and pro-mitogenic downstream transducers, RAS-ERK1/2 and PI3K-AKT, in myoblasts. Concomitantly, PDGF-AB and PDGF-BB enhanced myoblast migration and proliferation, which were inhibited by PDGFR inhibitor AG1296. However, PDGF-AA, which binds only to PDGFRα/PDGFRα homodimers, did not promote a noticeable cascade activation, migration, or expansion. Finally, PDGFR inhibition induces myoblast G0/G1 cell cycle arrest. Thus, we identified a potential signaling pathway to enhance tissue repair and regeneration after injury and disease.
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P-06.4-31
Unravelling the role of innate immune response to dsRNA in regulation of transgene expression from AAV9 vectors

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Adeno-associated viral vectors (AAVs) are one of the most efficient vectors for transgene delivery in clinical applications. Genome of AAVs consists of ssDNA or dsDNA – depending on the type of vector – and harbours the expression cassette flanked by inverted terminal repeats (ITRs). Although AAVs exhibit low immunogenicity, recent study has linked the decreasing transgene expression from AAV vectors in the liver with development of innate immune response against dsRNA. Such dsRNA can be formed in transduced cells due to active bi-directional transcription utilising ITR as a promoter. So far, the effectiveness of AAV application in treatment of cardiac diseases was limited and usually not sufficient to meet the therapeutic goals in patients. Thus, in this study, we aimed to elucidate the regulation of transgene expression in human cardiomyocytes, focusing on the impact of dsRNA in the process. As a model for our experiments, we used 2D and 3D cultures of cardiomyocytes differentiated from human induced pluripotent stem cells (hiPSC-CMs), which were transduced with AAV serotype 9 vector (AAV9) carrying GFP transgene under control of CMV promoter or ITR. Transduction efficiency was assessed by flow cytometry analysis 7 days after exposure of the cells to AAV vectors. Our findings confirmed that ITR can function as a promoter in hiPSC-CMs,

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maintaining approximately half of the GFP fluorescence signal when compared to CMV. What is more, we detected antisense transcript from AAV9 in transduced hiPSC-CMs. Its presence was associated with increased expression of dsRNA sensors, but did not induce interferon response. Activation of intracellular dsRNA immune response with lipid-complexed poly(I:C) significantly hampered transgene expression from AAV9. Currently, we are focusing our research on identification of proteins interacting with ITR-derived dsRNA in transduced cells and deciphering their impact on transgene expression.

P-06.4-32
Human-adipose derived stem cells interaction with natural-based composites for peripheral nerve regeneration

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Human adipose-derived stem cells (hASCs) have been largely explored for their therapeutic potential in diverse regenerative medicine applications. Recently, the capacity of these types of stem cells has been explored for their capacity to trans-differentiate towards non-mesenchymal lineages such as the neuronal lineage. Thus, our studies focus on developing varios biocomposites combined with hASCs for peripheral nerve regeneration (PNR). Gelatin meshes enhanced with carbon-based nanostructures, such as graphene oxide (GO) and magnetic nano-particles (MNPs) have been fabricated in order to test their biocompatibility with hASCs during one week of *in vitro* cell culture. Cell viability and proliferation was assessed using MTT test, whereas the materials' cytotoxic effects were tested by LDH assay. Visualisation of both live and dead cells in contact with these scaffolds was realised by Live/Dead staining and confocal microscopy. Cytoskeleton development and focal adhesions formation in contact with the materials was investigated by immunolabeling. Results indicated an overall good interaction between hASCs and gelatin scaffolds. Composites enriched with GO and MNPs displayed a better cell viability and proliferation rate as compared to the gelatin control. The addition of nano-components didn't induce significant toxicity, as LDH levels were found to be low. hASCs cytoskeleton presented an elongated shape and several adhesion proteins were evidenced, thus indicating that the structure of the material offers optimal conditions with a positive impact on cells behavior. Therefore, these materials present biocompatible characteristics and their features together with hASCs should be further investigated for PNR applications. This work was supported by PN-IIIP1-1.1-TE-2019-1191/MAGNIFICENT grant.

P-06.4-33
Reorganization of the proteasome pool in mesenchymal stem cells during ageing in culture

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Proteasomes hydrolyze most intracellular proteins and exist in several different forms. The 20S core proteasome subunit composition and its association with specific regulators contribute to the proteasome form heterogeneity. Most cells contain 20S proteasomes with constitutive catalytic subunits (β1, β2, β5) that might be replaced by so-called immune analogs (β1i, β2i, β5i), leading to the formation of immunoproteasomes (iPs). The iPs participate in stress adaptation and immune reactions, being abundantly present in the immune cells. Interestingly, the iPs were revealed in stem cells and their implication in the maintenance of embryonic stem cell pluripotency was reported. Here we investigated the dynamics of constitutive and iP subunits gene expression and levels of proteasome subunits and 11S proteasome regulator in primary murine mesenchymal stem cells (MSCs) during prolonged (56 days) cultivation. Using this MSC ageing model we have shown that the expression of constitutive proteasome subunit genes and the amount of constitutive proteasomes remained stable in cells cultured for 56 days. Conversely, a gradual decline of expression of iP subunit genes and number of iP subunits integrated into proteasomes was observed from 15th to the 56th day of culture. A similar dynamics was detected for the alpha subunits of the 11S proteasome regulator, which is frequently associated with the iPs. Obtained results indicate reorganization and depletion of the proteasome pool in MSCs during their ageing *in vitro*. In particular, the number of iPs and their regulators decreases, which might directly affect the functional activity of proteasomes and the profile of produced peptides. This in turn might be of key importance for the regulation of intracellular processes within the MSCs. This work was funded by a grant from the Ministry of Science and Higher Education Russian Federation (agreement No. 075-15-2020-773).

P-06.4-34
Comparison of 3D printed polylactic acid, poly-ε-caprolactone and polyethylene terephthalate scaffolds seeded with human dermal fibroblasts *in vitro*

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Skin tissue engineering is a growing scientific field that focuses on reconstructing the skin components, and improving wound healing. Scaffolds serve as a temporary base for cells until the natural extracellular matrix (ECM) is regenerated.3D printing

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has gained a great attention as an innovative tool that enables production of complex 3D tissue scaffolds by programming its pore size and shape. Dermal fibroblasts are located in the dermal skin layer that produce and organize ECM components.They play an essential role in skin wound healing. The aim of this study is to analyze the viability, adhesion. distribution and collagen IV expression of human dermal fibroblasts (HDFs) seeded onto 3D printed polylactic acid (PLA), poly-ε-caprolactone (PCL), and polyethylene terephthalate (PET) scaffolds *in vitro*. HDFs were seeded on scaffolds or tissue culture plastic (TCP) plates as control and were cultured for 1 and 3 days. 3D PLA, PCL, and PET scaffolds were prepared using a custom made fused deposition modeling printer. The cell viability was measured by WST-1 assay on days 1 and 3. The cell adhesion was evaluated by scanning electron microscopy (SEM). The distribution was analyzed by hematoxylin and eosin staining. Collagen IV expression was analyzed by immunohistochemical staining. The viability of HDFs on the 3D PLA scaffolds was significantly higher than PCL scaffolds on day 1. The viability of HDFs on 3D PLA and PET scaffolds was significantly higher than PCL scaffolds on day 3. SEM images showed that HDFs on 3D PLA scaffolds attached the surfaces, filled the inter-fiber gaps and maintained their tissue specific morphology on day 3 compared to PCL and PET scaf-folds. The distribution of HDFs on 3D PLA scaffolds was uniform on days 1 and 3. Collagen IV staining was more intense in HDFs on 3D PLA scaffolds on days 1 and 3. Our results show that 3D PLA scaffolds create a suitable environment for cell viability, adhesion, distribution and may provide a high advantage in wound healing.

P-06.4-35
Human adipose-derived stem cells secretome and hepatocyte growth factor (HGF) exhibit a beneficial effect on hepatic fibrosis reversion

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Liver fibrosis is a reversible pathology that can potentially lead to cirrhosis. The key cellular players are the activated hepatic stellate cells (HSCs) that start overproducing extracellular matrix (ECM) proteins. Transforming growth factor-β1 (TGF-β1)/SMADs is one of the main signaling pathways activated that upregulates fibrosis specific genes (α-sma, collagen type I). Alternative regenerative therapies are constantly investigated. Therapies based on stem cells derived from adipose tissue (ADSCs) could provide a novel strategy, as they are easily obtainable, have immunomodulatory activity, show multilineage differentiation potential and are capable of repairing damaged tissue. The aim of this study was to investigate the therapeutic antifibrotic effect of ADSCs combined with hepatocyte growth factor (HGF) on the reversal of aHSCs. TGF-β1 activated HSCs (aHSCs) were co-cultured with ADSCs for 24 h with/without 50 ng/mL HGF. Immunostaining of α-sma and Oil Red O staining were performed to confirm HSCs activation. To assess the antifibrotic effect of ADSCs with/without HGF, the expression of fibrosis markers (α-sma, col I, smad 2/3, TGF-β) was evaluated at gene (qPCR) and protein (immunofluorescence staining) levels. Activation of HSCs was confirmed by high expression of α-sma and lack of lipid droplets. aHSCs-ADSCs co-culture reduced α-SMA expression both at protein and gene level, suggesting ADSCs' positive role in reversing the activated state of HSCs to a latent-like state. Moreover, co-culture with ADSC inhibited SMAD 2/3 translocation in the nuclei of aHSCs, suggesting the ADSCs act upon TGF-β pathway. Furthermore, ADSCs effect on aHSCs was enhanced by HGF. Expression of col I and α-sma was further reduced and TGF-β pathway was inhibited. Co-culture of aHSCs-ADSCs showed potential to induce reversal of aHSCs. HGF enhanced the antifibrotic potential of ADSCs on aHSCs. This work was supported by PN-III-P1-1.1-PD-2019-0337 and PN-III-P2-2.1-PED-2019-3609 grants. *The authors marked with an asterisk equally contributed to the work.

P-06.4-36
Single cell RNAseq-based transcriptome profiling of mesenchymal stromal cell (MSC) subpopulations with different responses to profibrotic stimuli

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Fibrosis is an ineligible outcome of the tissue response to injuries. Multipotent mesenchymal stromal cells (MSCs) play a dual role in the fibrosis development: MSCs can contribute to a myofibroblast pool promoting the progression of fibrosis but they also could suppress fibrosis by producing anti-fibrotic cytokines and microRNAs. Therefore, MSC could contain distinct subpopulations which respond differently to fibrotic stimuli. To test this hypothesis, we cultured human adipose-derived MSCs for 4 days with a key profibrotic factor TGFb on fibrotic-like decellularized ECM produced by fibroblasts. Single cell RNA-seq datasets were created from MSCs under profibrotic versus standard conditions (10× Genomics) and analyzed using Loupe Browser, development trajectories, a graph of latent time (pseudo-time) and RNA-velocity. Cell phenotypes were predicted using cell marker libraries (BLUEPRINT, Human Protein Atlas, PanglaoDB) and customized software. We found that under profibrotic conditions MSCs could be segregated in 2 clusters, differing significantly by the expression of alpha smooth muscle actin (αSMA). aSMA+ cells were characterized by increased gene expression of contractile and ECM proteins (tropomyosin 1, filamin C, caldesmon 1, transgelin, etc.), protease inhibitors (PAI-1), insulin like growth factor binding protein 3 and fibroblast growth factor-2. In aSMA- cells transcripts of ECM proteins (dermatopontin (DTP), fibulin 1) and matrix-remodeling proteases (MMP11, urokinase) as well as factors involved in extracellular vesicle secretion were presented. Analysis of development trajectories, pseudo-time and RNA-velocity confirmed the identification of the cell cluster with myofibroblast markers within MSCs, while MMP11, DTP, sorting nexin-9, and cathepsin K genes were activated in αSMA- subpopulation of MSCs in profibrotic conditions. Additionally, the potential source of aSMA+ cells in control MSCs was predicted. The study was supported by RSF (#19-75-30007). *The authors marked with an asterisk equally contributed to the work.

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Bioinformatics and computational biology

P-06.5-01
Distinct age-associated transcriptome profiles in the trophocytes and oenocytes of worker and queen honey bees (*Apis mellifera*)

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Queen and worker bees share the same genome; however, trophocytes and oenocytes exhibit longevity phenotypes in queen bees and aging phenotypes in worker bees. To explore this phenomenon at the molecular level, we evaluated the transcriptomes of trophocytes and oenocytes in young and old worker and queen bees using high-throughput RNA-sequencing technology (RNA-seq). The results showed that (i) gene expression profiles were different between worker and queen bees; however, they were similar between young and old counterparts; (ii) worker bees exhibit high proportion of low-abundance genes, whereas queen bees display high proportion of moderate-expression genes; (iii) queen bees have higher up-regulated genes than worker bees, and (iv) aging-related candidate genes in worker bees and longevity-related candidate genes in queen bees were detected. These results provide new insights into the cellular senescence and longevity of trophocytes and oenocytes in honey bees. *The authors marked with an asterisk equally contributed to the work.

P-06.5-02
Evaluation of the therapeutic potential of dihydroartemisinin and its dimer as β-amyloid aggregation and β-secretase inhibitors

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Alzheimer's disease is characterized by the formation of plaques in the brain, which are commonly composed of insoluble forms of amyloid peptides as a result of aspartyl protease β-secretase expression. The interaction of artemisinin derivatives — dihydroartemisinin and its dimer, with the amyloidogenic peptides $5A\beta_{17\text{-}42}$, $12A\beta_{9\text{-}40}$ and β-secretase was studied in silico. The comparison was made with curcumin, which is in phase II of clinical trials. The presented work shows that the interaction of investigated ligands with all targets is characterized by fairly high binding energy values, with a maximum for dihydroartemisinin dimer. Studied ligands directly interact with amino acids, which are responsible for the formation, growth and stabilization of the peptides. So, dihydroartemisinin can prevent the formation of $5A\beta_{17\text{-}42}$, while dihydroartemisinin dimer and curcumin can affect its growth. All ligands can prevent the formation of the $12A\beta_{9\text{-}40}$, dihydroartemisinin dimer and curcumin can suspend its stabilization. For all ligands interactions with important amino acids of β-secretase are observed, dihydroartemisinin and curcumin interact with critical amino acids of the catalytic center of β-secretase. In this work, pharmacological characteristics, such as HIA and BBB were analyzed for the studied compounds. They can cross the BBB to one degree or another and have a very high absorbability. Based on our results, it can be concluded that dihydroartemisinin dimer can prevent the formation, growth and stabilization of amyloid peptides, and can modulate β-secretase activity. It interacts with peptides and β-secretase with higher affinity compared to other ligands. Thus, dihydroartemisinin dimer can be considered as a possible candidate for the treatment of Alzheimer's disease.

P-06.5-03
Interaction of artemisinins with quorum sensing regulator SdiA of enteropathogenic *E. coli*

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Enteropathogenic E. coli (EPEC) remains one of the most important pathogens infecting children and they are one of the main causes of persistent diarrhea worldwide. Virulence factors and biofilm formation are regulated by quorum sensing in E. coli. Suppressor of division inhibitor A (SdiA) is a quorum sensing receptor present in EPEC that detects acyl-homoserine lactones and autoinducers of other bacterial species. SdiA is regulated by 1-octanoyl-rac-glycerol (OCL), which serves as an energy source, substrate for membrane biogenesis. Artemisinins (ART) are known to have potent antivirulence effects against resistant strains by inhibiting the release of pro-inflammatory cytokines TNF-α and IL-6, as well as stimulate the accumulation of antibiotics in bacteria by inhibiting the AcrAB-Tol multicomponent multidrug resistance pump. We have shown that ART and dihydroartemisinin (DHA) at low concentrations less than 3 μM/mL have practically no effect on the growth of E. coli. The Markov modeling is used to predict the dynamics of SdiA protein. Obtained structures are used for molecular docking using MedusaDock and perform a comparative analysis of the ligands. We show that ART and DHA interact with most amino acids of the ligand-binding domain of SdiA to which OCL binds. ART and DHA bind with the crystal structure of SdiA with higher binding affinity than OCL (-29.63, -26.25, -24.75 kcal/mol, respectively). The C ring of ART and DHA with the endoperoxide bridge is involved in the interaction. The biological activity of artemisinins leading to cell death is mainly related to the endoperoxide bridge, which leads to the formation of ROS. Similar results were obtained from the docking of the same ligands with the centroid structure from the macrostates obtained by Markov modeling. Thus, artemisinins do not exhibit any antibacterial effect on the growth of E. coli at low concentrations, but they can bind to SdiA, which will reduce the virulence of the E. coli biofilm. *The authors marked with an asterisk equally contributed to the work.

P-06.5-04
Molecular mechanism of cationic antiseptics action revealed by coarse-grained molecular dynamics

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Antiseptics are antimicrobial compounds used in a clinical setting. Positively charged cationic antiseptics bind strongly to the

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bacterial cell walls and membranes because of their opposite negative charge. The bacterial plasma membrane is supposed to be the main target of antiseptic action. We created molecular dynamics (MD) coarse-grained models of four cationic antiseptics of different classes, including biguanides (chlorhexidine and picloxydine), pyridine derivatives (octenidine) and quaternary amines (miramistin). The model plasma membrane consisted of 180 POPE and 60 POPG molecules. To study the molecular mechanism of action of antiseptics at various concentrations, we designed molecular models of the membrane with antiseptic molecules, with antiseptic to lipid ratio of 1/24 (low concentration), 1/8 (middle concentration) and 1/4 (high concentration). The systems with low and middle concentration were simulated for 3 microseconds and the systems with high concentration – for 30 microseconds. To analyze the interaction of antiseptics with the model membrane, we estimated macroscopic parameters such as area per lipid, density profiles, order parameters and bilayer thickness for all systems. We have shown that octenidine, in comparison with other compounds, had the most pronounced disintegrating effect on the bacterial plasma membrane due to significant change in the lipid area and bilayer thickness. However, in addition to changing macroscopic parameters, one of the effects of antiseptics on the bacterial plasma membrane is its depolarization. This effect was revealed in molecular dynamics calculations with an extra electric potential applied to the membrane in the presence of antiseptics. We demonstrated that antiseptics induce faster membrane pore formation even at low values of electric potential. Acknowledgements: The reported study was funded by RFBR, project number 19-34-90045.

P-06.5-05
Identifying the mechanism of interactions between serine trimer and nucleotide dimer sequences by molecular modelling

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The side chains of amino acids have a pivotal role in intermolecular interactions between DNA and protein. Serine, a polar and hydrophilic amino acid, is a proton donor or an acceptor in forming hydrogen bonds due to the presence of –OH group. Thus, identifying of fundamental interactions between nucleotide and serine is a requirement for comprehensive understanding of the mechanisms of protein-DNA association. In this study, the non-covalent interactions between AG, CC, GG and AC nucleotide dimers in the single-stranded DNA, and the serine trimer were investigated by computational methods. For this purpose, Spartan 14 program was used to determine initial structures for the serine trimers and nucleotide dimers by conformational analysis. MOPAC2016 software with PM6-D3H4 method and Gaussian09 software at ωB97XD-6-311++G(d,p) level were used for optimizations and frequency calculations for each investigated system. Using the calculated results, the most stable structures were determined based on the relative energies. The non-covalent interactions between dinucleotide structures and serine trimer were demonstrated by using VMD with Multiwfn 3.6 Molecular structures were presented by Discovery Studio Visualizer 2019 software. The results revealed that the serine trimer can form hydrogen (–H) bonds and van der Waals forces with nucleotide dimer sequences. In addition, when the structures were examined, we observed that serine trimer interacts with both the sugar-phosphate backbone and the base on dinucleotide structure via –H bond. Most of the calculations were performed on TUBITAK-ULAKBIM Truba resources and this study was supported by Ege University Research Grants (BAP, Project No. 17-FEN-006). Keywords: Peptide; Nucleic Acids; Molecular Modelling

P-06.5-06
Molecular modeling of plastocyanin–cytochrome f complex formation in higher plants, green algae and cyanobacteria

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The interaction of proteins is an inalienable stage of most processes in the cell. The combined approach of Brownian and molecular dynamics and hierarchical cluster analysis were used to investigate the mechanisms of the complex formation between plastocyanin and cytochrome f in higher plants (Spinacia oleracea and Brassica rapa), green microalgae Chlamydomonas reinhardtii and two species of cyanobacteria (Phormidium laminosum and Nostoc sp.). In higher plants and green algae, electrostatic interactions force plastocyanin molecule close to the heme of cytochrome f. In the subsequent rotation of plastocyanin molecule around the point of electrostatic contact in the vicinity of cytochrome f, copper (Cu) atom approaches cytochrome heme forming a stable configuration where cytochrome f molecule behaves as a rather rigid body without conformational changes. In Nostoc plastocyanin molecule approaches cytochrome f in a different orientation (head-on) where the stabilization of the plastocyanin–cytochrome f complex is accompanied by the conformational changes of the G188E189D190 loop that stabilizes the whole complex. In cyanobacterium *P. laminosum*, electrostatic preorientation of the approaching molecules was not detected, thus indicating that random motions rather than long-range electrostatic interactions are responsible for the proper mutual orientation. We demonstrated that despite the structural similarity of the investigated electron transport proteins in different photosynthetic organisms, the complexity of molecular mechanisms of the complex formation increases in the following sequence: non-heterocystous cyanobacteria – heterocystous cyanobacteria – green algae – flowering plants. The research is carried out using the equipment of the shared research facilities of HPC computing resources at Lomonosov Moscow State University. This work was supported by RFBR grants No. 19-04-00999 and 20-04-00465.

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P-06.5-07
Investigating whole-transcriptomics alterations in early and proliferative stages of brain regeneration

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While mammals have a very limited capacity to regenerate their brain after an injury, the extensive regeneration capacity of the adult zebrafish brain makes zebrafish a useful model to understand the molecular programs that are necessary for central nervous system regeneration. Although several studies have been performed on zebrafish brain regeneration at the cellular level using histological methods, the way how underlying molecular mechanisms are controlled at the RNA level has not yet been elucidated. In this study, we used molecular biology methods and bioinformatics techniques together to investigate alterations on mRNA molecules in brain regeneration at its very early versus proliferative stages. For this aim, we generated stab lesions in adult zebrafish telencephalon to trigger brain regeneration. Next, we dissected lesioned and unlesioned hemispheres separetely and we performed RNA-Sequencing after isolating their RNAs. DESeq2 package of Bioconductor was used to analyze differentially expressed genes (DEGs). Disease (DO) and gene ontology (GO) enrichment and pathway analyses were performed based on the obtained DEG lists. Also, we performed quantitative PCR to check the expression levels of particular early and mature neuronal marker genes in samples before and after RNA sequencing. According to our results, while cell signaling, regeneration and development related GO terms were enriched in early stage, differentiation and homeostasis related terms were predominantly enriched in the proliferative phase. P53 and MAPK signaling and apoptosis related pathways were enriched in both stages. We believe that these results will greatly extend our knowledge about molecular mechanisms involved in brain regeneration and will pave the way to the development of new therapeutic approaches of traumatic brain injuries or neurodegenerative diseases. *The authors marked with an asterisk equally contributed to the work.

P-06.5-08
The catalytic mechanism of the human aldehyde oxidase

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The human aldehyde oxidase (hAOX) plays a crucial role in the metabolism of diverse compounds, either endogenous or xenobiotics. For that reason, the hAOX interferes in the pharmacokinetics of many drugs what makes it one of the enzymes with highest interest for the scientific and pharmaceutical communities. In our work, we studied the structure and the dynamics of this key metabolic[1] enzyme and unveiled, with atomistic detail, its full catalytic mechanism of oxidation of the N-heterocycle phthalazine to phthalazin-1(2H)-one, along with its associated energy profile. Moreover, the puzzling variations of the oxidation state of the molybdenum ion of hAOX throughout the catalytic cycle were carefully examined. We applied state of the art computational methods, namely a quantum mechanics/molecular mechanics scheme (QM/MM) within the ONIOM methodology. This methodology has been successfully applied to study catalytic mechanisms of many enzymes. The final energies were calculated at the B3LYP-D3/6–311++G(2df,2pd)/SDD:FF99SB//B3LYP/6–31G(d)/SDD:FF99SB. Our results indicate that the Lys893 has a fundamental role for the activity of the enzyme, for the favorable positioning of the substrate within the catalytic site and on the catalytic mechanism as well, as it must donate a proton to the substrate for the catalytic reaction to proceed. We found that the rate limiting step of the mechanism correspond to the transfer of a hydride from the substrate to the molybdenum sulfur ligand with a concomitant reduction of Mo from Mo^{VI} to Mo^{IV}. In addition, the unbinding of the products from the molybdenum cofactor seem to be more favorable with the Mo ion in its Mo^{V} state together with FADH•. In short, this work provided insights on the mechanism of oxidation of N-hetorcycles by the hAOX which is a valuable information for the study of the interaction of this enzyme with drugs and for the design of inhibitors. [1] Ferreira, P et al. (2019) Phys. Chem. Chem. Phys., 21 (25), 13545–13554.

P-06.5-09
SARS-CoV-2 Mpro as a challenging molecular target for small-molecule inhibitor design

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The pandemic of the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) constantly poses a serious global health threat. Still, no specific drugs are available. Among various molecular targets, the main protease (Mpro) from the SARS-CoV-2 appears to be the most promising one due to its key role in virus replication. Crystallographic structures of the SARS-CoV-2 Mpro revealed high similarity with the SARS-CoV Mpro structure; they differ by only 12 amino acids, but just one replacement is located in the proximity to the enzyme's active site. We analysed both Mpros structures using classical and mixed-solvent molecular dynamics (MD) simulations with small molecules tracking approach (AQUA-DUCT). In spite of a high level of sequence similarity, the active sites in both proteins show major differences in shape and size indicating that repurposing SARS drugs for COVID19 may be futile. The analysis of the

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pocket's conformational changes indicates its flexibility and plasticity. We also analysed the Mpro's binding site in terms of the potential mutability of the residues involved in inhibitors binding. Most of the mutations result in stable protein and advocate the high potential of Mpro further evolution. Moreover, we used molecular docking, toxicity profiling, and multiple MD protocols to assess the selectivity of 33 reported non-covalent inhibitors of SARS-CoV-2 Mpro against 8 proteases and 16 anti-targets. Several of the assessed compounds presented considerable off-target binding towards the panel of proteases and the selected anti-targets. The computational results are reflected in the experimental validation carried out using the MicroScale Thermophoresis technique. Even very similar compounds show large differences in binding to the active site, confirming that SARS-CoV-2 Mpro is a challenging molecular target. Previously published in: Bzówka M et al. (2020) Int. J. Mol. Sci. 2020, 21, 3099; Fischer A et al. (2021) Int. J. Mol. Sci. 2021, 22(4):2065

P-06.5-10
Deeper inside the specificity of lysozyme when hydrolyzing chito-oligosaccharides. A computational study

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Lysozymes that were used in numerous in vitro experiments of chitosan degradation were regularly from chicken egg-white. Human lysozyme provided to be more active than hen egg-white lysozyme when exerting its bacteriostatic effect and there is possible that the two enzymes have some different structural properties. Protein Data Bank contains structural files of complexes made by the human and hen egg-white lysozymes with the hexamer of N-acetyl glucosamine (GlcNAc) and it allows structural bioinformatics and molecular modeling studies. Consequently, the aim of this study was to compare the structural and physicochemical properties of the human and egg-white lysozyme and to use the molecular docking approach to gather more insight into chitosan hydrolysis by lysozyme. There is 60.47% of identity between the sequences of human and the hen egg-white lysozymes, but the amino acids that are involved in the interactions of considered lysozymes with (GlcNAc)6 are well conserved. Superimposition of the structures of investigated lysozymes reveals their structural similarity, the value of RMSD values being 1.198 Å for 118 equivalent carbon alpha atom pairs from 129. Even if there is a good structural similarity between the two enzymes, there are some local physicochemical properties, like the distribution of the electrostatic potential and the hydrophobicity of the catalytic cavities of enzyme, that are quite different. The interactions between the investigated chito-oligomers and the human and hen egg-white lysozymes seem to be dependent on the molecular weight, deacetylation degree and pattern.

P-06.5-11
Analysis of serotonin 5-HT3 receptor and its models: integration of X-RAY, EM, MD data

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Ion channels form a major class of integral transmembrane proteins involved in the regulation of different cellular processes. Serotonin receptors (5-HT3) directly regulate gut movement, and drugs that inhibit 5-HT3 function are used to control emetic reflexes associated with gastrointestinal pathologies and cancer therapies. However, the complex molecular architecture of eukaryotic ion channels, which include large non-membrane domains, is often an obstacle to structural studies by experimental methods. Thus, for comparative structural analysis we used one atomic X-ray structure of the pentameric ligand-dependent mammalian channel of the serotonin 5-HT3 receptor (pdbid 4PIR) and Cryo-EM structures (pdbid 6BE1, 6HIS, 6HIN, 6HIQ). Based on the available experimental data on this channel, the conformational state of this channel can not be determined. In this regard, the actual task is to compare the structure of serotonin 5-HT3 receptor and its models, built by modeling homology. Structures with pdb id 2BG9, 4AQ9 for modeling 5-HT3 receptor closed and open conformations respectively were used as templates for modeling. According to the molecular dynamics data obtained by us, hydrated sodium ions are unable to pass through threonine in the M2 helices form the area of the minimum radius of the pores of the channel of 5-HT3 receptor. Thus, the data obtained suggest that the structure of the 5-HT3 receptor (pdbid 4PIR) is more consistent with the closed conformation. The MD research has been carried out using the equipment of the shared research facilities of HPC computing resources at Lomonosov Moscow State University and computing resources of the federal collective usage center Complex for Simulation and Data Processing for Mega-science Facilities at NRC "Kurchatov Institute"(ministry subvention under agreement RFME-FI62117X0016), http://ckp.nrcki.ru/.

P-06.5-12
In silico screening of flavones and its derivatives as potential inhibitors of quorum-sensing regulator LasR of Pseudomonas aeruginosa

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Antibiotic resistance is a global problem nowadays and in 2017 the World Health Organization published the list of bacteria for which treatment are urgently needed, where Pseudomonas aeruginosa is of critical priority. Current therapies lack efficacy because this organism creates biofilms conferring increased resistance to antibiotics and host immune responses. The strategy is to "not kill, but disarm" the pathogen and resistance will be developed slowly. It has been shown that LasI/LasR system is the main component of the quorum sensing system in *P. aeruginosa*. LasR is activated by the interaction with its native autoinducer. A lot flavones and their derivatives are used as antibacterial drug compounds. The purpose is to search compounds that will inhibit LasR. This leads to the inhibition of the synthesis of virulence factors thus the bacteria will be vulnerable and not virulent. We performed virtual screening using multiple docking programs for obtaining consensus predictions. The results of virtual screening suggest benzamides which are synthetical derivatives of flavones as potential inhibitors of transcriptional regulator LasR. These are consistent with recently published experimental data, which demonstrate the high antibacterial activity of benzamides. The compounds interact with the ligand binding domain of LasR with higher binding affinity than with DNA binding domain. Among the selected compounds, by conformational analysis, it was found that there are compounds that bind to the same amino acids of ligand binding domain as the native autoinducer.

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This could indicate the possibility of competitive interaction of these compounds. A number of compounds that bind to other conservative amino acids ligand binding domain have also been discovered, which will be of interest for further study. Selected compounds meet the criteria necessary for their consideration as drugs and can serve as a basis for conducting further in vitro / in vivo experiments.

P-06.5-13
Gene functional network analysis of autophagy genes in colorectal cancer

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Autophagy is a cellular process in which intracellular components are degraded and recycled. Although some autophagic molecular regulators has been identified, the role of autophagy in cancer development and progression is still an appealing question. Based on the current literatures, autophagy seems to play dual roles in cancer progression. In this study, we investigated the functional networks of autophagy genes in colorectal cancer using the comprehensive analysis of transcriptomic datasets of the cancer genome atlas (TCGA). They has been normalized using variance stabilizing transformation. After running a well-controlled survival analysis on cancer tissues, we identified 40 autophagy genes correlated with survival (*P*-value <0.05). For the further filtering of these genes, we utilized a 5-fold cross-validated elastic net cox proportional hazard model. Based on the differential expression of the data across cancer and normal tissues, we found that the 18 autophagy genes were statistically significant (*P*-value <0.01). These genes seemed to be over-represented in the TP53 degradation pathway, and they were further characterized using protein-protein interaction and gene co-expression analysis. To sum, this network analysis suggests that autophagy could play a critical role in the progression of colorectal cancer. Key words: autophagy, TCGA, cancer progression, gene network analysis, colorectal cancer

P-06.5-14
A transcriptomic-based drug repurposing strategy for the identification of new SMN-independent skeletal muscle treatments for spinal muscular atrophy

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Spinal muscular atrophy (SMA) is a neuromuscular disorder (NMD) caused by loss of the survival motor neuron 1 (SMN1) gene. We have previously shown that administering prednisolone, a synthetic glucocorticoid (GC) to SMA mice improved muscle health and survival. As chronic use of prednisolone can lead to adverse effects in muscle, we wanted to determine its molecular targets to develop more selective therapeutic approaches. We performed RNA-sequencing on triceps from symptomatic untreated and prednisolone-treated SMA mice and healthy control littermates. The single RNA reads were then aligned to an mm10 Mus musculus genome through HISAT2 and gene read summarisation was calculated by FeatureCounts. DeSeq2 analysis estimated 3056 significant differentially expressed genes (DEG) (Log2 fold change >0.6, *P*-adj <0.05) in prednisolone-treated SMA mice muscle compared to untreated counterparts. Importantly, further comparison with untreated healthy littermates showed that prednisolone treatment in SMA muscle normalised the expression pattern of a large subset of DEG to healthy levels. Further analysis through iPathwayGuide of the gene targets in prednisolone treated SMA muscle, revealed several biological pathways ($P < 0.05$) associated with muscle metabolism, structure and function. Using various drug databases and published literature, we selected 2 clinically approved drug candidates (metformin and oxandrolone) that are predicted to similarly target the DEG and biological pathways in SMA muscle. Although metformin is commonly used for type 2 diabetes and oxandrolone for burn patients, both drugs have previously shown muscle-specific benefits in NMD, metabolic and muscle-wasting disorders. Our in silico work has thus identified potential new drugs that will be assessed in cellular and animal models. Importantly, this transcriptomic-based drug repurposing approach provides a less expensive and faster alterative for the development of new muscle-specific treatments for SMA.

P-06.5-15
Identification of novel single-nucleotide polymorphisms in chronic obstructive pulmonary disease (COPD) and lung cancer using genome-wide association analysis

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Chronic obstructive pulmonary disease (COPD) is a major public health problem associated with long-term exposure to toxic gases and particles. Patients with COPD have higher risk of developing lung cancer. Lung cancer is the leading cause of cancer-related deaths worldwide. Even though lung cancer is associated with tobacco smoking, genetic susceptibility plays an important role in disease etiology. Candidate susceptibility genes coding for enzymes involved in the activation, detoxification, and repair of damages caused by tobacco smoke as well as genes in inflammatory and cell-cycle pathways have been extensively studied. Immune system has been shown to be a determining factor during cancer initiation and progression. In recent years there is more and more focus on immune therapy, but still, genes that are involved in the regulation of the immune response haven't been fully identified/studied. Genome-wide association studies (GWAS) have a great capability of detecting genetic variants for complex diseases like COPD and lung cancer. In GWAS a cohort is genotyped and the resulting data is then analyzed in relation to disease or to quantitative trait phenotype. The results of the study are genetic variants, single nucleotide polymorphisms (SNPs). Each SNP is then analyzed according to minor allele frequency, *P*-value, biological significance and other factors. The aim of this study is to find new SNPs that are

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associated with COPD and lung cancer risk, with focus on immune genes. Using statistical analysis of GWAS data, two case-control studies (early and late COPD-control and lung cancer-control), we identified several SNPs related to immune response that could be important in developing COPD and lung cancer. Functional studies will be done on significant SNPs of interest to characterize them more in detail. Characterized SNPs could then be used as biomarkers for detection of lung cancer or they could be even used in development of immune and targeted therapies.

P-06.5-16
Profiling of squamous cell lung cancer on transcriptomic level

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Lung cancer is the leading cause of cancer-related death worldwide. Squamous cell lung cancer (LUSC) accounts for 25–30% of all diagnosed lung cancer cases. It is known that interactions between cancer, immune and stromal cells can impact tumor proliferation and progression, however immune microenvironment of LUSC is still not well characterized. Omics approaches to cancer research started a new era of precision medicine, allowing analysis of large pools of available data. Identification of new predictive and prognostic biomarkers for LUSC using this approach could help optimize therapy decision, since only few targeted therapies were approved for treatment of LUSC specifically so far. In this study we examined mRNA expression profiles of 23 treatment naive patients diagnosed with primary LUSC and 3 healthy controls using RNA-sequencing approach. We determined total of 1535 differentially expressed genes between patients and controls and interrogated the role of those genes with Gene Ontology including Biological processes, Molecular function and Cellular component analysis. To characterize tumor microenvironment of LUSC, we used ESTIMATE package in R. Both immune and stromal scores that represent the infiltration of immune cells in tumor tissue and presence of stroma in tumor tissue, showed different patterns in our samples. To elucidate this further we used GSVA (Gene Set Variation Analysis) to test enrichment of our samples using 17 immune-related gene sets. Our results showed that upregulated genes were associated with cell-cell signaling, transmembrane signaling receptor activity and DNA-binding transcription factor activity. Downregulated genes were associated with G-protein coupled receptor activity, response to stress, stimuli and immune response. GSVA analysis coupled with hierarchical clustering showed 4 distinct patients subgroups based on levels of immune cells infiltration.

P-06.5-17
Clostridioides difficile growth and toxin activity change after in vitro modulation of gut microbiota with polyphenol extracts

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Clostridioides difficile causes toxin mediated gastrointestinal infection (CDI) which is typically associated with a pre-disturbed gut microbiota. Better understanding of the mechanisms by which a healthy gut microbiota resists the development of CDI will improve current methods of the prevention and treatment of the disease. In the present study we used antibiotic clindamycin and polyphenol extracts from pomegranate and blueberries to modulate fecal microbial communities in the MBRA gut model (mini-bioreactor arrays). Modulated communities were subsequently inoculated with C. difficile vegetative cells (ribotype 027) followed by a periodical monitoring of C. difficile growth, activity of toxins TcdA and TcdB and bacterial community composition. Polyphenols affected multiple commensal bacterial groups and exhibited both synergistic and antagonistic effects in combination with clindamycin. Exposure to either clindamycin or polyphenols led to the loss of colonization resistance against C. difficile. Communities modulated with pomegranate polyphenols were able to decrease toxin activity despite allowing a successful growth of C. difficile. We demonstrated that an extracellular heat-labile component synthesized by Clostridium sporogenes decreases the activity of both toxins TcdA and TcdB. This newly discovered C. sporogenes trait is interesting as a potential treatment for CDI or prevention of recurrent infections.

P-06.5-18
Evaluation of kinetic parameters of paraoxonase-1 from the high-curvature region of progress curves

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The kinetic parameters Km (Michaelis constant) and Vmax (limiting rate) remain the basic means to quantify the functioning of enzyme-substrate pairs. Here, we describe the advantages of evaluating them from entire progress curves rather than from initial rates. The advantage of the former is that the progress kinetics analysis is faster and more accurate. [1] However, calculating Km based on the entire progress curve has a drawback, namely that not all points in the curve carry the same amount of information. The highest ratio of signal to noise ratio is carried by the points around the region of maximal curvature. This was noted by Stroberg and Schnell [2]. They presented an equation for estimating the high-curvature region of the progress curve based on predictable Km and Vmax values. Based on their work we have developed an iterative approach to determine Km and Vmax from the high-curvature region of progress curves when none of these parameters is known accurately at the beginning. To demonstrate this practically, we used recombinant paraxonase 1

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enzyme (rePON1). The high-curvature region of progress curves was analysed with the iFIT program developed by Goličnik and Bavec for fitting and determination of Km and Vmax. In comparison to other programs which can fit only whole progress curves, the advantage of iFIT is that it can iteratively adjust the region of data used for fitting the model equation. The results obtained by using this approach were similar to whole fitting progress curves method, but the confidence intervals received for Km values were smaller. PON1 is a human serum enzyme involved in antioxidative metabolism and has been noted as a potential diagnostic marker for different medical conditions. Hence, we propose that any diagnostic tests based on determining Km values of PON1 should include our evaluation procedure. [1] Goličnik M, Bavec A. (2020) J Enzyme Inhib Med Chem. 35, 261–264. [2] Stroberg, W and Schnell, S. (2016) Biophys Chem 219, 17–27.

P-06.5-19
Transcriptomic approach to identification of possible interactions of skin fibroblasts and cancer cells co-cultured in 3D collagen gel

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The development and metastasis of tumors, as well as their response to therapy, are largely determined by the tumor stroma. An important component of the tumor stroma are cancer-associated fibroblasts (CAFs). As a result of the activation by cancer cells, these fibroblasts acquire a phenotype characterized by the expression of CAF marker genes, active remodeling of the extracellular matrix (ECM), and the production of cytokines and growth factors that promote tumor metastasis. Currently, interactions between cancer and stromal cells are actively studied in vitro due to the difficulties of in vivo assessment of this problem. One of the convenient in vitro models for this study is the co-cultivation of cancer and stromal cells in 3D collagen gel. The aim of this work was to identify possible interactions of cancer cells and fibroblasts, which occur during their co-cultivation in a 3D collagen gel. Colorectal cancer cells (lines HT29 or SW480) and pre-stained skin fibroblasts were co-cultured in collagen gel for 5 days, after which they were separated using FACS. RNA was isolated from the collected cells and was subjected to RNA-seq analysis; differential gene expression was determined relative to monocultures of cancer cells and fibroblasts cultured under the same conditions. Analysis of the RNA-seq data showed the activation of skin fibroblasts in these conditions in ways similar to the processes occurring during the recruitment of fibroblasts into the tumor. Also, based on the expression of genes of known ligands and receptors in co-cultured cells, we identified interactions that may exist between cancer cells and fibroblasts. Among these interactions, we found the specific interactions, existing only between fibroblasts and cancer cells. Many of such specific interactions are activated in cancer. Thus, the potential interactions between fibroblasts and cancer cells were found and analyzed. The reported study was funded by RFBR, project number 20-015-00447 A.

P-06.5-20
Analysis of substrate exchange between bulk solvent and buried enzyme active site via multiple molecular tunnels using high-throughput simulations

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A majority of enzymes have their active site buried in the hydrophobic core connected to the surface by transport paths known as tunnels. Understanding how small molecules utilize transport tunnels is of growing interest in the field of structure-based drug design and protein engineering. Haloalkane dehalogenase LinB belongs among typical representatives of enzymes with buried active sites. Recently, a mutant of this enzyme was constructed by closing a primary tunnel and designing a novel tunnel, creating a valuable model system to study ligand transport. In the current study, we have proposed a methodology to effectively study the transport process of a substrate molecule in this model system using high-throughput molecular dynamics guided by Markov state models. We evaluated four schemes for selecting the initial seed positions of ligands to start the adaptive simulations, ranging from randomly placed substrates in the bulk solvent to gradually more knowledge-based positioning of the substrate into the locations relevant for the transport process. This approach enabled us to highlight the robustness of various schemes and rigorously compare the rates connected with the usage of individual tunnels for ligand transport, understand non-trivial paths taken by transported molecules including exploration of high-affinity sites outside the tunnels. Overall, one of the schemes resulted in more kinetically meaningful Markov state models in a very cost-effective manner, facilitating more efficient and accurate analyses to study ligand transport mechanisms in enzymes. This research was supported by POWR.03.02.00-00-I006/17 project; and National Science Centre, Poland (grant no. 2017/26/E/NZ1/00548). Calculations were performed at Poznan Supercomputing and Networking Center.

P-06.5-21
Markov modeling and molecular simulations reveal open and closed state of quorum sensing regulator LasR of *P. aeruginosa*

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Pseudomonas aeruginosa, one of the most dangerous superbugs, is responsible for both acute and chronic infections. Bacterial virulence and biofilm formation are regulated through a system called quorum sensing, which includes transcriptional regulators LasR and RhIR. These regulators are activated by their own natural autoinducers. It is known that LasR's L3 loop in the ligand-binding domain may govern receptor stability, ligand exchangeability, and signal transduction from the ligand-binding domain to LasR's DNA binding domain. So far the available crystal structures of LasR only show closed conformations of L3 loop and ligand-binding domain in the presence of the native autoinducer. In this work, molecular dynamics and Markov state modeling are employed to characterize the L3 loop motion of LasR

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protein without the native autoinducer. The results indicate that the L3 loop is flexible, which can result in an open and closed state, and are crucial for ligand specificity and interaction. The results confirm the high mobility of the L3 loop and the detailed analysis of the loop can help us to understand better how transcriptional regulator LasR interacts with ligands.

P-06.5-22
Building a probabilistic ensemble model of the intrinsically unstructured regions of the post-synaptic density scaffold protein GKAP

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The Guanilate Kinase-Associated Protein (GKAP) is an essential part of an intricate protein network called the Postsynaptic Density (PSD), which is concerned with the transmission of signals between neurons in the brain. The GKAP is a scaffold protein taking part in the regulation of the activity of glutamate receptors and hence the activity of the whole synapse. According to predictions, the GKAP protein is largely intrinsically disordered, save for its GH1 domain, for which there is an experimental structure available. We have developed a pipeline in order to build a structural model of the intrinsically disordered regions of GKAP by creating an ensemble of atomistic three-dimensional structures. Our approach is similar to previously published methods using backbone diamides as building blocks. We are using sequential neighbour dependent probabilities to determine the dihedral angles of the peptide bonds. With this method we have built an ensemble of structures reflecting the local structural preferences of the intrinsically disordered regions. This ensemble model is suitable for subensemble selection and further analysis. The latest results of the analysis will be presented in the conference.

P-06.5-23
Qualitative and quantitative analyses of postsynaptic density complexes

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The postsynaptic density is a dense network of proteins. Its organization and especially reorganization upon stimuli is believed to play a key role in directing and modulating information flow from the synapse towards the cell body. Our main objective is the modeling and analysis of complexes formed by selected postsynaptic proteins in different brain areas containing variable amounts of the constituent proteins. We combine multilevel approaches in order to analyze the organization of the postsynaptic density. We have applied stochastic modeling based on the Gillespie algorithm to predict the emergence and abundance of different protein complexes showing that the presence and prevalence of complexes can not be trivially inferred just from the abundance of component proteins, thus, dedicated modeling provides substantial added value to the understanding of the organization of the PSD. Our approach exemplifies a system-level analysis and provides insights how changes in protein abundance can lead to perturbations of signal processing and transduction. Furthermore we believe that a three dimensional integrative model of the complexes in the postsynaptic density can provide us with a better understanding of their functions and how they affect neurological disorders.

P-06.5-24
A new SINE repeats search method based on the nucleotide correlation

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SINE repeats (short interspersed elements) originated from transposons that can move around a genome and create new copies. It is known that SINE are quite ancient repeats. The copies can differ greatly from the original sequence due to a large number of mutations that occurred after the insertion. This complicates their search by standard similarity-based methods. Our method is based on the correlation of pairs of bases in the studied sequence. The correlation of neighboring bases is considered at the stage of constructing a position-weight matrix and at the stage of the subsequent search. This approach allows to detect distant similarity up to 3 substitutions per position. Previously we used a similar method to search for amino acid repeats and reading frame shifts in genes (Pugacheva VM et al (2016), Suvorova YM et al. (2018)). To evaluate the method, we constructed a set of test sequences. Copies of a SINE repeat with different levels of similarity (the number of random substitutions per base) were inserted into a shuffled chromosome sequence. We inserted full-length copies of the repeat as well as the repeat parts. These chromosomes with insertions were submitted to the input of our program and the RepeatMasker program. On the dataset where inserted sequences had more than 0.75 substitutions per position, our method found 100% of the inserted copies and RepeatMasker found 65% of the full-length copies and only 29% of the incomplete. On the dataset with a substitution level of more than 1.0 per position, RepeatMasker found only 12% of full-length repeats and 3% of partial copies, while our program detected 99% and 44%, respectively. Next, we applied the developed method to search for new copies of SINE repeats in the rice genome (Oryza sativa Japonica). The OsSN1 sequence from SINEbase was used as the initial sequence. As a result of scanning, we found 255 new copies that were not previously described as repeats. The level of false positives was less than 4%.

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P-06.5-25
Effect of disease-causing germline mutations on coiled-coil regions of the human proteome

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Coiled-coils are alpha-helices twist into a superhelical structure, where two or more helices wrap together either in parallel or antiparallel orientations. Their length and rigidity provide the unique functions: they act as molecular spacer helping oligomerization or they play a role in molecular motors, receptors, and signaling molecules. As a result of next-generation sequencing, thousands of Single-Nucleotide Variations have been identified. They can be classified based on their differences in their inheritance to germline and somatic mutations or based on their phenotypic effect on polymorphisms or disease-causing mutations. In the past several years mutational effect on protein structures has been widely studied, but the investigation of coiled-coils from this aspect was not accomplished yet. Our research focused on the mutational effects of disease-causing germline mutations (DMs) in coiled-coils from a structural point of view using sequence-based predictions and structural data. Our results show that DMs accumulate in the N-terminal segments of coiled-coil and that the heptade positions aiding the formation of coiled-coils are affected to a varying degree in different oligomerization states. Furthermore, the structural analysis revealed that most DMs occur in homooligomeric coiled-coils, where they subtly destabilize the structure. Our outcome also suggests that impaired coiled-coils are mostly responsible for central nervous system diseases.

P-06.5-26
Interdomain interactions of the full-length prion protein studied by molecular dynamics simulations

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Prions are protein infectious agents, which cause transmissible neurodegenerative diseases. Prion infection occurs as a result of the post-translational conversion of a normal cellular protein PrPC to a pathogenic and a prone to aggregation PrPSc isoform. PrPC consists of two domains: the unstructured N-terminal and the globular C-terminal. The N-domain includes two charged clusters (CC1 and CC2), a hydrophobic domain (HD), an octarepeat region (OR), and several proteolytic sites (α-, β- and γ-cleavages). Due to the high mobility of the N-domain, the structure of the full-length PrPC has not yet been obtained by experimental methods. In this work, we implemented de novo structure prediction and the molecular dynamics (MD) simulation method to study interactions between the functional domains of the full-length PrPC. The NMR solution structure of the C- domain was taken from the Protein Data Bank (ID code: 1dx0) and models of the N-domain were predicted in I-TASSER. Five top-scoring models were selected and merged with the C-domain to create five starting conformations for 300 ns- long MD simulations of PrPC in water. Interdomain contacts were analyzed using the CONAN package. According to our results, CC2 and α-cleavage regions of the N-domain are in contact with the C-domain for the majority of the simulation time, while OR and HD regions contribute to the interdomain interactions to a lesser extent. The contacts of the α-cleavage and HD are located closer to the C-terminus of the molecule (residues 180–200) and CC2 binds closer to the central part (residues 140–150). Our findings may be used for theoretical descriptions of pathological conformational transitions of the prion protein. The authors acknowledge funding from the Russian Science Foundation (grant № 19-74-20055). Previously published in: Mamchur A.A. et al. (2020) Lobachevskii Journal of Mathematics 41, 1502-1508.

P-06.5-27
Tyrosine phosphatase-inspired redesign of an abzyme bioscavenger

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Recently we developed a potent bioscavenger abzyme against organophosphates, A17-L-L47K. However, to further advance its capabilities by turning it into a full-cycle organophosphatase, a mechanism to reprotonate catalytic tyrosine must be introduced. Taking inspiration from tyrosine phosphatases, we introduced H-A106E substitution to act as a general base. Multiscale modeling demonstrated stable pairing of E106 with the catalytic tyrosine as well as its ability to acquire and retain the tyrosine's proton until the reactivation stage. What is more, the calculated free energy barrier of the inhibition stage of 16 kcal/mol is the lowest of all A17 variants constructed to date, highlighting the importance of a well-behaved catalytic diad in bioscavenger design. We performed multiple Rosetta-powered design iterations to further stabilize the diad and optimize substrate binding. The resulting variants demonstrated a potent ability to bind paraoxon in a productive pre-reaction conformation in silico in addition to low reaction barrier and proton retention. Therefore, our work not only produced promising bioscavenger variants but also paved the way for the future design of a full-cycle abzyme organophosphatase. The reported study was funded by RFBR, project number 19-34-51043, and supported by a grant 21-74-20113 from the Russian Science Foundation. The research is carried out using the equipment of the shared research facilities of HPC computing resources at Lomonosov Moscow State University [2]. 1 Mokrushina, … Lerner, R. A. (2020). Multiscale computation delivers organophosphorus reactivity and stereoselectivity to immunoglobulin scavengers. PNAS USA, 117 (37), 22841–22848. 2 Supercomputer Lomonosov-2: Large Scale, Deep Monitoring and Fine Analytics for the User Community. (2019). Supercomputing Frontiers and Innovations (Vol. 6, Issue 2).

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P-06.5-28
Allosteric effects of antibiotics and resistance modifications in the exit tunnel of bacterial ribosome revealed by MD simulations

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The ribosome is a protein biosynthesis machine and a target of significant amount of clinically used antibiotics. About a half of ribosomal antibiotics interact with the nascent peptide exit tunnel (NPET) and inhibit peptidyl transferase reaction (PTR). Mechanism of allosteric impact of antibiotics bound in the NPET on the PTR is still unknown, however, it is proposed to be connected with the decreased affinity of the A-site to an aminoacyl (aa)-tRNA. We investigated 70S E. coli ribosomes in different conformational states using molecular dynamic (MD) simulations. Introduction of erythromycin or chloramphenicol into the NPET of prevoisly obtained A/A, P/P ribosome system before the PTR induced destabilisation of interactions between the CCA-end of aa-tRNA and the A-site through reorientation of the Ψ2580 residue. This is in line with the published mutagenesis studies of Ψ2580. Additional introduction of so-called stalling peptides, which are necessary for translation arrest in the presence of antibiotics, inhanced this effect and provided severe mutual disorientation of functional groups participating in the PTR. To the contrary, resistance modification of the m2A2503 residue in the NPET, particularly its 8-monomethylation, did not affect any peptidyl transferase center (PTC) or NPET residue conformation except the proximate neighbors of this residue. However, in the P/P, E/E state of the ribosome, where the A-site conformation completely prohibits the aa-tRNA binding, this resistance modification allosterically returns certain NPET residues into their A/A, P/P state, where the ribosomal A-site is adjusted for the aa-tRNA binding. These residues were previously described as putative NPET-PTC allosteric pathways paricipants according to mutagenesis studies. The calculations were performed using Lomonosov-II supercomputer of Lomonosov Moscow State University. The study was funded by the Ministry of Science and Higher Education of the Russian Federation (FENU 2020-0019).

P-06.5-29
A comparative analysis mechanisms of vascular calcification between coronary artery and aorta from human clinical data

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Introduction. Vascular calcification is a deficit associated with classical age-related diseases such as atherosclerosis and type II diabetes. It manifests as deposition of calcium phosphates in arteries thus increases odds of stroke. Purpose. We aimed to compare molecular mechanisms of vascular calcification in aorta and coronary artery in subjects from GTEx dataset. Methods. First, hematoxylin and eosin (H&E) staining of aorta and coronary artery were visually validated for presence of calcification and grated accordingly. Samples with non-vascular tissue contamination were excluded, which left 157 aorta samples and 88 coronary artery samples for further analysis. Change in expression of protein-coding genes was tested versus calcification severity using sex and cause of death as covariates. Statistical analysis was performed in R. Results. BMP2, a driver of calcification, was up-regulated, while MGP, calcification inhibitor, was down-regulated in both tissues, thus confirming the validity of our approach. Aorta and coronary artery shared 7 and 10 upregulated and downregulated genes, accordingly. Given the high number of differentially expressed genes (DEGs) in calcified aorta (162) and artery (1568), the tissues poorly overlap in transcriptome changes. However, both tissues largely intersect by KEGG pathways. We observed down-regulation of metabolic pathway and up-regulation of inflammation which was previously suggested to drive vascular calcification. Conclusions. Calcified aorta and coronary artery are poorly overlapped by DEGs, however, largely overlap by pathways, and demonstrated inflammatory response as a possible driver in both tissues. The publication was prepared with the support of the "RUDN University Program 5-100".

P-06.5-30
In silico study of nisin/lipid II molecular recognition

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Given the challenge of global antibiotic resistance, the development of new medications is indispensable. Lipid II - bacterial cell wall precursor – is a promising pharmaceutical target for innovative antibiotics, whereas lantibiotic nisin, effectively capturing lipid II's conservative pyrophosphate group, is a potential prototype of a new generation of antibiotics. Because the structure of membrane-bound lipid II/nisin complex is lacking, we studied their recognition via molecular dynamics (MD) simulations. As a result, the medium-driven dynamics of both partners in their parent environments was explored. The N-terminal 11-residue fragment of nisin, which recognizes lipid II in bacterial membrane, adopts a unique closed-ring conformation only in water solution - this was proven by recent NMR study. In this state, the peptide NH groups of the ring A orient toward a common center, forming a pool of H-bond donors. Based on MD data, it was shown that nisin in this conformation forms the most stable complex with pyrophosphate analogues mimicking the binding determinant of lipid II. Here, we describe the results of the detailed in silico study of nisin1-11 structure and dynamics in different solvents. Efficient conformational sampling and clustering of the MD states based on backbone coordinates and dihedral angles was performed. The results obtained were found to be environment-dependent. These findings may be further employed to improve the peptide structure in order to design its new pharmaceutically applicable forms. Acknowledgements: This research was supported in the framework of the Basic Research Program at the National Research University Higher School of Economics and Russian Academic Excellence Project '5-100'. Supercomputer calculations were sponsored by the Russian Science Foundation (19-74-30014).

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P-06.5-31
Molecular docking of small molecules that inhibit vimentin-plectin interaction

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Vimentin forms a major type of cytoskeleton intermediate filaments that participate in many cell processes including cell migration. Particularly, it was shown that vimentin determines migration directionality both in filament form and in the form of ULF (unit-length filament), possibly due to its interaction with adaptor protein - plectin. Therefore, potentially, vimentin-plectin interaction is a new target for searching and testing small molecules inhibiting this interaction in order to lower the migration capacity of the cells. According to literature, the interaction sites of vimentin and plectin were both identified: the fifth plakin repeat domain (PRD) and a plectin linker from the plectin side and a 1B segment (PDB ID 3UF1) from the vimentin side. Here, the homology model of the fifth PRD and the linker of plectin were constructed based on structures PDB ID 5DZZ and PDB ID 4Q28. The protein-protein docking was performed and the amino acids that participate in the interaction of vimentin and plectin were identified. Based on the determined binding site, a library of small molecules (around 1 million compounds) was docked to vimentin and the substances were ranked according to the docking score and MM-GBSA parameters. Additionally, the docking scoring function was calculated for evaluation and verification of substances. Finally, two molecules, Amikacin and Paromomycin, were chosen for further in vitro experiments to verify their influence on cell stiffness and cell migration. The research was carried out using the equipment of the shared research facilities of HPC computing resources at Lomonosov Moscow State University. The reported study was funded by RFBR, project number 19-34-90178.

P-06.5-32
Extracting enzyme kinetic parameters from time-course data with a novel iterative automated procedure

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To one who wishes to quantify the functioning of an enzyme, the kinetic parameters Km (Michaelis constant) and Vmax (limiting rate) remain the basic means to assess an enzyme-substrate pair. These parameters are still widely determined in terms of initial rate measurements at different substrate concentrations, and plotting the results in a linearized form, usually as the Lineweaver-Burk diagram. An alternative approach for determination of these parameters is based on the entire progress curves by direct fitting of reaction model on time-course data. However, determining Km based on the entire progress curve has a considerable weakness, namely that not all points in the curve carry the same amount of information. The points at the initial linear part and those at the plateau of the progress curve carry less information compared to noise, while the points around the maximal curvature have the best signal-to-noise ratio. An equation for estimating the high-curvature region of the progress curve based on predictable Km and Vmax values was presented by Stroberg and Schnell. Based on their theoretical work we have developed an iterative approach, a computer program iFIT, to determine Km and Vmax from the high-curvature region of progress curve when none of these parameters is known accurately at the beginning. Other fitting programs such as Dynafit or GraphPad can fit only whole progress curves, therefore the advantage of iFIT is that it can iteratively adjust the region of data that are used for fitting model equation. The results obtained by using this iterative approach are similar or even more accurate to whole fitting progress curves method. In comparison to other fitting programs, the iFIT can quickly process many progress curves and thus is appropriate tool for large scale biological data analysis, especially in working environments, such as diagnostic and pharmaceutical laboratories where speed and accuracy matter.

P-06.5-33
The spatial structure of the mutant form of carboxypeptidase T, which mimics the site of primary specificity of carboxypeptidase B

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Carboxypeptidase T from Thermoactinomyces vulgaris is a bacterial carboxypeptidase that has broad substrate specificity compared to mammalian carboxypeptidases, for example, carcinoma peptidase B from porcine pancreas. To determine the structural foundations of the substrate specificity of carboxypeptidase T, we previously performed five mutations in the pocket of the primary specificity of carboxypeptidase T: G215S, A251G, T257A, D260G, T262D. However, kinetic studies have shown that substrate specificity has not changed with the introduction of these mutations. In this study, we present the structure of a mutant form of carboxypeptidase T, which further mimics carboxypeptidase B in the active center loop: G215S, Q249G, A251G, T257A, D260G, T262D, Ins253T, L254I. The spatial tructure of this mutant is even closer to carboxypeptidase B and, possibly, will reveal the missing determinants of substrate specificity of carboxypeptidase T. The study was partially supported by the Federal Space Program of Russia for 2016–2025 (the development project, "International Space Station, the Multipurpose Laboratory Module Nauka" and by the Russian Foundationfor Basic Research (RFBR №19-0400220). *The authors marked with an asterisk equally contributed to the work.

P-06.5-34
The bioinformatic identification of potential antimicrobial peptides from the metagenome profiling of Hirudo medicinalis

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The global problem of microbial resistance requires the development of new therapeutic agents. Antimicrobial peptides (AMPs) are a promising solution to the problem on account of they

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effectively destroy microorganisms and have a number of advantages over antibiotics. The microbial community of the medicinal leech Hirudo medicinalis is involved in digestion, protection from pathogens, and regulation of immunity. Our investigation of the H. medicinalis genome carried out previously [Babenko VV et al. (2020) BMC Genomics 21, 20–21] allowed for sequencing and annotation of the H. medicinalis metagenome. We applied a bioinformatic algorithm to de novo assembled microbiome proteins to discover new AMPs. Proteins with potential antimicrobial properties were retrieved using the DBAASP v2. server. Further, the AMPA server was used to define the amino acid sequence in proteins responsible for the putative antimicrobial effect. The antimicrobial properties of peptides were estimated by using the available online algorithms (ADAM, CAMP–R3, iAMP–2L, AmPEP). The prediction of the secondary structures of candidate peptides was fulfilled by using the I–TASSER–MR server. As a result, we choose five peptides with the highest antimicrobial propensity scores, which can adopt the α–helical secondary structure. Testing the activity of the peptides against different bacteria (E. coli K12 substr MG1655, B. subtilis 168HT, S. aureus ST 88 14.8, S. haemolyticus 527 14.8) showed that the peptides actually exhibited bactericidal activity. The method implemented in our study may be applied to screen data of other organisms to identify new antibacterial agents. The research was supported by the Russian Science Foundation (project № 20–15–00270).

P-06.5-35
Molecular modelling of TATA-box related short nucleotides

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Nucleic acids are important molecules to store and use the information to synthesize the functional molecules like proteins or enzymes for the sustainibility of the metabolic functions. Interestingly, some of these synthesized proteins bind to short DNA sequences in promoter region to initiate RNA synthesis. In current work, we investigated short single nucleotide (di-tetramer) chains that are related to TATA box, a protein-binding site. The Spartan'14 software was used to obtain the initial structures for all possible combinations of the di, tri and tetramer structures of the TATA sequence. PM6-D3H4 method in MOPAC2016 was used for optimizations to reduce the number of structures. Selected structures were optimized with Gaussian09 program at ωB97XD/6-311++G(d,p) level and frequency calculations were carried out at the same level. The results revealed the most stable structures and possible intramolecular interactions of the investigated systems. The main observation was the formation of–H bond between the –OH group of the ribose ring and -PO4 group of the nucleotide structure. Additionally, we observed interactions between nucleobase conjugates (A=T) in tetramers and CH−π non-covalent interaction due to nucleobases. Most of the calculations were performed on TUBITAKULAKBIM Truba resources and this study was supported by Ege University Research Grants (BAP, Project No. 18 FEN007).

P-06.5-36
Single cell RNA sequencing of MSC in profibrotic microenvironment reveal senescence-associated transcriptomic pattern

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The local signals from the microenvironment play a crucial role in the development of fibrosis, and accumulated data indicate the important contribution of induced cell senescence into these processes. Multipotent mesenchymal stromal cells (MSCs) are able to suppress fibrosis due to the antifibrotic effects of their secretome. However, profibrotic microenvironment, including excessive amount of extracellular matrix (ECM), specific cytokines and growth factors, could severely affect MSC properties by triggering cell senescence. To test this, we cultured human adipose-derived MSCs for 4 days in the presence of a key profibrotic factor TGFb on fibrotic-like decellularized ECM produced by fibroblasts. Single cell RNA-seq databases were created from the samples and processed (10 × Genomics). Using Loupe Browser, we analyzed the senescence-related genes differentially expressed in MSCs under profibrotic versus standard conditions. We found that profibrotic microenvironment initiated in the most of MSCs cell cycle arrest (reduction of cyclins and CDKs mRNA with an increase in cell cycle inhibitors: p15, p16, p57), which was confirmed by an increase of p21 protein expression. Other transcriptomic changes also indicated MSC senescence: redistribution of chromatin in the nucleus (loss of laminB1 mRNA), changes in epigenetic enzymes (↓DNA-methyltransferases), maintenance of histone methylation proper for euchromatin (↓DOT1L,↑KDM6B). The specific metabolic activity of MSCs in profibrotic conditions was characterized by an increase in transcript of mTORC1/2 inhibitor DEPTOR coincided with an increase in SA-β-gal activity. Expression of senescence-associated secretory phenotype (SASP) components, such as IGF-1 and MMP-2, was also increased under profibrotic stimuli. Thus, we demonstrated the ability of the profibrotic microenvironment to induce the senescence of MSCs. Whether it affects MSC antifibrotic effects needs further research. The study was supported by RFBR (#19-29-04172).

P-06.5-37
Data integration pipeline for the reconstruction of context-specific genome-scale models

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Omics data can be integrated with a reference model using various model extraction methods (MEMs). to yield context specific genome-scale metabolic models. This process is marred with challenges such as choice of MEM, choice of threshold to determine which genes are actively expressed, etc. We set out to assess the impact of MEMs and thresholding on the model outcome and to propose a data integration pipeline for an effective analysis of omics data using GEMs. We integrated mouse transcriptomic

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data from a Cyp51 knockout mice diet experiment (GSE58271) using five MEMs (GIMME, iMAT, FASTCORE, INIT and tINIT) in a combination with iMM1865 model. Two kinds of thresholding were applied, i.e within a sample for all the genes and within a gene for all the samples. four thresholds in each type were set at different percentiles. Principal component analysis and Jaccard index on present/absent reaction matrix were used to assess the efficiency of MEMs and impact of thresholds. ANOVA and student's t-test were used for statistical analysis. Except for INIT and tINIT, the size of extracted models varied with a MEM used (t test: P-value <0.001). iMAT models varied the most, in which jaccard index spanned from 0.27 to 1.0. PC1 from iMAT explained the most variability (40%) in the data followed by FASTCORE at 14%. The highest variance explained by PC1 was achieved by thresholding at the 80th percentile per sample. Of the three factors (diet, gender and genotype) under study in the experiment, gender explained most of the variability (>90%) in PC1 for FASTCORE. In iMAT, each of the three factors explained less than 40% variability within PC1, PC2 and PC3. Based on gender, only FASTCORE captured the true variability in the data. Efficient use of MEMs requires one to apply various MEMs and thresholds in order to choose the MEM that best captures the true variability in the data. Our future work is to develop a computational pipeline for efficient analysis of GEMs.

P-06.5-38
DNAmoreDB, a database of DNAzymes

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Deoxyribozymes, DNA enzymes, or simply DNAzymes are single-stranded DNA molecules that, like proteins and ribozymes, possess the ability to perform catalysis. Although DNAzymes have not yet been found in living organisms, they have been isolated in the laboratory through in vitro selection. The selected DNAzyme sequences have the ability to catalyze a broad range of chemical reactions, utilizing DNA, RNA, peptides, or small organic compounds as substrates. DNAmoreDB is a comprehensive database resource for DNAzymes that collects and organizes the following types of information: sequences, conditions of the selection procedure, catalyzed reactions, kinetic parameters, substrates, cofactors, structural information whenever available, and literature references. Currently, DNAmoreDB contains information about DNAzymes that catalyze 20 different reactions. We included a submission form for new data, a REST-based API system that allows users to retrieve the database contents in a machine-readable format, and keyword and BLASTN search features. The database is publicly available at https://www.genesilico.pl/DNAmoreDB/. Ponce-Salvatierra A, Boccaletto P, Bujnicki JM. (2021) NAR 49(D1), D76–D81.

P-06.5-39
Development of the systems biology model of platelet cell death for the study of hematological disease pathogenesis

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Human platelets are non-nuclear blood cells, responsible for the preservation of the blood system integrity. Upon blood vessel wall disruption, fibrillar collagen becomes exposed. Collagen initiates thrombus formation via platelet activation through the GPVI receptor. The main blood coagulation protease, thrombin, also strongly activates platelets through PAR1 and PAR4. Strong platelet activation induces mitochondrial-dependent necrosis, which is essential for blood coagulation (Sveshnikova et. al. JTH 2016). Signaling pathways leading to platelet necrosis are not yet established. Here we developed the comprehensive systems biology model of the platelet activation by collagen and thrombin and characterization of the platelets of patients with hematological diseases. The model comprised collagen-induced GPVI activation, resulting in PLCγ2 activation and thrombin-induced PAR1 and PAR4 activation, resulting in PLCβ activation. PLCβ and PLCγ2 produced IP3, leading to calcium release from the intracellular stores. Cytosolic calcium could be sequestered in the mitochondria, which eventually overloaded with calcium thus inducing platelet necrosis. The underlying differential equations were solved using the LSODA method. The model validation was performed against flow cytometry (BD FACS Canto II) data on platelet calcium signaling. 10 patients with the Wiskott-Aldrich Syndrome, 10 patients on therapy with BH-3 mimetics, and 10 healthy donors were studied. The model revealed that the increased platelet cell death in the studied patients was associated with the mitochondria being more prone to collapse. This was in agreement with published data on platelet functioning and signaling in such patients. Thus, the developed systems biology model of platelet signaling is applicable for the determination of the disease pathogenesis. The reported study was funded by RFBR and the Royal Society of London (RS), project number 21-51-10005 and Russian Presidential Fellowship SP-2675.2019.4

P-06.5-40
Whether neutrophil movement around growing thrombi is related to chemotaxis?

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Thromboinflammation, the interplay between thrombosis and inflammation, is thought to be driven mainly by the interactions

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between neutrophils and blood platelets. When activated at the site of injury, platelets secrete several factors regulating the coagulation and immune response including chemoattractants for neutrophils – CXCL1-7, IL-8. The movement of neutrophils in the area of growing thrombi was observed both *in vivo* and *ex vivo* [1]. The aim of this work is to answer the question of whether the movement of neutrophils could be guided by chemoattractants secreted by platelets. In this work, the previously described approach [1] was used to image thrombi in whole blood using flow chambers. Image processing and calculations were performed using ilastik software (www.ilastik.org). We have constructed a computer model of the distribution of a platelet-released chemoattractant (corresponding to IL-8 in concentration of 10 pM and diffusion coefficient D = 300 $\mu m^2/s$) in a flow chamber using COMSOL Multiphysics (www.comsol.com). Using Python, we have constructed a model of neutrophil chemotaxis in the field of chemoattractant. We showed that the movement of neutrophils in the flow chamber does not have a preferred direction, and, therefore, is not caused by the liquid flow. We also showed that, on average, neutrophils are located closer to thrombi than if they were distributed randomly. A theoretical analysis of the direction of movement of neutrophils showed that it partially occurs in the direction of an increase in the concentration of chemoattractants. The constructed mathematical model of neutrophil movement is capable of describing the experimental trajectory of a neutrophil in 60% of cases. Thus, we suggest that the movement of neutrophils around growing thrombi could be considered as chemotaxis. The study was supported by Russian Science Foundation (Grant 21-74-20087). [1] Previously published in: DS Morozova, et al., bioRxiv 2020.07.13.199174, 2020.

P-06.5-41
Transcriptome profiling of polyunsaturated fatty acids cascades genes in neurodegenerative diseases

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Polyunsaturated fatty acids (PUFAs) cascades include the metabolism of w-3/ w-6 acids, i.e. elongation and desaturation processes, transport, degradation, as well as synthesis of oxylipins via the lipoxygenase (LOX), cyclooxygenase (COX), and epoxygenase (CYP) branches of transformation into prostaglandins, leukotrienes and other compounds acting through nuclear and membrane receptors. PUFAs and oxylipins are active participants of inflammatory processes and therefore they are under intensive investigations associated with the study of the systemic disorders biology, including neurodegenerative diseases. The aim of this work is to evaluate changes in the expression of PUFAs cascades genes in various tissues of patients with neurodegenerative diseases. Datasets were taken from GEO and PDBP databases. We analysed the following datasets: GSE135036 (Parkinson's disease); GSE138614 (multiple sclerosis); GSE125583 (Alzheimer's disease); GSE124439 (amyotrophic lateral sclerosis). Salmon software (v1.0.0) was used for wicked-fast transcript quantification from RNA-seq data. The expression was summarized at the gene and transcript levels using R tximport. All available metadata about patients is included in the design as covariates. When evaluating a differential expression, 2 boundaries of the adjusted value of the pplotDispEsts significance level (0.05 and 0.01) were selected. When studying the distribution of gene expression by autosomes and X chromosome, the Core preprocessor package R for quantum normalization and the Kolmogorov-Smirnov pair test was used to test the differences between samples with high and low XIST gene expression. The involvement of PUFAs cascade genes in the inflammatory processes accompanying studied diseases was estimated. The dependence of expression parameters and patients clinical data is discussed. This study was supported by the RFBR research project № 19-29-01243.

Biochemistry of toxins

P-07.1-01
Impact of selected pesticides on cholinesterase efficiency

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Residual presence of pesticides in a variety of fruits, vegetables, and agricultural crops can nowadays often exceed maximum residue limits. Exposure to organophosphorus pesticides through direct contact, ingestion, or inhalation can have a deleterious effect on human health; additional residue in food also contributes to the risk of exposure. The neurological system is particularly susceptible to adverse effects from either direct pesticide exposure or through food. Selected pesticides such as methamidophos, fenamiphos, ethoprophos, phosalone, and acetamiprid are pesticides with the possible potential to inhibit acetylcholinesterase (AChE), the essential enzyme for neurotransmission. Disruption of AChE biosynthesis process by certain pesticides has also been evidenced and was considered as one of the mechanisms for the long-term effects of these compounds. In order to fully understand the action of these pesticides, we investigated their effects and mechanisms on the enzymatic activity of both AChE and its analog enzyme butyrylcholinesterase (BChE). The objective of this study was to reveal alterations caused by selected pesticides on the enzymatic activity and expression of AChE in selected cell lines. Moreover, a possible detoxication mechanism was tested, taking into account BChE as an endogenous bioscavenger of xenobiotics. Acknowledgment: This study was supported by the Croatian-Chinese Scientific and Technological Cooperation (2019–2021) and partially by the Croatian Science Foundation (IP-2018-01-7683).

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P-07.1-02
The inhibitory effect of Moroccan cobra *Naja haje legionis*' venom and its toxic fraction on Hedgehog-dependent medulloblastoma DAOY cell growth

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The cobra *Naja haje legionis* (Nhl) is the only representative of the elapid family in Morocco. It belongs to the *Naja haje* species which can be prominently found in North Africa and is mainly known for its composition in various molecules especially enzymes and toxins responsible for neurotoxic, cardiotoxic and myotoxic effects. In the present work, we investigated the proteome of *Naja haje legionis*' crude venom and isolated toxic fraction for their potential antiproliferative activity on hedgehog-dependent human medulloblastoma cancer cells (DAOY) as well as a non-cancerous cell line (MEF) as control. To this end, we conducted proliferation and mortality assays using increasing concentrations of the Nhl crude venom and its toxic fraction (6.25, 12.5, 25, 50 and 100 μg/mL). The results were observed after 24, 48 and 72 hours. The results showed that the Nhl crude venom had an effect on cell growth in both cell lines from the lowest concentration (6.25 μg/mL) as well as inducted a fast increase in cell mortality rates as soon as 24 hours into the experiment. As for the toxic fraction, no growth inhibition was observed on the non-cancerous MEF cells as opposed to the DAOY cancer cells where a decrease in cell growth was achieved from the lowest concentration (6.25 μg/mL). As of yet, this study is the first report showing the potential of the *Naja haje legionis* venom as an alternative therapeutic approach for cancer therapy and will prospectively further explore the venom to specifically identify the molecules responsible for the antitumoral activity.

P-07.1-03
Interaction between group IIA secreted phospholipases A2 and mitochondria

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Group IIA secreted phospholipase A_2 (GIIA) is a mammalian orthologue of ammodytoxin (Atx), a β-neurotoxic GIIA (β-ntxs) from the snake venom. It plays both physiological and pathological roles in mammalian brain. Physiologically, it is involved in regulation of neurotransmission, neuritogenesis and mitochondrial homeostasis, while pathologically, it is implicated in neurodegenerative and cerebrovascular diseases. The action of GIIA in these pathologies is far from being clear on the molecular level. To reveal them, β-ntxs may be very useful as they inflict apparently identical damage to neuronal mitochondria as GIIA. Using Atx, we identified a high affinity $sPLA_2$ membrane receptor in neuronal mitochondria as the subunit II of cytochrome c oxidase (CCOX-II), the essential constituent of the respiratory chain. We have shown in isolated mitochondria as well as on rat brain tissue sections that Atx inhibited the enzymatic activity of CCOX. Interestingly, the observed inhibitory effect of Atx was not dependent on its phospholipase activity, as also the enzymatically inactive mutant of Atx was able to induce it. Using heterologous competition assay, we demonstrated that mammalian GIIA binds to CCOX-II with a 100-fold lower affinity than Atx. Homologous competition assay using ^{125}I-GIIA, however revealed that endogenous $sPLA_2$ primarily targeted another binding protein in mitochondria, which is lighter than CCOX-II. Taken together, our results suggest the explanation of the mechanism by which β-ntxs hinder production of ATP in the poisoned nerve ending and open an important direction of study to advance the understanding of the involvement of the mammalian GIIA in mitochondrial function and dysfunction.

P-07.1-04
Structure-function relationship of broad-range phospholipase C from *Listeria monocytogenes*

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Broad-range phospholipase C (PC-PLC) is a metalloenzyme and a crucial virulence factor of *Listeria monocytogenes* (Lm). Crucial step in Lm pathophysiology is the escape from the lipid encased phagosome after internalization. Disintegration of the phagosome membrane is facilitated by three key bacterial proteins: pore-forming cholesterol dependent cytolysin listeriolysin O (LLO), and two listerial phospholipases C: PC-PLC and PI-PLC. PC-PLC can hydrolyse a variety of lipid substrates, including those having phosphatidylcholine, phosphatidylserine, phosphatidylethanolamine headgroups as well as sphingomyelin. We recombinantly expressed the enzyme and purified PC-PLC to high concentrations, enabling us crystallisation and determination of its crystal structure at 2.0 Å resolution. With structure solved, we searched for mutants retaining wild-type protein fold, while exhibiting lower enzymatic activity. The prime candidate was N-terminal Trp1 residue, which coordinates one of the Zn^{2+} ions and anchors the N-terminus in the hydrophobic core of the protein. We prepared mutants where W1 was replaced by A, E, F, or K or was deleted (dW1S2). While all mutated proteins retained wild-type fold, they had mostly reduced enzymatic activity: wild-type W1 ~ W1F >> W1A > W1E ~ W1K ~ dW1S2. To decipher the interplay between LLO and PC-PLC, we pre-incubated POPC/SM/cholesterol lipid vesicles with PC-PLC. PC-PLC caused significant increase in LLO binding to liposomes and LLO induced vesicle leakage, while PC-PLC on its own did not cause any permeabilization. Preincubation with less active PC-PLC mutants resulted in reduced LLO binding and lower vesicle leakage. Those findings suggest that activity of PC-PLC may increase the availability of membrane cholesterol. Further structure-based functional studies of PC-PLC with LLO are in progress, aiming towards a better understanding of the mechanism and interplay between these two toxins of Lm.

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P-07.1-05
Phospholipase A2 way to hydrolysis: bee venom PLA2 pulls apart lipid polar heads on bilayer surface

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Phospholipase A2 (PLA2) from bee venom has a wide range of biological effects and attracts a lot of attention of researchers. Two sites are involved in manifestation of enzymatic activity: catalytic site (CS), responsible for substrate binding and fatty acid cleavage from the sn-2 position of a glycerophospholipid, and interface binding site (IBS), responsible for the protein binding to lipid membrane. IBS is formed by positively charged and hydrophobic amino acids on the outer surface of the protein. Understanding the mechanism of PLA2 interaction with the lipid membrane is the most challenging step in biochemistry of this toxin. We used combination of experimental (fluorescent spectroscopy, FRET measurements) and computer simulation (full atom molecular dynamics) techniques to investigate molecular details of bee venom PLA2 interaction with lipid bilayer formed by palmitoyloleoylphosphatidylcholine or dipalmitoylphosphatidylocholine. We report that upon initial interaction the enzyme induces the formation of large hydrophobic areas on surface of the fluid phase bilayer, in contrast to bilayer bending with hydrophobic tails disordering, for gel phase bilayer. The process is driven by amino acids capable of the formation of hydrogen bonds with phosphate groups of the lipids. We assume that relative ability of a bilayer to come through lipid redistribution process defines the rate of the initial stages of the catalysis. Thus, gel phase bilayers are known to be hydrolyzed more slowly, due to lower lateral lipid mobility, and the formation of hydrolysis products accelerates the reaction, as fatty acids and lyso-lipids promote lipid redistribution. For more details: Alekseeva et al., BBA-Biomembranes, 1863 (1), 183481, 2021 (10.1016/j.bbamem.2020.183481). The work was supported by the Russian Science Foundation (project no. 19-75-00101).

P-07.1-06
Chronic low-dose exposure of human granulosa cells to the mixture of endocrine disruptors alters steroidogenesis in human granulosa cells *in vitro*

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Endocrine disrupting chemicals are present in numerous human body fluids, including follicular. In the past decades, the individual effects of those chemicals were studied and there are evidences that they have negative influence on ovarian granulosa cells' function. However, the prevalence of some disorders cannot be explained by the influence of only one endocrine disruptor. Studying effects of chronic exposure to low doses of chemical mixtures is necessary to mimic the real-life exposure. The aim of this study was to investigate the effects of chronic exposure to chemicals at concentrations found in human follicular fluid on the function of human granulosa cells (HGrC1). The mixture (MIX) was made of bisphenol A [2 ng/mL], polychlorinated biphenyl 153 [70 pg/mL], benzo[a]pyrene [1 ng/mL] and perfluorooctanesulfonate [100 pg/mL]. Treatment lasted for 4 weeks, and the granulosa cells' function was evaluated every week. The results showed that chronic exposure to MIX had no effects on cell morphology, but transiently decreased cell viability after 3 weeks of exposure, which returned to control values by the end of treatment. Production of estradiol decreased after 2 and 3 weeks, returning to the control level after 4 weeks of exposure. The mRNA levels of CYP19A1, enzyme responsible for estradiol biosynthesis, increased after 2 weeks of MIX treatment. However, CYP19A1 protein levels decreased after 2 weeks, explaining decline in the estradiol production in the MIX-exposed HGrC1. MIX increased the production of progesterone after 3 and 4 weeks of treatment, without affecting the mRNA or protein levels of steroidogenic acute regulatory protein (StAR), protein that regulates rate-limiting step in the production of progesterone. These results indicate that chronic exposure to low doses of endocrine disruptors mixture leads to imbalance in estradiol and progesterone production, which could have negative impact on ovarian function and fertility.

P-07.1-07
Modulation of platelet functions by European toad (*Bufo bufo*) skin secretions components

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A growing number of reports indicate that amphibian skin secretions may have a remarkable medical importance; however, the effects of the components of some dermal secretions on blood platelets and hemostasis are inadequately recognized. Since our previous studies demonstrated that the active fraction (Superdex G200 pg, ion-exchange chromatography on DEAE-C) of *B. bufo* skin secretions induced platelet aggregation in platelet rich plasma, this work was designed to study the effects of this active fraction on the adhesion, activation, and aggregation processes of the isolated rabbit platelets *in vitro*. The effects of the fraction on the adhesion of blood platelets to fibrinogen, collagen, and albumin were studied using acid phosphatase assay. The studied fraction was shown to induce platelet adhesion onto fibrinogen-coated surface. The effects of this fraction were also examined on some processes that involved in platelets activation: $[Ca^{2+}]_i$ mobilization, Akt phosphorylation, and serotonin secretion. The results demonstrated that the studied fraction facilitated mobilization of intracellular ionized calcium: the released $[Ca^{2+}]_i$ level in platelets treated with the fraction was the same as in the platelets stimulated by collagen. Moreover, the fraction attenuated platelets Akt phosphorylation, but had no effect on platelet serotonin secretion. It was also confirmed that the studied fraction in a dose-dependent manner induced aggregation in the isolated platelets. To make sure that no platelet damage, which could lead to misinterpretation of the data, occurred, membrane integrity was determined using lactate dehydrogenase assay. No increased LDH release was recorded while testing the studied fraction. The results suggest that the components of European toad skin secretions may be a promising source of natural compounds which can modulate platelet functions and might be used for the treatment of diseases that involve aberrant platelet function.

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P-07.1-08
Characterization of VaaMPIII-3, the first member of a novel P-IIIe subclass of snake venom metalloproteinases

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Snake venom metalloproteinases (SVMPs) typically interfere with hemostasis and cause hemorrhage in the prey. They are classified into three classes (P-I, P-II, P-III) according to their structure. P-III SVMPs consist of a metalloproteinase (MP), a disintegrin-like (D) and a cysteine-rich (C) domain, while the other two classes emerged by the loss of the C and/or D domain. A protein named VaaMPIII-3, consisting of only a truncated D domain and a C domain, was found in the venom of the nose-horned viper (*Vipera ammodytes ammodytes*). Regarded as products of autolysis of P-III SVMPs, similar proteins have been found previously in other viperid venoms; however, the origin of VaaMPIII-3 was shown to be different. Resulting from an evolutionary MP domain loss, VaaMPIII-3 defined a new P-IIIe subclass of SVMPs (Leonardi A et al. (2019) J Proteome Res 18, 2287–2309). The aims of this study were to biochemically characterize the protein and to describe its effects on blood. An efficient isolation procedure, including size-exclusion, cation-exchange, and covalent chromatography, was developed to purify VaaMPIII-3 from crude snake venom. Consistent with its primary structure, the existence of a free cysteine residue in VaaMPIII-3 was confirmed. This Cys was responsible for formation of a covalent dimer, nevertheless, the predominant form of VaaMPIII-3 in the venom was monomeric. VaaMPIII-3 is a highly glycosylated 21 kDa protein present in 6 isoforms with isoelectric points between pH 4,5 and 5,1. It inhibited platelet aggregation induced by ADP, collagen, or arachidonic acid, but not platelet agglutination triggered by ristocetin. This makes VaaMPIII-3 interesting as a starting compound for development of innovative antithrombotic drugs. To further investigate structural determinants underlying the physiological effects of VaaMPIII-3, a 3D model of its structure was constructed and procedure to produce the recombinant molecule and its mutants established.

P-07.1-09
Visualization of unusual oligomers of Cyt2Aa toxin from *Bacillus thuringiensis* on model membrane systems and in aqueous solutions

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Bacillus thuringiensis (Bt) is a Gram-positive bacterium, which produces insecticidal proteins during the sporulation phase. These toxins are located in parasporal crystals consisting of two delta-endotoxin families, Crystal (Cry) and Cytolytic (Cyt) toxins. *In vitro*, Cyt toxins display cytolytic activity toward bacterial, insect and mammalian cells. Although Bt is being used as a bioinsecticide, the mechanism by which Cyt proteins disrupt the membrane remains unknown. There are two proposed models of Cyt membrane integrity disruption: the pore-forming model, where multiple monomers come together and form an oligomeric pore in the membrane, and the detergent-like model, where monomers aggregate on the membrane surface until they reach a critical concentration, which causes membrane disintegration in a detergent-like manner. In an attempt to shed light on the mechanism of this potentially commercially important protein, we have focused our research on visualization of oligomerized Cyt2Aa on model membrane systems such as multilamellar lipid vesicles (MLVs) and planar lipid membranes. When incubated with lipid vesicles the protein forms oligomers on the lipid bilayer. We visualized the oligomers with Native-PAGE, conventional negative stain transmission electron microscopy (nsTEM) and cryo-EM. nsTEM revealed chain-like Cyt2Aa oligomers bound to the vesicles but we did not find any classical ring-shaped pores. At longer incubation times, oligomers were also present in the area surrounding the vesicles. We prepared solubilized oligomers by incubating Cyt2Aa with MLVs and then dissolving the vesicles and studied them with cryo-EM, which revealed chain-like oligomers similar to those observed with nsTEM. Our results reveal new details on the unconventional mode of lipid membrane disruption by Cyt toxin. With additional biophysical experiments and structural studies, we plan to deliver further details to explain the mechanistic background of Cyt2A toxin action.

P-07.1-10
Altering the cholesterol selectivity of cholesterol-dependent cytolysins with ribosome display technique

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Cholesterol-dependent cytolysins are one of the most studied groups of pore-forming proteins. They are important virulence factors of several bacterial genera. Understanding their mechanism of action is crucial for the rational design of their inhibitors and for their potential application in therapy, biotechnology, and synthetic biology. The aim of our study was to test whether the ribosome display technique can be used to study the selectivity of cholesterol-dependent cytolysins binding to the lipid cholesterol embedded in a membrane. We randomized seven codon positions in domain 4 of perfringolysin O (PFOD4) at sites supposedly involved in cholesterol recognition/binding. Those positions were 401, 403, 458, 460, 468, 490, and 491, looking at the whole PFO protein, and composed an aminoacid sequence AVETRTL in the wild type PFO. After four rounds of ribosome display, we gained several groups of small protein binding scaffolds based on PFOD4 that bound to the membrane with different kinetic parameters. The wild type PFOD4 interacted with the membrane with a minimum of 50% of cholesterol with affinity (K_D) in a nanomolar range. Unnatural variants of PFOD4 retained the same affinity, but differed from the wild type in other binding characteristics. PFOD4 variant with the randomized aminoacid sequence WVVTHVW bound to cholesterol less specifically, presumably through hydrophobic interactions, whereas the variant with the randomized aminoacid sequence WVVTHSL bound less selectively, as it bound also to membranes with a lower share (30%) of cholesterol in the membrane. Ribosome display, in combination with established techniques for measuring interactions, proved useful as an additional tool for studying protein-membrane interactions.

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P-07.1-11
Connecting the dots in toxic NLP protein-membrane interaction

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Necrosis and ethylene-inducing peptide 1-like proteins (NLPs) are produced by a variety of phytopathogens. They trigger leaf necrosis and immunity-associated responses in dicot plants. Because NLPs are structurally similar to pore-forming toxins actinoporins from sea anemones, and able to induce leakage from plasma membrane vesicles, pore formation was proposed to be the underlying mechanism of their observed cytotoxicity. Glycosylinositol phosphorylceramides (GIPC), the most abundant class of plant sphingolipids, were shown to be receptors for NLP binding to membranes. A model of early steps of NLP membrane interaction was already presented, but the exact mechanism of disruption remains unclear. We are exploiting various model lipid systems, composed of plant-isolated GIPC, e.g. cell-sized vesicles, which are conveniently followed with confocal microscopy. Visual information after NLP–membrane interaction about localised toxin binding, changes in morphology of the vesicles and differential leakage of different-sized probes allows to make speculations about membrane-damaging mechanism. Further, have molecular dynamics studies revealed that C-terminal α-helix of NLP_{Pya} undergoes conformational change during interaction. We are tackling this clue by designing different kind of mutants, among which cysteine ones are of special interest. Introduced cysteines at different locations will be labelled with IANBD probe which allows to monitor the insertion of this residues into the lipid bilayer by changing its fluorescence. Double cysteine mutants will have C-terminal α-helix locked to the core of the protein upon disulfide formation. Reduced cytolitic activity will be the sign that flexible C-terminal region is indeed a prerequisite for proper membrane interaction. Our study will contribute to understanding of the nature and mechanism of interaction of NLP proteins with lipid membranes, be it either via pore formation or some other membrane destabilizing process.

P-07.1-12
Toxic and genotoxic effects of natural surface water in laboratory mice

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Natural surface water is the habitat of many species of organisms and the necessary resource in human life. The current period of development of the biosphere is characterized by global environmental pollution by environmentally hazardous factors, many of which have toxic, mutagenic and carcinogenic activity, in most cases due to the formation of free radicals and inhibition of the repair system. As a result of an increase in the generation of free radicals, an increase in lipid peroxidation (LP) is observed. The aim of the research was to study the prooxidant activity of surface natural waters in the body of experimental animals. The prooxidant and genotoxic activity of the studied waters was determined by the content of lipid hydroperoxides and malondialdehyde in the liver of mice using the extraction-spectrophotometric method and the method of DNA comets. The research material was samples from 26 water sources of the Almaty region of the Republic of Kazakhstan. It was found that surface waters statistically significantly enhanced LP in intoxicated animals compared with intact ones. In mice that received water from different sources studied repeatedly (7 days), the lipid hydroperoxide content was statistically significantly higher than the control values (2.1–6.1-fold, $P < 0.05$–0.001). An increase in the content of the LP secondary product – malondialdehyde – was also noted; its level was statistically significantly higher by 3.3–5.4-fold ($P < 0.01$–0.001). Using the DNA-comet assay, single-stranded DNA breaks were detected in the cells of the liver, kidneys, spleen, and bone marrow of the experimental animals with a frequency statistically significantly higher than the spontaneous level. Thus, the studies revealed the prooxidant and genotoxic activity of natural surface waters, which indicates the pollution of water sources with toxic and mutagenic substances. The research was conducted within the framework of the MES project AP05130546.

P-07.1-13
Activation of (un)regulated cell death as a new perspective for oxime activity research

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Compounds known as oximes are tested as antidotes against the toxic effects of organophosphates (OP). Oximes have diverse scaffolds, created with the purpose to fit the active site of the acetylcholinesterase-OP conjugate as their main target in the organism. After primary screening, candidates which meet the in vitro kinetic parameters of action as antidotes are promoted to further evaluation, while others are discarded. Our previous research showed that some of the oximes display toxic effects on cell level which could be explored beyond their main mechanism of action. To investigate this further, we performed an *in vitro* cell-based evaluation of four structurally diverse groups of oximes at concentrations of up to 0.8 mM, identifying specific biomarkers. We tested the effect of oximes on several *in vitro* cell models: skeletal muscles, kidneys, liver, and neurons. As our results indicate, the effect of oximes was consistent in all cells tested. Compounds with quinuclidine and imidazolium core induced unregulated cell death caused by cell burst, with increased levels of reactive oxygen species formation, but without antioxidant scavenging activation. On the other hand, oximes with a pyridine or pyridinium core activated apoptosis and specific caspases-3, -8, and/or -9. Interestingly, some of the compounds even had a synergistic effect. We generated a pharmacophore model for each series and identified ligands from public databases that map to generated pharmacophores. Several interesting hits were obtained including well known chemotherapy agents, gene expression modulators, antibiotics, cytochrome P450 modulators, etc. Even though the exact mechanism by which oximes act to trigger observed cell effects needs to be explained, our findings should open up a whole new perspective for oxime research. Acknowledgement: This work was supported by the Croatian Science Foundation under the project UIP-2017-05-7260 and by Croatian-Slovenian bilateral grant 2020-2021.

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P-07.1-14
Effect of dose dependent organophosphate compound on interleukin-6 and insulin receptor substrate-1 levels in rat liver

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Organophosphates are highly toxic pesticides with a high chemical mortality ratio. This compounds constitute 50% of the total recorded cases of pesticide poisoning amongst the pesticide family. Malathion is one of the earliest developed organophosphate insecticides. By affecting carbohydrate metabolism, malathion may lead to inflammation, oxidative stress and tissue damage. In the study, 24 Wistar albino (weight ~230 g) female rats were used. The animals were randomly divided into four groups of 6 animals each. Group 1 (Control group) was given corn oil. Group 2, Group 3, and Group 4 were administrated 100, 200 and 400 mg/kg malathion, respectively. The rats were sacrificed 24 hours after malathion administration. The liver tissues were removed and washed with serum physiologic solution. Tissue samples were kept at -80^0C until homogenization. After homogenization, liver tissue Interleukin-6 and Insulin Receptor Substrate-1 levels were measured using commercially available ELISA kits. Acute administration of malathion led to a significant increase in liver tissue Interleukin-6 level. In addition, Insulin receptor substrate-1 levels slightly increased except high dose group. Recent studies are proposed that enhanced levels of advanced glycation end products may lead to insulin resistance or Type 2 Diabetes, by increasing oxidative stress, inflammation and/or affecting insulin receptor substrate 1 (IRS-1). When we evaluate this study with our previous results, it is supported that acute malathion administration seems affect insulin resistance

P-07.1-15
Non-competitive inhibition of Vibrio alkaline phosphatase with cyclohexylamine: an X-ray crystal structure versus blind docking

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Enzyme inhibitors have biological effects as drugs or toxins. They also find applications in studies of enzyme mechanism. The discovery of such interactions is often fortuitous. Here, we report on the inhibition of alkaline phosphatase by cyclohexylamine, a component of a commercially sold substrate salt. The inhibition of a cold-active Vibrio alkaline phosphatase (VAP) fitted a non-competitive kinetic model (K_m was unchanged and V_{max} reduced) with an IC_{50} value of 45.3 mM at the pH optimum of 9.8. This was unchanged by adding 500 mM NaCl. APs from *E. coli* or calf intestines were less sensitive to cyclohexylamine, whereas an Antarctic bacterial AP was similar to VAP in this respect. We compared blind-docking using AutoDock Vina with the results obtained from a 2.2 Å crystal structure that we obtained after soaking crystals with cyclohexylamine for locating the binding site(s). Only two out of the four sites suggested by the blind-docking procedure were observed in the crystal structure. Two of these were broadly in common, although the details were different. One location was in the active site, but different to the substrate binding location. The other binding site was at the interface between subunits. Since the rate determining step in hydrolysis of p-nitrophenylphosphate by VAP is product release, binding of cyclohexylamine in locations at the dimeric interface and/or in the active site may influence cooperative movement in the dimer that drives conformational changes through intersubunit communication causing a delay in product release or reduction in the rate of catalytic step(s).

P-07.1-16
Basal transcription factor 3 (BTF3) on etoposide-induced cell death in cervical cancer cells

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Basal Transcription Factor3 (BTF3) was identified as one of general transcription factors (TFIIs) and also confirmed as the identical protein with β-nascent polypeptide-associated complex (β-NAC). Most of BTF3 researches have been focused on the protein targeting. BTF3 is involved in the control of protein targeting to ribosome and mitochondria. We examined the roles of BTF3 and its molecular mechanisms on chemotherapeutic drug-induced cell death in cancer cell lines. Hela (cervical cancer) cells were transfected with BTF3 siRNA (siBTF3) and cytotoxicity assays (MTT) were performed with various chemotherapeutic drugs. siBTF3-transfected (BTF3 K/D) cells showed significant resistance against the cytotoxicity in cells treated with etoposide, compared with that of control cells. The resistance in the BTF3 K/D cells was blocked with pretreatment of Erk1/2 inhibitor (SCH772984). Increase of Erk1/2 phosphorylation was shown in BTF3 K/D cells in immunoblot analysis. Assays with co-transfection of siBTF3 and siErk1 showed the corresponding findings in MTT and immunoblot analyses. Baseline Bcl-xL protein level increased in BTF3 K/D cells and a higher Bcl-xL was maintained in the cells treated with etoposide. The resistance in etoposide-treated BTF3 K/D cells was blocked in cells pretreated with Bcl-xL inhibitor (A1155483) and in the cells co-transfected with siBcl-xL. Immunoblot analysis showed that Erk1/2 phosphorylation in BTF3 K/D cells was dependent on the Bcl-xL. Our data suggest that BTF3 has an inductive role on etoposide-induced cell death via down regulation of Bcl-xL expression and Erk1/2 activity in cervical cancer cells.

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P-07.1-17
Phospholipase A2 from *Macrovipera lebetina obtusa* venom shift cellular bioenergetics to a more oxidative and glycolytic phenotype

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With advances in pharmacology and quantitative biology, animal toxins targeting the physiological mechanisms of cellular bioenergetics have attracted major interest, revealing considerable potential as carriers of molecular cargo and probes for meddling therapeutic mechanisms for research and medical benefit. To analyze such an impact on cellular metabolism, *Macrovipera lebetina obtusa* snake venom was used in this study with blocked phospholipase A2 (svPLA2) and Zn-metalloproteinases (SVMP). The assessment of the mitochondrial respiratory (based on the oxygen consumption rate) and glycolytic (based on the extracellular acidification rate) rate and capacity by respective stress tests through Seahorse technology (Agilent, USA) enabled determination of the bioenergetic phenotype of venom-processed cells. Irrespective of the cellular metabolic background, venom pre-incubation induced a shift of the bioenergetic state of epithelial Vero cells to a higher oxidative and glycolytic level. Interestingly blocking of the major venom component PLA2 totally inhibits the ability of the venom cocktail to induce a significant increase in metabolic activity. While the similar blocking of the other basic set of components of the venom, which are the PI and PIII Zn-metalloproteinases appeared to be rather negligible for venom-induced stress. These changes are developing in a dose-dependent manner and very well correlate with the experimental data of chemulimenscent analysis of the free radical generation in a real-time mode and lipid peroxidation tests. The study suggests that the capacity of viper venom to induce metabolic alterations could evolve mainly due to the lipid membrane damage in course of the natural envenomation and the acidic svPLA2 is the main responsible component of these changes.

P-07.1-18
Post-translational modifications of cobra venom toxins and their relation to toxicity

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Snake venoms are complex mixtures of proteins and peptides, sometimes including hundreds of different components. This diversity is determined primarily by differences in amino acid sequences. However, post-translational modifications (PTMs) also contribute to this versatility. To identify PTMs in cobra *Naja kaoutia* toxins, a proteomic analysis of the venom from several cobra specimens carried out. About 190 proteins were quantitively determined in each venom sample by the method of label-free quantitative proteomics. The total content of 35 major protein components of the venom of each individual was shown to comprise about 98% of the total amount of venom proteins. A number of PTMs in the cobra toxins was found and about 1960 peptides with PTMs were revealed. While the glycosylation is quite common PTM in snake venoms, other PTMs found are quite unusual. Four types of PTM, namely, acetylation, formylation, glycosylation and phosphorylation were analyzed in details. The large quantity of peptides with acetylated and formylated N-terminal amino acid residues may indicate the presence of processed and modified molecules of the toxins in the venom, herewith acetylated peptides show the highest abundance of this PTM in cobra venom toxins. N-formylated, glycosylated and phosphorylated toxins were less plentiful. It should be noted that most of peptides with PTM found are fragments of three-finger toxins or phospholipases A_2. One of the reasons for the toxin PTMs may be putative alteration of their biological activity. Interestingly, the number of toxins with PTMs in venoms of male snakes is higher than in females. Thus, analysis of the profile of posttranslational modifications of N. kaouthia toxins revealed previously unknown sites for phosphorylation, glycosylation, acetylation, and formylation. This study was funded by RFBR according to the research project № 20-54-00033. Partially published in: Ziganshin RH et al. (2019) Russ J Bioorgan Chem 45, 107–121.

P-07.1-19
Cytotoxic and genotoxic effects of lead and cadmium on HL-60 and Jurkat cell lines

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HL-60 cells and Jurkat cells were used as models to explore the single and combined cytotoxic effect of lead (Pb) and cadmium (Cd) on immune cells. Also, the genotoxic potential of each individual metal was analyzed. Lead (10–100 μM) and cadmium (2.5–10 μM) were introduced individually and as mixtures in cell lines for 24 hours. The concentrations of Pb and Cd for the analysis of mixture interaction were selected from their individual cytotoxic effects. The effect additivity method was utilized, in which concentration of each compound, equivalent to its IC20, was calculated from single metal response curve. For the assessment of cytotoxicity, cells were incubated for 2 hours with Presto blue reagent and the absorbance was measured at 570 nm. Statistical analysis was performed according to Student's t-test; *P* values below 0.05 were considered significant. To determine genotoxicity of Pb and Cd, indirect immunofluorescence method with primary anti-phospho-hystone H2A.X antibody was used. The genotoxic effects were evaluated by the chi-square test; *P* values below 0.05 were considered significant. Lead and cadmium caused cytotoxic effects in both cell lines. Cadmium exhibited higher cytotoxic potential. The most pronounced cytotoxic effect was observed at the lowest concentrations of metals (2.5 μM for Cd and 25 μM for Pb). Further increase in metal concentration did not lead to proportional decline in cell viability, in both lines, respectively. Metal mixtures showed synergistic effect in HL-60 cells and antagonistic effect in Jurkat cells, compared to individual metals. Cadmium exhibited more potent genotoxic effect than lead, and showed dose dependence in both cell lines. The combined effects should be considered in the risk assessment of heavy metal toxicity. Previously published in: Dautović E et al. (2019) Genetics & Applications 3, 57–64. Lau K, McLean WG, Williams DP, Howard CV. (2006) Toxicol Sci 90, 178–187. *The authors marked with an asterisk equally contributed to the work.

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P-07.1-20
Biochemical characterization of BDH2 – a novel candidate for metabolite repair enzyme

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Previous reports identified 3-hydroxybutyrate dehydrogenase type 2 (BDH2) as an enzyme acting in ketone bodies metabolism as well as in mammalian siderophore synthesis. In our recent investigation, we have found that BDH2 exhibits the activity of 4-oxo-L-proline reductase (EC 1.1.1.104). The enzyme converts 4-oxo-L-proline to cis-4-hydroxy-L-proline with concurrent oxidation of NADH. The extensive biochemical characterization of the 4-oxo-L-proline reductase activity was performed including determination of the pH optimum and the substrate specificity of the homogenous BDH2. The kinetic parameters of the reaction were determined in a direct spectrophotometric assay. Among all tested compounds, 4-oxo-L-proline was the only reasonable substrate, and the enzymes revealed high activity and moderate affinity towards it ($\approx$ 41 μmol min^{-1} mg^{-1} and 28 μmol min^{-1} mg^{-1}; $K_M \approx$ 480 μM and 390 μM for rat and human, respectively), whereas cis-4-hydroxy-L-proline was the only product of BDH2 activity as verified by HPLC-MS analysis. Finally, human HEK293T and monkey COS7 cell lines, which endogenously express BDH2 enzyme, efficiently metabolized 4-oxo-L-proline to cis-4-hydroxy-L-proline. Intriguingly, the presence of 4-oxo-L-proline in the culture medium led to an increase in the content of trans-4-hydroxy-L-proline as well. To conclude, our results clearly indicate that BDH2 is a 4-oxo-L-proline reductase. Most importantly, BDH2 is the first mammalian enzyme shown to produce cis-4-hydroxy-L-proline *in vivo*. Obtained results raised questions regarding the physiological importance of BDH2 and its endogenous substrate(s). This investigation was supported by the DSM 501-D114-01-1140100 from the Polish Ministry of Science and Higher Education.

P-07.1-21
RANKL/RANK/OPG axis is involved in the development of vitamin D deficiency-associated hepatotoxicity in rats induced by glucocorticoid prednisolone

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Hepatotoxicity, the least explored side effect of glucocorticoid (GC) treatment, may be associated with impaired vitamin D (VD) metabolism. We hypothesized that RANK (receptor activator of NF-κB)/RANKL (RANK ligand)/OPG (osteoprotegerin) axis, which plays a role in glucose homeostasis and liver function, can be regulated by VD. We assessed GC-induced changes in RANKL/RANK/OPG axis in liver depending on VD status. Female Wistar rats received GC prednisolone (5 mg/kg b.w.) with or without VD (1000 IU/kg b.w.), 30 days. Flow cytometry, western blotting, RT-PCR and ELISA were used. GC increased protein poly-ADP-ribosylaton, TNFα in liver and the number of annexin-positive hepatocytes, indicative of inflammation and cell death. Moreover, GC administration led to deregulated angiogenesis confirmed by a 2-fold decrease in VEGF level and lowered level of lipid metabolism regulator PPARα. These changes were accompanied by a 2-fold reduction of RANK level. GC elevated the RANKL level in the liver with simultaneous 1.27-fold increase in serum RANKL, while serum OPG level declined. Impairments of RANKL/RANK/OPG signaling in liver were associated with D-deficiency (66% reduction of 25OHD), downregulated CYP27B1 expression and elevated glucose level (7.3 ± 0.6 vs 5.3 ± 0.4 mmol/L in control). VD administration diminished poly-ADP-ribosylaton and cell death in liver that can be related to improved D status. VD did not affect TNFα and PPARα mRNA levels, nevertheless enhanced VEGF expression. After VD treatment partial normalization of RANK mRNA and protein levels was observed, while membrane-bound RANKL declined with simultaneous rise of OPG in liver and blood. Prevention of disturbances in the RANKL/RANK/OPG signaling by VD also resulted in glucose level restoration (4.9 ± 0.5 mmol/L). Thus, VD administration might be perspective in amelioration of GC-induced hepatotoxicity due to restoration of VD status, glucose level, angiogenesis and modulation of impaired RANKL/RANK/OPG signaling.

P-07.1-22
A novel kinase provides self-resistance to isocoumarin antibiotic amicoumacin A in Bacillus

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The increasing number of infection cases caused by antibiotic-resistant strains of pathogens challenges the scientific community to discover new antibiotics. This calls for the application of new platforms for antibiotic activity screening. We developed the innovative microfluidic platform for ultrahigh-throughput screening allowing deep functional profiling of bacterial communities. This platform was applied for efficient isolation of microorganisms displaying antibacterial activity. We discovered that the oral microbiome of the East Siberian brown bear contains a population of *Bacillus pumilus* strains inhibiting the growth of common human pathogen *S. aureus*. The genome mining revealed a new biosynthetic gene cluster of antibiotic amicoumacin A (Ami) responsible for the anti-*S. aureus* activity of these strains. Here we performed the exhaustive proteomic and metabolomic analysis of B. pumilus associated with the activation of Ami production. These data display dramatic differences in protein levels of AmiA-AmiM biosynthetic enzymes described previously, as well as unknown putative kinase AmiN and phosphatase AmiO. We observed a rapid phosphorylation of Ami by purified AmiN kinase *in vitro*, while purified AmiO demonstrated phosphatase activity toward phosphorylated Ami. In turn, the phosphorylation resulted in a more than 400-fold decrease in the antibacterial activity of Ami. The biological function of AmiN was further elucidated using *E. coli* BL21(DE3) and *B. subtilis* 168 ΔyerI strains. The heterological expression of amiN provided the resistance of model microorganisms toward Ami. We found that the Ami cluster is present only in a few *B. pumilus* reference genomes, while the presence of amiN and its homologs is almost ubiquitous among Bacillus. We believe that AmiN and its homologs are particularly important for the ecology of these bacteria and their survival in the wild. This work is supported by RFBR grant 19-14-00331.

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P-07.1-23
Engineered polycationic receptor binding domain of colicin N increased its cytoxicity against human lung cancer cells

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Colicin N (ColN) is a bacteriocin secreted by *Escherichia coli* (*E. coli*) to mediate cell killing by forming ion channels in the inner membrane of Gram-negative bacteria. Apart from antibacterial activity, recent findings showed that ColN can also selectively induce apoptosis in human lung cancer cells via the suppression of integrin modulated survival pathway. However, ColN showed a mild toxicity against human lung cancer cells which could be improved for further applications. To achieve this, the protein resurfacing strategy was chosen to engineer ColN by the substitution of solvent-exposed residues on ColN. The highly accessible Asp and Glu on wild-type ColN ($ColN^{WT}$) were replaced by Lys in order to create polycationic ColN ($ColN^{+12}$). Increasing in positive charges on proteins previously showed the enhancement of mammalian cell penetration as well as interaction with negatively charged surface of cancer cells. Those solvent-exposed residues of ColN were identified by Rosetta and AvNAPSA (Average number of Neighboring Atoms Per Side-chain Atom) approaches. Polycationic resurfaced $ColN^{+12}$ were then engineered at selected residues and produced in E. coli. The structure and stability of $ColN^{+12}$ and $ColN^{WT}$ were studied by biochemical and biophysical approaches. The selectivity and cytotoxicity of ColN were also tested on human lung cancer cells and normal cells. The findings revealed that the structural features and stability of $ColN^{+12}$ determined by circular dichroism were similar to $ColN^{WT}$. Furthermore, the toxicity of both ColN was cancer-selective. Human lung cancer cells, H460 and H23 ,were sensitive to ColN but human dermal papilla cells were not. $ColN^{+12}$ also showed more potent toxicity than $ColN^{WT}$ in cancer cells. This confirmed that polycationic resurfacing method has enabled us to improve the anticancer activity of ColN towards human cancer cells.

P-07.1-24
Phototoxicity of ambient particulate matter against human keratinocyte cell line (HaCaT)

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Air pollution is known to trigger adverse effects against the respiratory, nervous and circulatory system but recently researchers' interest has begun to arouse on the impact of ambient particulate matter on the skin – a natural barrier of the body. Although photochemically active components like benzo[a]pyrene and heavy metals are found in particulate matter samples and their contact with skin cells is inevitable not a single study has considered the potential role of light in the toxicity induced by this atmospheric pollutant. It should be emphasized that despite a low intensity of sunlight during winter months, the amount might be sufficient for triggering certain potentially dangerous photochemical processes. In this study, we have characterized photoreactive properties of fine particulate matter ($PM_{2.5}$) collected in Cracow during different seasons of the year. To understand the role of light in $PM_{2.5}$-mediated skin toxicity we have incubated HaCaT cells with particulate matter samples and irradiate them using a solar simulator (340–800 nm) that gives a unique opportunity to resemble conditions occurring in life-like situations. We have performed an array of experiments to examine the effect of photoexcited particulate matter on cell viability, gene expression and oxidative damage to cell organelles. We have also studied the potential mechanism of phototoxic action. Our results suggest that solar radiation might significantly increase the toxicity of ambient particulate matter against human keratinocytes and should be considered as an important factor in future studies. This research was supported by National Science Center (NCN) grant Preludium- 2020/37/N/NZ1/01054.

P-07.1-25
Heavy metals from the aquatic environment change the activity of organ-specific enzymes: an example of the brown trout

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Presence of the heavy metals in the environment has an impact on the overall health of the fish, so it is important to manage monitoring and water quality assessment. The aim of this study was to determine impact of the heavy metals on biochemical status in the brown trout (*Salmo trutta* morpha *fario*) and to correlate concentrations of the heavy metals in the selected tissues with activities of the organ-specific enzymes. In this study, we analysed the concentrations of the heavy metals in the liver, gills and muscle samples of brown trout (n = 20) collected from the pond supplied with water from the River Tilava, in the period from April–July 2018. Analysis were performed at the Faculty of Science, University of Sarajevo, Bosnia and Herzegovina. The concentrations of Ni, Cd, Pb and Zn were determined by the FAAS. Activities of AST, ALT, CK, and LDH in the serum were determined using spectrophotometric methods. One-way ANOVA test and Spearman's correlation coefficient were used to analyse statistical differencies and positive/negative correlations. Observed concentrations of all analysed heavy metals, except for Zn, were the highest in the liver. The concentrations of Cd (0.07 ± 0.04 mg/kg) and Pb (0.43 ± 0.29 mg/kg) in the muscle tissue were over the maximum permissible limit, as well as in the water samples. In general, AST, LDH and CK activities were elevated (149.40 U/L, 345.31 U/L, and 1372.50 U/L, respectively). High negative correlations between level of Cd in the muscles and activities of AST and LDH were found (ρ = -0.737 and ρ = -0.702, respectively). The highest positive correlation coefficients were determined between CK activity in serum and Cd concentration in the liver (ρ = +0.371) and muscles (ρ = +0.306). The obtained results indicate that the increased concentrations of heavy metals with no physiological function in the body (Cd and Pb) had a negative effect on the activity of the organ-specific enzymes, and therefore on the overall health of the brown trout.

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P-07.1-26
Rationalization of enantioselectivity Ig-mediated substrate reaction by QM/MM calculation

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Enantioselectivity is one of the important characteristics of enzyme-substrate and ligand-receptor relationships. Narcotic and nerve agents, including lethal organophosphorus (OP) esters, express stereoselective performance in their action in homo. Engineered Ig molecule is one of the promising biological antidotes due to their potential capability to act as covalent OP trap. Previously, set of catalytic antibodies were selected toward racemic OP substrate and enantioselectivity was not defined. Here we utilized hybrid QM/MM and funnel metadynamics approach to rationalize the enantioselectivity of two antibodies for (R)- and (S)-isomers of the same OP agent as identified by x-ray structure analysis of the product complexes. Experimental Western-blot and kinetic analysis of the reaction matched with in silico generated prediction. The results obtained in this study demonstrate possibility of QM/MM maturation method not only to create antibodies covalently binding OP toxins but also to evolve enzymes as well as chimeric antigen receptors acting by the mechanism of covalent catalysis. This work was supported by RSF project 17-74-30019 and ICGEB grant CRP/RUS18-01.

P-07.1-27
Di (2-ethylhexyl) phthalate affects the adipogenic differentiation of human adipose derived stem cells

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Di (2-ethylhexyl) phthalate (DEHP) is an endocrine disrupting chemicals like and is ubiquitously present in the environment. This phthalate belongs to the subgroup of obesogens, as it is able to interfere with adipogenesis. Adipose tissue, which is a preferred storage site for lipophilic endocrine disruptors, including DEHP, is one of the important reservoirs of mesenchymal stem cells. These cells could be the target of molecules capable of interfering with their viability and their differentiation. The aim of this study was to investigate the effect of DEHP on the viability and the adipogenic differentiation of the human adipose derived stem cells (ADSCs). Pre-adipocytes were exposed to DEHP in environmentally relevant concentrations and the cytotoxicity was monitored by cell viability, ROS generation, antioxidant enzyme expression and cell cycle evaluation. On the other hand, pre-adipocytes were then exposed to DEHP and differentiated into mature adipocytes. At different stages of differentiation, mRNA expression of adipogenic marker genes like peroxisome proliferator-activated receptor gamma (PPARγ), lipoprotein lipase (LPL), leptin and adiponectin were quantified as well as functional marker like triglyceride content was measured in mature adipocytes and compared with corresponding unexposed cells. We demonstrated that treatment of undifferentiated cells with DEHP decreased cell viability, increased reactive oxygen species and blocked the cell cycle in the S phase which lead to apoptosis. Moreover, we observed a significantly lower expression level of adipogenic markers and a reduction in triglyceride accumulation level in the supernatant of treated adipocytes. In conclusion, DEHP led to lower grade of adipogenic differentiation in human ADSCs under the chosen conditions.

P-07.1-28
Natural deep eutectic solvents affect acetylcholinesterase kinetics

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The pivotal role of acetylcholinesterase (AChE) in hydrolyzing the neurotransmitter acetylcholine can be disturbed by inhibitors like organophosphates. The formed adducts are not efficiently hydrolyzed by water and AChE loses its catalytic function. Consequent overstimulation of cholinergic receptors can lead to death. To overcome the life-threatening state, antimuscarinic atropine and an oxime reactivator must be administered promptly. Oximes, as nucleophiles stronger than water, restore phosphylated AChE activity, but due to their positive charge, standard oximes poorly pass the blood-brain barrier (BBB) and fail to reactivate synaptic AChE. Lately, zwitterionic molecules with a sufficient neutral species at physiologic pH to cross the BBB have been investigated. Synaptic AChE is then reactivated by cationic species formed upon establishing pH equilibrium in the brain. This was proven for oxime RS-194B. But, RS-194B has low solubility in water. Natural deep eutectic solvents (NADES) can dissolve compounds of low water solubility, and besides being considered as safe for human use, often have beneficial features like antioxidative activity. With the aim to test the suitability of such solvents for oxime formulation, RS-194B was dissolved in NADES composed of glucose and citric acid with 30% water. The oxime was simultaneously dissolved in water, and the effect of such prepared oxime formulations on AChE kinetics was compared. The results indicate that NADES induces conformational changes of the AChE enabling easier embedding of the oxime in the active center which results in higher AChE affinity for the RS-194B/NADES than for RS-194B/H_2O. Yet, it seems that RS-194B/NADES orientation or vicinity to the phosphylated AChE's catalytic serine was unfavorable, and the reactivation rate was higher with RS-194B/H_2O. This NADES has been shown to inhibit AChE in fractions greater than 0.2%, implying its limited benefit; however, further studies on other NADES are planned

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P-07.1-29
Lipid-binding aegerolysin from the entomopathogenic fungus *Beauveria bassiana*

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Fungi have a remarkable impact on ecosystem functioning; they act as decomposers, symbionts and pathogens. Among other things, fungi are the most common pathogens of insects and thus important regulators of their populations. Rapid advances in nucleotide sequencing technology have accelerated our understanding of entomopathogenic fungi by determining the genomic sequences of several species from the genus *Metarhizium*, as well as *Beauveria*, *Cordyceps*, and *Ophiocordyceps*. Proteins from the aegerolysin family have characteristic compact beta-sandwich folds, low isoelectric points, and low molecular weights, and are stable over a wide pH range. Some aegerolysins are known to interact with either biological or artificial lipid membranes. They act alone or in concert with membrane attack complex/perforin (MACPF)-like domain-containing proteins to form pores with cytolytic or hemolytic activity. Adapted to the lifestyle of the organisms that produce them, they can be involved in various biologically relevant processes, such as: attack and defence against other organisms, ontogenetic development or cell cycle regulation. We have identified aegerolysins in different kingdoms of life, with the largest number of representatives found in fungi and bacteria. Aegerolysins are encoded in genomes of Dikarya fungi including Sordariomycetes, which include most genomes of entomopathogenic fungi, but they are also absent in some fungal clades. Paring with MACPF-like domain- containing proteins appears to be characteristic of entomopathogenic fungi, as well. We have performed an in-depth bioinformatic analysis of aegerolysins in Fungi, with a particular focus on entomopathogenic fungi. From this group of fungi, we selected a candidate aegerolysin from *B. bassiana* and expressed it as a recombinant protein in *Escherichia coli* and purified it to further determine its functional and structural properties, including lipid-binding ability, and to compare it with functionally related proteins.

P-07.1-30
How ammonia effects the biochemical parameters of cultured fish?

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Sea Bream (*Sparus auratus*) is one of the cultured marine species in Mediterranean and in Turkey. 99.730 ton of sea bream was produced in 2019. As a result of the high density of the intensive culture, the fish are exposed to nitrogenous compounds mainly with ammonia. The aim of the study was to observe the plasma biochemical alterations in cultured sea bream that was exposed to ammonia. Fish were placed in tanks containing 500L of sea water. NH_4Cl was added to the tanks at 5 mg/L (total ammonia nitrogen). Water samples were taken as soon as dosing was done and at the end of the experiment to determine the amount of ammonia. Water temperature was around 18°C, pH 7.8. Blood samples were collected from the caudal vein with heparinized syringes and separated after centrifugation at 12,000 rpm and frozen until analysis at 24th and 48th hours interval. Plasma cortisol (ng/mL) was measured using a commercial ELISA kit (DRG Diagnostics) and glucose (mg/L), calcium (Ca, mg/dL), inorganic phosphorus (P, mg/dL), sodium (Na, mMol/L), potassium (K, mMol/L) and chlorine (Cl, mMol/L) were determined using commercial kits (Olympus AU 400). Hematocrits were immediately determined by routine methods. 24th and 48th hour sample results were compared to the control group. K was increased (5.09 ± 0.77) and Ca was decreased (9.14 ± 1.9) in 24th hour group compared to control (3.65 ± 0.76 and 11.85 ± 0.8 respectively, $P < 0.05$). P level (9.7 ± 0.65) was found to be higher in 48th hour group compared to control (8.52 ± 0.76) ($P < 0.05$). 24th and 48th hour groups cortisol (18.36 ± 0.69, 19.15 ± 0.7, respectively) and glucose levels (101.35 ± 8.9, 97.32 ± 8.2, respectively) were higher compared to the control group (cortisol: 8.24 ± 1.0, glucose: 84.5 ± 5.9) ($P < 0.05$). As a conclusion, ammonia exposure represents a potential stress on sea bream that can be directly related to environmental health deterioration. *The authors marked with an asterisk equally contributed to the work.

P-07.1-31
Bacterial production of Soricidin, inhibitor of TRPV6 channels, possessing antitumor activity

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TRPV6 is a highly selective calcium ion channel localized in various epithelial tissues including breast, kidney, intestine, pancreas, and prostate. TRPV6 is over-expressed in ovary, breast, colon, prostate and thyroid cancers and implicated in tumor development and progression. Selective TRPV6 inhibitors are considered promising compounds for the development of anticancer therapy. Soricidin is a paralytic 54 a.a. neurotoxin isolated from the submaxillary saliva glands of the northern short-tailed shrew. Soricidin inhibits calcium input via the TRPV6 channel and demonstrates anti-tumor activity on epithelial cancer cells. The structure and mechanism of Soricidin/TRPV6 interaction are poorly studied. Here we report high-efficient bacterial expression systems for production of full-length Soricidin and its 13 a.a. C-terminal fragment (Sor13). Both peptides were produced in the form of hybrid proteins with thioredoxin, and were isolated after fusion hydrolysis by BrCN. The Soricidin variants were characterized by NMR-spectroscopy, HPLC, and MALDI. Anticancer activity was confirmed using K562 leukemia cells. Both peptides effectively inhibit the growth of cancer cells upon 72 h incubation. Developed expression systems open new perspectives for structural and functional studies of Soricidin interaction with the TRPV6 channel and for investigation of the peptide activity on other cancers associated with a high TRPV6 expression. The work was supported by the Russian Science Foundation (project # 21-14-00392).

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P-07.1-32
Faster toxin production methods for the discovery of high potency drug products

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Innovation in botulinum neurotoxin (BoNT) drug discovery challenges drug designers to produce large numbers of candidates while working safely with Containment Level 3 (CL3) working procedures. Indeed, BoNTs are the most potent known bacterial toxins, with lethal doses of 1 ng/kg in humans. BoNTs are modular proteins made of three domains, one involved in binding of membrane receptors, the second in translocation into the cytoplasm of the catalytic activity present in the third domain, which acts on intracellular targets to inhibit secretion. The modular organization and the secretion inhibitory properties of BoNTs make them attractive engineering targets for drug discovery. Efforts to re-target the catalytic activity of BoNTs to different cells have led to the design of targeted secretion inhibitors (TSIs), where the binding domain is substituted by alternative targeting moieties. Automation offers obvious advantages for producing panels of TSIs (safety, throughput, reproducibility). However, installing instruments in microbiological safety cabinets presents challenges such as size constraints or resistance to the stringent chemical treatments used to disinfect the cabinets. In this poster, we present our efforts to increase production throughput of engineered TSIs. We achieve automated, parallel generation of expression vectors by Golden-Gate assembly using a liquid handler and automatized script-writing/data analyses. Medium-scale *E. coli* expression, chemical lysis, batch purification, and endotoxin reduction processes have been developed to meet the needs of downstream testing. With this workflow, fully adapted to CL3 procedures, we have increased the throughput of protein production 8-fold over less automated methods of cloning, expression and purification. Novel technologies such as these will continue to open new possibilities for automation of expression and purification at the CL3 safety standards.

Mechanisms of microbiome–host interactions

P-07.2-01
Engineering *Lactococcus lactis* to target tumor cell lines

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Lactococcus lactis is a food-grade and a model lactic acid bacterium (LAB). It is often used as a cell factory for recombinant protein expression and represents a promising vector for *in vivo* delivery of bioactive proteins. Engineered LAB have already been suggested for therapy of colorectal cancer by surface displaying protein binders of pro-inflammatory cytokines, by delivering antioxidant molecules, and by secreting apoptosis-inducing proteins; however the therapeutic activity was non-specific. By surface-display of proteins targeting tumor antigens, directed binding of *L. lactis* to cancer cells could be achieved. In the present study, we focused on the development of a system for targeted binding of *L. lactis* to colorectal tumor cell lines. We applied proteins with affinity for three tumor antigens (EpCAM, Her2 and Gb3), which are typically overexpressed in tumor cells of colorectal cancer. Genetic constructs for surface display included genes for affitin with affinity towards EpCAM, for affibody with affinity towards Her2 and for B subunit of Shiga toxin with affinity towards Gb3. To enable concomitant imaging of bound bacteria, we simultaneously expressed infrared fluorescent protein (IRFP) in bacterial cytoplasm, by using plasmid for dual protein expression. Surface display of FLAG-labelled targeting proteins was confirmed by flow cytometry, while expression of IRFP fluorescent protein was determined by measuring fluorescence. Furthermore, we confirmed, with flow cytometry, binding of soluble tumor antigens, EpCAM and Her2, to bacteria displaying their respective targeting proteins. L. lactis with surface-displayed proteins targeting EpCAM and Her2 strongly and selectively bound to HEK293 cells overexpressing EpCAM and Her2 receptors, respectively. *L. lactis* with surface-displayed targeting proteins were also able to bind to human Caco-2 and HT-29 tumor cell lines. Our work demonstrates the ability of engineered *L. lactis* to selectively target cancer-cells.

P-07.2-02
Is there life after macrophages? AIEC's ways to survive

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In the last decades, *Escherichia coli* (*E. coli*), especially adherent-invasive *E. coli* (AIEC) pathotype has been implicated in the pathogenesis of Inflammatory bowel disease (IDB) and Crohn's disease (CD). CD is a severe chronic immune-mediated granulomatous inflammatory disease of the gastrointestinal tract that can affect all its parts, starting from the mouth and ending with the rectum. Macrophages are part of the body's first line of defence, eliminating pathogens by phagocytosis or by releasing a broad array of inflammatory mediators, such as cytokines, chemokines, and proteases. To survive, intestinal pathogens use various mechanisms, which include the induction of inflammation, the direct or indirect destruction of commensals, or the use of alternative carbon sources. This study discusses the mechanisms of action by which AIEC can survive in macrophages. To determine proteins of CD-isolates that are significant for survival in the internal environment of macrophages, we performed a proteome-wide LC-MS analysis of E.coli colonies obtained at the stage of bacterial phagocytosis by macrophages (0 hours) and after 24 hours after infection. This is the first attempt to describe proteome-wide events occurring in AIEC infection in macrophages. Comparative analysis revealed that we obtain some changes in two *E. coli* lines (0 hours and 24 hours). Our results allow us to propose which pathogens proteins during specific infection phase may serve as targets for therapy of intracellular infections. This work was supported by the Russian Science Foundation (project no. 16-15-00258).

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P-07.2-03
Study of the viability and structural changes of representatives of the human microbiota after exposure to physical factors

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The study was conducted on representatives of the skin microbiota – *S. aureus* and human intestines *E. coli*. To assess the viability and structural changes of lipids and proteins of microorganisms, we used suspensions of *S. aureus* and *E. coli* with a concentration of 1.2 ×104 cells/mL, which were processed in sterile plastic Petri dishes with a diameter of 40 mm for 60–600 s. UV radiation of mercury lamp and gas-discharge plasma radiation were selected as physical factors. Viability was evaluated after sowing "solid lawn", incubation for 24 hours at a temperature 37°C and counting CFU. Phospholipid fractions were studied by thin-layer chromatography, the state of the lipid bilayer using the excimer coefficient of the fluorescence probe of pyrene, and the modification of bacterial proteins by the fluorescence of tryptophan and tyrosine. The control was untreated suspension of microorganisms. It was shown that with an increase in the time of exposure to physical factors on microorganisms, the number of surviving microorganisms decreased. By 60 seconds of treatment, UV radiation mercury lamp already reduced the number of colonies by 96% for *S. aureus* and 98% for *E. coli*. After exposure to gas-discharge plasma radiation, the viability of *E. coli* and *S. aureus* decreased gradually with increasing exposure time and reached an absolute bactericidal effect by 600 s. It was shown that with an increase in the exposure time by physical factors, the microviscosity of the lipid bilayer and the zone of protein-lipid contacts decrease for Gram-positive and Gram-negative bacteria. Despite the high bactericidal effect of the studied physical factors, some single cells of *S. aureus* and *E. coli* remain viable and exhibit resistance to UV radiation. Under the influence of studied factors, the phospholipid composition, the structural organization of the phospholipid bilayer in the region of protein-lipid and lipid-lipid contacts change, and proteins *S. aureus* and *E. coli* are modified.

P-07.2-04
Metabolic changes in hepatocytes during HCV infection: a study in the physiological media

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Hepatitis C virus (HCV) is a wide spread and dangerous human pathogen that leads to liver fibrosis, cirrhosis, and cancer. Clearance of the infection with direct acting antivirals does neither completely prevents liver fibrosis in all patients nor reduces incidence of liver cancer to the general population. Thus, investigation of mechanisms of HCV pathogenesis is still needed. Altered metabolism of the infected hepatocytes is considered to contribute to virus pathogenesis. Its investigation is hampered by imprint of common non-physiological culture media. Our aim is to study metabolic changes in the infected hepatocytes using human plasma-like "Plasmax" media described in 2019. We show that substitution of Dulbecco's Modified Eagle Medium (DMEM) with Plasmax led to change in morphology of Huh7.5 cells that are permissive for HCV, as well as to moderate decrease in proliferation speed. It was accompanied by changes in expression of the genes from various metabolic pathways including glycolysis and glutaminolysis, pentose phosphate pathway, urea cycle, and serine/glycine biosynthesis towards more physiological levels. The Huh7.5 cells in Plasmax medium exhibited significantly higher rates of mitochondrial respiration and spare respiratory capacity. It was accompanied by assembly of fragmented mitochondria into networks. In lines with this, the cells were much more sensitive to inhibitors of respiratory complex II and ATP synthase. Finally, Plasmax reduced replication levels of hepatitis C virus both in non-infectious replicon model and in infectious cell culture (HCVcc) system. Metabolic changes induced by the virus are currently under investigation. The study was supported by the Russian Science Foundation grant No. 19-74-10086.

P-07.2-05

Abstract withdrawn

P-07.2-06
Structural and functional insight into the cell wall proteins from *Clostridium difficile*

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C. difficile is a leading cause of antibiotic-associated diarrhea that can lead to life-threatening complications. While cell surface proteins play an important role in the colonization, which is the first required stage of the infection that is followed by disease-causing toxin production, scarce structural data limit our understanding of their interactions within the bacterial cell wall and with their environment. Recently, the crystal structures of multi-domain cell wall proteins (CWPs) Cwp8 and Cwp6 from *C. difficile* revealed the cell wall binding type 2 (CWB2) module that is shared among 29 CWPs of *C. difficile* 630 and represents one of the two evolutionary conserved surface layer (S-layer)-anchoring modules in Gram-positive bacteria. The aim of our studies is to gain structural insight into the CWPs to understand the functions of these proteins acting either alone or as a part of the S-layer assembly. The mature Cwp5 (i.e. without the signal sequence) from *C. difficile* 630 was overexpressed in *Escherichia coli* BL21(DE3), purified by Ni-affinity and size exclusion chromatography. After spontaneous degradation of Cwp5 the resulting C-terminal fragment was identified by mass spectrometry and crystallized by sitting-drop vapor-diffusion technique using optimised commercial screens. Platinum derivatives of the crystals were prepared by soaking. Single-wavelength anomalous dispersion method revealed a two-faced right-handed β-helix crystal structure of the functional domain of Cwp5. Site-directed mutagenesis showed that Cwp5 undergoes intramolecular autoproteolysis, most likely similar to the maturation mechanism of CwpV, indicating a possible common autoprocessing mechanism of *C. difficile* CWPs and other CWB2 module-containing S-layer proteins of Gram-positive bacteria.

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P-07.2-07
Effect of pumpkin pectin supplement on microbiome composition and adhesion of probiotic *Lactobacillus* species

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It is widely known, that animals assemble and maintain a diverse gut microbial community that enormously contribute to their general health status. One of the factors that influence the microbial composition of the gut, especially in the small intestine, is the bacterial ability to penetrate or adhere to the intestinal surfaces. Although there are many aspects influencing bacterial adhesion, the most general one is a dietary fiber composition of the daily diet. In this work, the evaluation of the effect of pumpkin pectin on intestinal microbiome community was performed. Pumpkin pectin has a molecular weight range of 90–120 kDa, the content of polygalacturonic acid of 75% and the high degree of esterification of 72%. Firstly, the *in vivo* evaluation of the effect of pumpkin pectin on intestinal microbiome was performed in Wistar rats. The control group of animals was kept on a general diet; the diet of the experimental group contained 4% of pumpkin pectin. After 28 days, fecal samples were collected from all groups. Total DNA was extracted, and 16S rRNA sequencing was performed following the Illumina MiSeq Protocol. The statistically significant increase in the number of intestinal *Lactobacillus* (5 times) and decrease in the number of *Helicobacter* (1.7 times) were determined. Secondly, the ability of pumpkin pectin to increase the adhesion of *Bifidobacterium adolescentis* and *Lactobacillus rhamnosus* was demonstrated on the dog's kidney cell line MDCK 1. The total count of live bacteria which have adhered to, or internalized into, the MDCK1 cells after 2 h incubation was measured. It was shown, that pumpkin pectin increased adhesion of *B. adolescentis* and *L. rhamnosus* by 40 and 30–50% compared to the control. This research was funded by Russian Science Foundation, Grant No. 16-16-00094.

P-07.2-08
Safety assessment of lactic acid bacteria isolated from traditional fermented products of Russia and South Africa

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Although lactic acid bacteria (LAB) comprise only a minor part of the human intestinal microbiota, their proportion is considered to be an important health influencing factor. Given a small amount of LAB constantly present in a human gut, some researchers even considered possibility to reassess their gut-autochthonous status. Indeed, the regularly introduced into the habitant allochthonous species can form as stable population as autochthonous one. Hence, at least for some LAB (nomadic species) their regular consumption is very important. Currently, fermented food is a main source of LAB that can reach human intestine. However, it was established that some strains of LAB traditionally used for fermentation can harbor antibiotic resistant (AR) genes. Since these genes can be horizontally transmissible to pathogens, some investigators have questioned the safety of LAB used in commercial food preparation. Additionally, it is known that LAB characterized by excessive activity of the so-called fecal enzymes (mainly hydrolases and reductases) may lead to disorders in the functioning of the digestive tract or even be the reasons of the colon cancers. In this work several strains of LAB were isolated from the traditional fermented products of Russia (kefir) and South Africa (amasi and mahewu), and their AR profile, presence of AR genes and enzymatic activities were assessed. While all tested strains did not harbor transmittable AR genes, their AR profiles were remarkably different e.g.: for *Lactococcus lactis*, strain from amasi was not resistant to any antibiotic, while the strain from mahewu showed resistance to 7 of them; two *Lactobacillus paracasei* strains from kefir demonstrated almost opposite AR profiles. Generally, enzymatic activities of the strains from the same species were similar and only minor strain specific differences were observed. The reported study was funded by RFBR and NRF according to the research project № 19-54-60002

P-07.2-09
Bacteroidales and Clostridiales are the dominant orders in the rumen community of the Russian Arctic reindeers

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In the period 2017–2018, a series of expeditions were organized in the Yamalo-Nenets Autonomous District (Kharp, forest-tundra natural and climatic zone) and the Nenets Autonomous District with the aim of colecting rumen content from reindeer of various ages, including calves. A total of 38 samples were taken from reindeer (*Rangifer tarandus*) of the Nenets breed. The phylogenetic affiliation of the constituents of the microbial community of the reindeer rumen was determined using the NGS 16s rRNA gene. Data analysis was performed in QIIME software. The dominant position was occupied by the phyla Bacteroidetes and Firmicutes. In total, they occupied from 65 to 88% of all bacteria in the reindeer rumen. Representatives of phyla Cyanobacteria took the following position. The proportion of Cyanobacteria increased in the winter. If in the summer period their share was from 0.5 to 3.3%, then in the winter from 1.5 to 12.2%. These differences are significant in the Nenets Autonomous District - the proportion of cyanobacteria there was significant in the winter from 6.7 to 12.2% ($P = 0.02$), while in the Yamalo-Nenets Autonomous District it did not exceed 5% in the winter. Verrucomicrobia occupied from 0.2 to 4% in the rumen community. Bacteria of other phyla (Tenericutes, SR1, TM7, Spirochaetes, Chloroflexi, Proteobacteria, Elusimicrobia, Actinobacteria, Fibrobacteres, Fusobacteria and others) were less represented in the reindeer rumen community. The core community included representatives of the orders Bacteroidales (from 10 to 40%) and Clostridiales (from 8 to 25%), the proportion of these bacteria was more dependent on the season, so in summer the proportion of Bacteroidales was higher than in winter. This is probably due to a more favorable composition of the summer diet compared to the winter one, in which lichens and shrub branches occupy a large share. This research was supported by a grant from the Russian Science Foundation № 17-76-20026.

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P-07.2-10
Alpha biodiversity of reindeer's rumen bacteria of the Russian Arctic

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The object of the study was 38 individuals of reindeer (*Rangifer tarandus*) of the Nenets breed – calves and adult individuals. Samples of the content of the rumen were colected in the summer-autumn and winter-spring periods of 2017–2018 in the Yamal-Nenets Autonomous District and the Nenets Autonomous District. The composition of the community of microorganisms in the reindeer rumen was determined using the NGS of 16SrRNA gene. Among alpha biodiversity parameters operational taxonomic units or species (OTUs), Chao1 and Shannon indices were calculated. The number of sequenced sequences varied from 5753 to 113156 per sample. The OTU number ranged from 746 to 7665 per sample. In the Yamalo-Nenets Autonomous District, the Chao1 index was 403.01 ± 23.23, the Shannon index was 7.64 ± 0.09. In the Nenets Autonomous District, the Chao1 index was 492.27 ± 21.82, the Shannon index was 7.98 ± 0.08. Significant differences are revealed between samples from different regions when comparing the alpha biodiversity indices of Chao1 ($P = 0.004$) and Shannon ($P = 0.01$). Samples taken in the winter-spring time significantly differed from the samples of the summer-autumn period in the Chao1 index ($P = 0.01$). In summer samples, the Chao1 index was 503.31 ± 28.28, the Shannon index was 7.94 ± 0.13. For winter samples, the Chao1 index was 411.21 ± 18.07, the Shannon index was 7.73 ± 0.05. There were no other significant differences when comparing other groups of samples. The number of unique OTUs was on average higher in the summer-fall season, and significant differences in the number of OTUs were observed between regions. Age and gender did not significantly affect the amount of OTU. Thus, the main structure-forming factors influencing the composition of the microbial community were the place of selection and the season of the year. This research was supported by a grant from the Russian Science Foundation № 17-76-20026.

P-07.2-11
The protective effect of microbiota metabolite 4-hexylresorcinol on peroxidase proteolysis

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4-hexylresorcinol (4-HR) is a resorcinolic lipid of plant/bacterial origin circulating in the blood in micromolar concentrations. 4-HR is known to induce dormancy in bacteria and exhibit local anesthetic, antiseptic, antioxidant, anti-inflammatory, and antitumor activities. Our study aimed to determine whether the 4-HR at physiological concentrations exhibited protection of a protein molecule (horseradish peroxidase, HRP) from proteolysis. Chromatographically purified HRP (Sigma, USA) was immobilized on a nitrocellulose membrane, incubated with 0.2–200 μM of 4-HR (Sigma, USA), washed, and treated with trypsin for 1.5 hours. The protective effect of 4-HR on peroxidase hydrolysis was detected by chemiluminescence and visualized using ChemiDoc MP (Bio-Rad, USA) imaging system. Treatment of HRP with 4-HR at a large concentration (200 μM) abrogated chemiluminescence of HRP. However, at much fewer concentrations (0.2–2 μM) that corresponded to physiological levels in human serum, the 4-HR led to a 3.5-fold increase of the level of chemiluminescence of HRP compared to control HRP samples not treated with 4-HR ($P < 0.05$, three repeats). Therefore, at physiological concentrations, 4-HR protects HRP from enzymatic proteolysis. To confirm the protein protection by 4-HR, the experiment was performed in a solution. HRP was incubated with 0.2–2 μM 4-HR in a buffer, and after dilution 1:10 the solution was incubated with proteinase K. After boiling, samples were subjected to SDS-PAGE using 15% polyacrylamide gel, and the bands were assessed by silver staining. Additional data confirmed that 4-HR protected HRP from proteolytic cleavage. Therefore, the plant/microbiota-derived lipid molecule 4-HR, when employed at a low physiological concentration, preserves the proteins from a proteolytically active environment. The protective capability of the compound may explain, at least in part, the facilitation of antioxidant and antimicrobial activities of various proteins by 4-HR. *The authors marked with an asterisk equally contributed to the work.

P-07.2-12
Investigation of the mutator effects of currently used TB drugs provides insight into the mechanism of drug resistance development in Mycobacteria

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Recent WHO report estimates that there were over a half million new tuberculosis cases in 2018 with resistance to rifampicin – the most effective first-line drug - of which 78% had multidrug resistance. Globally, 3.4% of new TB cases and 18% of previously treated cases were multidrug-resistant or rifampicin-resistant. In most bacteria, horizontal gene transfer is a major molecular mechanism playing important role for driving evolution of drug resistance. However, mycobacteria acquire antibiotic resistance merely by single point mutations. Interestingly, in contrast to the remarkable genomic diversity displayed in isolates from tuberculosis patients, the basal mutation rate in mycobacteria is very low *in vitro*, suggesting that genotoxic stress and harsh conditions within the host cell may elicit transient or constitutive mutator phenotypes. We investigated the effects of several genotoxic stress factors the bacteria facing in the host, like currently used tuberculosis drugs and different environmental stress conditions in *Mycobacterium smegmatis*. We measured the changes in the expression pattern of the DNA repair enzymes and cellular dNTP concentrations upon genotoxic stress. We also analyzed the mutation rates and the spectra of mutations using whole-genome sequencing upon long term treatment. We found that first line combination antibiotic treatment (rifampicine, ethambutol, isoniazid and pyrazinamide) caused severe effects in the bacteria. SOS response and error-prone polymerases were highly induced in the treated strains, while concentrations of purine nucleotides (dATP, dGTP) were decreased. These effects were not traceable in strains treated of the same drugs separately, therefore the

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combination of these drugs definitely has synergistic effects. Correlating these data with the defined mutator effect will yield an unprecedented insight into the mechanisms underlying stress-induced mutagenesis and will hopefully allow better management of current therapeutics.

P-07.2-13
Probing the substrate preference of peptidylarginine deiminase from human pathogen *Porphyromonas gingivalis*

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Human oral opportunistic pathogen *Porphyromonas gingivalis* is considered to be the prime factor driving the development and progression of periodontitis. Among a number of various virulence factors produced by this pathogen, one of the most attractive and best studied is peptidylarginine deiminase (PPAD), the enzyme which converts arginine to citrulline. Citrullination by PPAD inactivates antibacterial pathways and is proposed as a factor stimulating destructive chronic inflammation. Unlike mammalian citrullinating enzymes, PPAD modifies preferentially C-terminal arginine residues, and to much lesser extent, free arginine. The general catalytic mechanism and the active site architecture is similar in mammalian and bacterial peptidylarginine deiminases, and only a few residues seem to decide the enzyme specificity. Based on the high-resolution crystal structure of PPAD, we sought to mutate those residues in an attempt to change PPAD substrate preference. To accomplish this, we engineered *P. gingivalis* strains secreting mutated PPADs. Modified enzymes were purified and their activity screened using a panel of peptide substrates derived from human and bacterial proteins. We have also determined thermal stability and thermostabilization of PPAD variants by a substrate and an inhibitor. Our results show that the removal of residues constituting the potential spatial interference for amino acids following non-C-terminal arginine was not sufficient to affect PPAD preference for C-terminal arginine. Mutated enzymes remained active, albeit at the lower rate than wild type PPAD. Interestingly, they were thermostabilized by the substrate or the inhibitor, suggesting that introduced mutations may enhanced substrate binding. Our results indicate that the affinity of PPAD to C-terminal arginine is defined by the tight network of interactions with the substrate, including the carboxyl group of the C-terminal arginine rather than by the spatial limitations of the binding pocket.

P-07.2-14
Molecular bases of slow growth of *Mycoplasma hominis* on different energy sources: proteomic level

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Mycoplasma hominis is an opportunistic urogenital pathogen of vertebrates. It is a nonglycolytic species, producing energy through arginine degradation. Among the genital mycoplasmas, *M. hominis* is most commonly reported to play a role in systemic infections and can persist in the host for a long time. However, it is unclear how *M. hominis* proceeds under arginine-limitation. The recent metabolic reconstruction of *M. hominis* demonstrates the ability to catabolize deoxyribose-phosphate to produce ATP. In this study, we cultivate *M. hominis* on two different energy sources (arginine and thymidine) and demonstrate the difference in growth rate, antibiotic sensitivity, and biofilm formation. Using label-free quantitative proteomics, we compare the proteome of *Mycoplasma hominis* under these conditions. A total of 466 proteins are identified from *M. hominis*, representing approximately 85% of the predicted proteome. While the levels of 94 proteins change significantly. As expected, we found changes in the level of metabolic enzymes. The energy source strongly affects the synthesis of enzymes related to RNA modifications and ribosome assembly. The translocation of lipoproteins and other membrane-associated proteins is impaired. It is the first global characterization of the proteomic switching of *Mycoplasma hominis* in arginine-deficiency media illustrates energy source-dependent control of pathogenicity factors like lipoproteins. This study can help to find out mechanisms underlying of interaction between growth rate and fitness of genome-reduced bacteria. This work was supported by the Russian Science Foundation (project no.19-75-10124).

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Cellular organization

P-07.3-01
MKK6-p38γ/δ signaling is essential to control cardiac electrical activity

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Maladaptive cardiac hypertrophy can progress to heart failure, a worldwide leading cause of mortality. However, knowledge about the signaling pathways implicated in how cardiomyocyte growth could induce ventricular arrhythmias remains incomplete. Our group recently showed that p38MAPK signaling pathway has been implicated in the promotion of cardiac hypertrophy. p38MAPKs are activated by the upstream kinases MKK3 and MKK6. However, the specific function and the control of the activation of the different p38MAPK isoforms by these kinases in cardiomyocytes remains elusive. To further explore this, we generated MKK6-KO and MKK3-KO mice. We saw that MKK3 is the main activator of p38γ/δ in the heart, whereas MKK6 activates cardiac p38α. Echocardiography, histology, and immunoblot show that lack of MKK6-p38α activation leads to MKK3-p38γ/δ hyperactivation and increased mTOR signaling, resulting in physiological cardiac hypertrophy. In addition, on patch-clamping experiments ventricular cardiomyocytes from MKK6-KO mice showed spontaneous calcium release events, formation of early afterdepolarization and increased arrhythmia susceptibility on programmed electrical stimulation, particularly in the presence of β-adrenergic stimulation. We also demonstrate that p38γ/δ control several pathways involved in heart failure and arrhythmogenesis: they promote hyperphosphorylation of RyR2 and the scaffold protein SAP97, together with increased expression and activation of CaMKII. Using co-immunoprecipitation experiments, we define a macromolecular complex comprising RyR2, p38γ/δ, PKA and CaMKII together with SAP97 and AKAP79. We show that p38γ/δ phosphorylation of RyR2 increases its binding to PKA, SAP97, p38γ/δ and AKAP79 resulting in RyR2 hyperphosphorylation. In conclusion, this work shows for the first time the importance of cardiomyocyte MKK6-p38γ/δ signaling in cardiac hypertrophy and how p38γ/δ hyperactivation predisposes to stress-induced arrhythmias and sudden death. *The authors marked with an asterisk equally contributed to the work.

P-07.3-02
3D hepatic tumor cell culture plasticity mediated by YAP-mTOR axis

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Cell culture in monolayer on standard plastic dish (so-called 2D cell culture) is a well-established system, which is inexpensive and easy to analyze. Unfortunately, current 2D cell culture systems do not represent real cell conditions; and especially in drug testing are not always predictive. However, so-called 3D cell culture systems offer more physiologically relevant and so far more reliable model than 2D cell culture. Furthermore, with 3D cell cultures we can get more realistic view on tumor cells growth and behavior. Thus, adding one dimension extra provides environment, which could better mimic natural *in vivo* conditions. In this study, we used collagen scaffold as a model of soft 3D environment to alter extracellular conditions and study the mechanotransduction of hepatic tumor cell lines (HepG2 and Alexander). We found that cell mechanics, guided by the physical constraints of 3D collagen scaffolds greatly affect cellular size and morphology, proliferation, cytoskeleton organization and molecular signaling. Additionally, we identified YAP-mTOR pathways as a downstream effector in 3D cell culture mechanotransduction.

P-07.3-03
Calcium binding properties of human α-actinin-1

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α-actinin-1 is a major actin filament cross-linking protein in stress fibers and focal adhesions where it also acts as a scaffold for the assembly of junction complexes. It is ubiquitously expressed in virtually all cell types. α-actinin-1 contains 4 EF-hand motifs (together they form the calmodulin-like domain – CaMD – of α-actinin-1), but only EF1 is able to bind Ca^{2+}. Research suggests that Ca^{2+} binding to α-actinin-1 weakens its ability to cross-link actin filaments and therefore acts as a regulator of its function. Recently the model of regulation of actin-binding activity of α-actinin-1 was proposed whereupon Ca^{2+} binding α-actinin-1 obtains a more closed conformation that prevents it from cross-linking actin filaments, but the exact molecular details have not yet been determined. To asses the effect of neighboring domains on Ca^{2+} affinity of EF1 we used two different constructs of α-actinin-1: isolated calmodulin-like domain (CaMD) and a half dimer where the CaMD is in the same environment as in the full-length α-actinin-1 dimer. Ca^{2+} binding affinities are 127 ± 5 and 20.9 ± 6.9 μM for the calmodulin-like domain (CaMD) and half dimer, respectively, as measured using isothermal titration calorimetry. Based on our results we conclude that the presence

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of other domains and contacts enhances Ca^{2+} binding affinity of EF1 in α-actinin-1. Next, we performed limited proteolysis assays of full-length wild type α-actinin-1 (closed form) and a full-length α-actinin-1 mutant with impaired Ca^{2+} binding ability (open form). Proteolysis with trypsin and thermolysin showed that α-actinin-1 is more resistant to digestion in the presence of Ca^{2+} (closed form), which is in agreement with the proposed model where Ca^{2+} binding fastens CaMD to the neck region between actin-binding domain and spectrin region and thereby limits accessibility for proteolytic cleavage. Detailed insight will be provided by the crystal structure of Ca^{2+}-bound and -free α-actinin-1 (in progress).

P-07.3-04
Relationship between intrinsic pathway of cardiomyocyte apoptosis and morphological changes of the myocardium in acute focal ischemia

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Coronary heart disease is one of the most common causes of death worldwide. The formation of heart failure in case of focal myocardial ischemia is associated with heart remodeling which has no specific pathogenetic concept despite many investigations performed for the last decades. The experiment was carried out on 16 adult male Chinchilla rabbits. The animals were divided into 4 groups: 1 control group (intact) and 3 experimental groups (rabbits with 1, 3 and 5-day focal ischemia of the left ventricular myocardium). Focal ischemia was modelled by ligation of the descending branch of the left coronary artery. The content of the proteins regulating the intrinsic pathway of apoptosis (BCL-2 and BAX) in cardiomyocytes was evaluated using the immunohistochemical assay of the peri-infarction area. Morphological and morphometric examination of histological sections of the myocardium was also performed. On day 1 the content of BCL-2 increases, on day 3 it keeps increasing, but on day 5 decreases (27.0 ± 4.3, 43.0 ± 4.3 and 23.0 ± 3.8 vol.% respectively vs 0.04 ± 0.02, $P < 0.05$). On day 1 of the study a significant increase in the level of BAX is observed which then decreases on days 3 and 5 (42.4 ± 4.0, 20.3 ± 3.4 and 4.1 ± 1.5 vol.% respectively vs 1.0 ± 0.2, $P < 0.05$). The ratio of BCL-2/BAX progressively increases during all the periods of myocardial ischemia (0.63, 2.10 и 5.65 respectively, $P < 0.05$). The increasing trend in BCL-2/BAX ratio had a strong positive correlation with the increasing destructive changes in the myocardium (0.91) and a strong negative correlation with the decreasing number of morphologically intact myofibrils (-0.92) and nuclear-cytoplasmic ratio (-0.96) in all the terms. Thus in case of focal ischemia inhibition of apoptosis at the later stages of the experiment was preceded by the earlier induction of the intrinsic pathway of apoptosis. This phenomenon may be due to the increasing destructive changes in the myocardium of the peri-infarction zone.

P-07.3-05
Expression of heat shock protein HSP60 and morphological changes of the myocardium under combination of diabetes mellitus with hypertension

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Heat shock proteins (HSP) are also called stress-induced proteins due to their ability to stabilize and repair proteins during their synthesis in case of cell stress. Heat shock proteins are involved in the mechanisms of apoptosis, playing both pro- and anti-apoptotic role depending on their location in the cell. These processes are often implemented with the participation of heat shock proteins, activation of which can guide the process to the activation of pro-apoptotic program or the way of cells survival. The expression of HSP in cardiomyocytes in association with morphological disorders of the myocardium under diabetes mellitus and hypertension were the main objective of our study. The experiments were carried out on 20 male 26 week Wistar-Kyoto and SHR (spontaneously hypertensive rats). The animals were divided into 4 groups: Wistar-Kyoto rats (control); Wistar-Kyoto rats with diabetes mellitus (DM); SHR rats; SHR rats with diabetes mellitus. Diabetes mellitus was induced in the animals by a single intraperitoneal administration of Streptozotocin. The animals were selected for further experiments if their glucose blood levels were higher than 18 mmol/l. The expression of HSP60 in cardiomyocytes was investigated using immunohistochemical assay of the left ventricular (LV) myocardium. Morphological examination of histological sections of the myocardium was also carried out. It was found that LV myofibrils were hypertrophied in both SHR and SHR+DM rats. In Wistar-Kyoto rats with DM and SHR+DM rats considerable changes of myocardial blood vessels were seen including their wall thickening. HSP60 was less expressed in the SHR and Wistar-Kyoto diabetic rats compared to the controls with no considerable differences between the experimental groups. It can be concluded that both single hypertension or DM and their combination are accompanied by the inhibition of HSP60 production to approximately similar levels.

P-07.3-06
Oocyte specification in Drosophila depends on genetic interaction of orb and aPKC

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Drosophila translational regulator Orb, homolog of vertebrate CPEB proteins, is a key regulator of multiple steps of oogenesis. Orb is necessary to determine oocyte identity by positive 3'UTR-dependend autoregulatory loop. On later stages, orb interacts genetically aPKC and cdc42 genes encoding Par proteins that direct oocyte polarity. In this study, we investigated the role of genetic interactions of orb and aPKC in determination of oocyte

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identity. We used females after insertion of partially functional XN fragment of the orb 3'UTR in orbΔ3'UTR mutants. These flies have delayed oocyte specification and disordered oocyte polarity, what leads to block of further embryonic development. We analyzed effect of mutations of aPKC on orb-XN 3'UTR genetic background on oocyte specification. Hypomorphic aPKC mutations resulted in decreased number of the laid eggs, and in case of null mutation of aPKC the eggs were absent. Further microscopic analysis of immunostained ovaries showed that the aPKC null-mutation on orb-XN 3'UTR genetic background resulted in absence of oocyte in the egg chambers. Our results demonstrated that genetic interaction of aPKC and orb is necessary for oocyte specification during Drosophila oogenesis. The work was supported by the Russian Science Foundation (grant №18-74-10051).

P-07.3-07
The actin regulators Spire1 and Myosin Vc localize to endothelial Weibel-Palade Bodies and facilitate calcium evoked von Willebrand factor surface presentation

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Weibel-Palade Bodies (WPB) are endothelial cell-specific storage granules that participate in the regulation of vascular hemostasis by releasing the platelet adhesion receptor von Willebrand factor (VWF) following endothelial stimulation. The cigar shaped WPB form at the trans-Golgi network and further mature upon transport to the cell periphery, where regulated exocytosis occurs upon elevation of intracellular Ca^{2+} or cAMP levels. Several small GTPases have been implicated to participate in the maturation and exocytosis of WPB, most notably Rab27a and 3b. By applying proximity proteomics via the ascorbate peroxidase APEX2 targeted to WPB by fusion with Rab27a or Rab3b, we identified several novel WPB associated proteins in human vein endothelial cells (HUVEC). These included the actin-binding proteins Spire1 and Myosin Vc (MyoVc) which are of special interest as WPB exocytosis and regulated VWF release have been linked to reorganization in the cortical actin cytoskeleton. MyoVc has previously been implicated to play a role in the trafficking of recycling endosomes and melanosomes. Other members of the MyoV family have been described to form a complex with Rab GTPases and Spir proteins facilitating de novo actin synthesis, thus linking actin nucleation and myosin force generation to specific cellular sites. Here, we show how both MyoVc and Spire1 localize only to mature WPB and that disruption of WPB maturation abolishes MyoVc and Spire1 recruitment. MyoVc positively regulates the number of WPB, possibly via Rab3 interaction, and promotes the Ca^{2+} dependent secretion of VWF. Spire1 also participates in histamine evoked WPB exocytosis, but the role seems to be restricted to a specific subset of WPB fusion events that assemble actin rings for efficient VWF expulsion.

P-07.3-08
Molecular model of primary protein digestion and absorption of amino acids and peptides in the midgut cell microvilli of *Musca domestica* larvae

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Digestion of proteins and absorption of products occur along the *Musca domestica* midgut. To disclose their molecular bases, the expression and localization of selected enzymes and transporters were analyzed by transcriptomics and microvillar membrane proteomics. A total of 18 trypsins (Try) and 27 chymotrypsins (Chy) were found; 9 Try and 12 Chy are more expressed in anterior midgut, whereas 8 Try and 14 Chy in the posterior midgut. One Try and one Chy are more expressed in middle midgut. Four Chy and Try have predicted transmembrane domains, and a few Try have predicted GPI-anchors. However, 8 Try and 12 Chy were found by proteomics associated with microvillar membranes in an unknown way. Ten lysozymes and 8 cathepsins D were highly expressed in the anterior and middle midgut and one of each is more expressed in carcass. Eight H^+-coupled amino acid transporters (PAT), one H^+-peptide transporter (PepT), 6 Na^+-dependent amino acid transporters (NAT) and 2 cationic amino acid transporters (CAT) were found, most of them at the cell microvilli. PATs and PepT are expressed along the midgut. NATs with a narrow spectrum are found at the beginning and at the end of the midgut, whereas NATs with a broad spectrum only at the end. NATs are powered by an apical proton pump and cation-H^+ exchanger. Amino acids could be also absorbed in a facilitated way at anterior and mainly posterior midgut by CAT. *M. domestica* larvae feed on dung with low free protein content but rich in microorganisms. Protein digestion begins with the hydrolysis of free protein at anterior midgut by Try and Chy. After killing bacteria and fungi by lysozymes and low pH at middle midgut, protein released are digested there by luminal cathepsins D and by another set of Try and Chy at posterior midgut. Absorption of amino acids and peptides occurs at anterior and mainly posterior midgut cell microvilli. Supported by FAPESP (Procs.ns.: 2017/08103-4; 2019/22451-0); CNPq; INCT-EM.

P-07.3-09
Band 3 protein-mediated chloride gradient is the driving force for ammonia/ammonium influx in human red blood cells

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Ammonium/ammonia (AM) Rhesus transporters are expressed in tissues involved in AM generation, secretion, and excretion, however, the physiological role of erythroid RhAG glycoprotein is still unclear. AM concentration in red blood cells (RBCs) is approximately three times higher than in plasma, and RBCs are the only blood cells that swell and lyse in isotonic NH_4^+ media,

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indicating that AM is transported inside the cell. However, the real driving force for AM influx in RBCs is still not clear. RhAG and anion exchanger 1 (band 3, AE1) form a structural complex in erythroid membrane, therefore we hypothesized that AE1 might be involved in AM transport in RBCs. Washed human RBCs were resuspended in HEPES buffer (NaCl, 140mM) and analyzed by flow cytometry and laser diffraction. To evaluate the AE1 role in AM transport, we (i) inhibited AE1 by DIDS in NH_4^+ media (NH_4Cl, 140 mM), (ii) added HCO_3^- (0–25mM) equimolarly to isotonic NH_4^+ media, (iii) dose-dependently substituted Cl^- by glutamate in NH_4^+ media until complete disposal of the Cl^- gradient. In isotonic NH_4^+ media the AE1 substrate HCO_3^- dose-dependently increased hemolysis rate, and inhibition of AE1 completely blocked hemolysis. In isotonic NH_4^+ media where Cl^- was substituted by glutamate altering of the Cl^- gradient dose-dependently decreased hemolysis rate until the complete prevention of cell swelling and hemolysis in absence of Cl^- gradient. Our data strongly indicate that erythroid RhAG and AE1 are functionally connected, and AE1-mediated Cl^- gradient drives AM transport against its gradient via RhAG. Taken together our data revealed the new mechanism of AM transport against the gradient in RBCs and suggested that RBCs in addition to main functions are involved in regulation of AM concentration in plasma. This study was supported by the RFBR, grant No. 19-315-60015 to JS.

P-07.3-10
The effect of Notch signaling pathway on spermatogenesis in Sertoli cells of azoospermic men

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Sertoli cells found in seminiferous tubules of testis contribute to germ cell differentiation by regulating the spermatogonial stem cell niche. Notch signaling pathway takes an important place in asymmetric stem cell division. The studies on rodents showed that NOTCH1 and NOTCH2 genes were necessary for germ cell differentiation, protecting stem cell pool. In this study, our aim was to investigate the role of these genes in azoospermia etiology in humans. Testis tissue samples obtained from obstructive (OA, n = 5; control group) and non-obstructive (NOA, n = 5) azoospermic men were enzymatically digested and primary Sertoli cell (SC) cultures were prepared with and without recombinant FSH (rFSH) supplementation. Three groups were designed: OA primary SC cultures (I), rFSH-treated NOA primary SC cultures (II) and non-FSH treated NOA primary SC cultures (III). Gene expressions were analyzed by quantitative PCR method. NOTCH1 gene expressions in group II and group III were determined 1.59 and 1.23 times higher than group I ($P < 0.05$) however, the NOTCH2 gene expressions were 0.51 and 0.73 times less, respectively ($P < 0.05$). NOTCH1 and NOTCH2 have been shown to have different contributions in certain cellular, developmental and disease contexts. It was shown that constitutive activation of NOTCH1 signaling in Sertoli cells induced premature differentiation of spermatogonia in rodents. In studies on different cancers it was shown that NOTCH2 signaling contributed to anti-apoptotic processes. Our results indicated that higher expression level of NOTCH1 and lower expression levels of NOTCH2 in Sertoli cells might be one of the important mechanism in the etiology of azoospermia, by inducing premature differentiation of spermatogonia that will result in apoptosis. Besides, FSH treatment on Sertoli cells does not show a significant change in the Notch signaling pathway. Advanced researches on this signaling pathway should reveal their importance as potential biomarkers in NOA.

P-07.3-11
Investigation of MFN2 gene expression in Sertoli cell cultures of azoospermic men

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Spermatogenesis is a multi-step process that begins with spermatogonia in the basal membrane, continues with spermatocytes and ends with haploid spermatozoa when it comes to the tubular lumen. The number, morphology, localization and membrane potential of sperm mitochondria have important properties during the differentiation steps of germ cells. Mitofusin 2 (MFN2) is an outer membrane protein that contributes to the fusion, maintenance, and order of the mitochondrial pathway as well as regulation of mitochondrial membrane potential. Under the aids of MFN2 mitochondria in the Sertoli and germ cells are arranged with fusion and fission events reaching finally to mature forms. Our aim was to investigate basal MFN2 gene expressions in Sertoli primary cell cultures obtained from nonobstructive (NOA) and obstructive azoospermic (OA) patients as control, and evaluate the effects of recombinantFSH (rFSH) supplementation on expression status. Testicular tissue samples were first enzymatically digested and then the cell suspension was prepared for primary Sertoli cell culture. MFN2 gene expressions were evaluated by real-time PCR analysis. MFN2 was found to be expressed 0.54 fold less in primary Sertoli cells of NOA patients, than those of OA ($P < 0.05$). After rFSH supplementation into Sertoli cell cultures, MFN2 expression in NOA cases showed a 0.23 fold decrease ($P < 0.05$). Our findings indicate that loss of MFN2 expression in Sertoli cells impairs maintenance of spermatogenesis due to disassembled mitochondrial organization and so do not fulfill sufficient mitochondrial dynamics to germ cells. We suggest that the role of MFN2 function in male infertility will be a step for future clinical studies on spermatogenesis defects and provides important clues to the role of mitochondria in testicular physiology. Keywords: Mitofusin 2, Sertoli cells, Male infertility, Azoospermia

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P-07.3-12
Relationship between lipid bilayer composition and gasdermin D activity

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Gasdermin D (GSDMD) was identified as an essential effector of pyroptosis. Pyroptosis is a form of programmed cell death that occurs most frequently upon infection with intracellular pathogens. Inflammatory stimulus triggers caspase activation, production of proinflammatory cytokines, and eventually leads to cellular swelling and death. After caspase cleavage, N-terminal domain of GSDMD interacts with acidic lipids in the plasma membrane, oligomerizes and forms pores. Formation of pores allows the release of highly inflammatory molecules from cell, resulting in membrane damage and consequently cell death. Although recent studies significantly expanded our knowledge on GSDMD structure, the molecular aspects underlying the mechanism of GSDMD pore formation are poorly characterized and need further elucidation. In our study, we investigated the effect of lipid composition on GSDMD-N binding to liposomes, oligomerization and pore formation. To obtain information about the affinity of GSDMD-N to the lipid membranes, surface plasmon resonance and the vesicle cosedimentation assay were employed. As expected, the presence of cholesterol inhibited interaction of GSDMD-N with cardiolipin/DOPC liposomes, but enhanced with cardiolipin/DPPC-containing liposomes. Measurement of carboxyfluorescein release from liposomes demonstrated that the presence of cholesterol also enhanced pore-forming activity of GSDMD toward cardiolipin/DPPC liposomes. Surprisingly, GSDMD-N mutant (R137A/R151A) with impaired pore-forming activity toward cardiolipin/DOPC liposomes induced release of carboxyfluorescein from cardiolipin/DPPC-containing liposomes, both in the presence or absence of cholesterol. These data indicate that membrane fluidity plays an essential role in pore-forming activity of GSDMD. Further studies focusing on structural insight into the properties of GSDMD mutants can expand our knowledge on the molecular mechanism of GSDMD-N pore formation.

P-07.3-13
Blockade of myosin II activity affects focal adhesion morphology

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Focal adhesions' (FAs) formation and maturation are closely linked to actomyosin contraction and tension. Altered interaction between FAs and actomyosin complex affects the migratory potential and can be the basis of metastatic invasion. We evaluated the role of actomyosin tension in regulation of FA parameters in non-cancer 3T3 fibroblasts and osteosarcoma U2OS cells using inhibitory analysis, immunocytochemistry and time-lapse fluorescence microscopy. Myosin II inhibition (45uM blebbistatin) diminishes FA median area and intensity in both cell lines. In 3T3 cells median FA area decreased to 0.37 μm^2 (0.13–1.17) compared to 0.57 μm^2 (0.21–3.06), Median FA intensity declined from 7313 a.u. (3944–20254) to 4293 a.u. (2576–8291). Median FA area in U2OS cells decreased from 0.65 μm^2 (0.12–6.54) to 0.5 μm^2 (0.5–2.87), median FA intensity dropped from 3098 a.u. (398.1–12752) to 2329 a.u. (42.87–6293). Inhibition of ROCK (10uM Y-27632) led to the decrease of FA area and intensity in both cell lines. In 3T3 fibroblasts median area decreased from 0.85 μm^2 (0.08–5.45) to 0.77 μm^2 (0.07–2.53). Median intensity in 3T3 declined from 2636 a.u. (577.2–6259) to 1366 a.u. (253–3361). Median FA area in U2OS cells decreased from 1.10 μm^2 (0.2–9.06) to 0.56 μm^2 (0.13–4.37), median FA intensity declined from 3085 a.u. (103.7–10751) to 2404 a.u. (178.4–7413). Blebbistatin and Y-27632 differentially affected the actin cytoskeleton of modal cell lines. Blebbistatin treatment led to disassembly of the stress fibers and arches in both cell lines, but thin actin fibrils network remained intact. In Y-27632-treated cells we visualized only short ventral SF. For cells under both inhibitors phosphorylated myosin is not associated with actin filaments and is dispersed along the cytoplasm. Thus, phosphorylation of myosin II is necessary to maintain FA structure indirectly through the systems of actin cytoskeleton.

P-07.3-14
Cell respiration of ovaries *Drosophila melanogaster* increases after simulated microgravity

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The need to maintain reproductive health during a long space flight for the deep space exploration in the future poses the problem of studying the effect of microgravity conditions not only on a single cycle of egg maturation, but also on the full cycle of gametogenesis. The aim of this study was to determine the cell respiration of *Drosophila melanogaster* ovaries after exposure to a modelling microgravity during the oogenesis cycle and during the full gametogenesis cycle. All the experimental procedures were approved by the Commission on Biomedical Ethics of the SSC RF IBMP RAS (Minutes No. 521 dated September 25, 2019). After exposure to conditions simulating the effects of microgravity during one cycle of oogenesis and during the full cycle of gametogenesis, the rate of cell respiration of *Drosophila melanogaster* ovaries, measured by polarography, increased. Substrate inhibitory analysis showed that an increase in the rate of oxygen uptake for exposure during the full cycle of gametogenesis is associated with the I complex of the respiratory chain, since when this complex was inhibited and then the substrate II of the respiratory chain complex was added, the rate of cell respiration did not differ from the control group. This effect is apparently associated with an increase in the intensity of oxidative phosphorylation. Under the effect of simulated microgravity during the oogenesis cycle, an increase of the rate of cell respiration is probably associated with a change in the work of not only I, but also II complex of the respiratory chain, capable of oxidizing succinate from the tricarboxylic acid cycle to fumarate, which, possibly, has an adaptive character under conditions of altered gravity. This work was financially supported by the program for fundamental research SSC RF – IBMP RAS 65.4 and Strategic Academic Leadership Program.

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P-07.3-15
Redistribution of actin isoforms in mouse oocytes under simulated microgravity

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The processes of fertilization and embryogenesis in animals under microgravity conditions, especially in the perspective of long-term space flights, are still poorly understood. The aim of the work was to evaluate in mouse oocytes one of the parameters associated with the formation of the contractile ring in early embryos - beta and gamma actin. Oocytes were obtained by stimulating superovulation in mice, and they were randomly divided into two groups: control and simulated microgravity for 6 hours. The assessment of the beta and gamma actin content was carried out by the immunohistochemical staining followed by fluorescence intensity. The images were analyzed using the Fiji software package (https://imagej.net/Fiji). All the experimental procedures were approved by the Commission on Biomedical Ethics of the SSC RF IBMP RAS (Minutes No. 521 dated September 25, 2019). It follows from the obtained results that the total content of beta-actin in mouse oocytes after simulated microgravity did not change, since the fluorescence intensity did not differ between the control and experimental groups. However, the distribution of fluorescence in the control and experimental groups was different: in the control group – mainly along the periphery of the cell, in the experimental – more evenly. A numerical assessment of this effect indicates that the parameter characterizing the steepness of the parabola describing the fluorescence distribution was significantly lower in the experimental group ($P < 0.05$). At the same time, the relative content of gamma-actin significantly ($P < 0.05$) increased in the cell after exposure to microgravity, but the distribution of fluorescence did not differ from the control. Thus, it can be assumed that these changes may be significant for the subsequent formation of the contractile ring during zygote cleavage. This work was financially supported by the program for fundamental research SSC RF – IBMP RAS 65.4 and Strategic Academic Leadership Program.

P-07.3-16
The organization of the cytoskeleton in the endometrium of patients with various types of endometriosis

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The decrease in reproductive potential among women of fertility age is increasingly associated with ectopic growth of the endometrium. However, the pathogenesis of various types of endometriosis, despite intensive study, is still not entirely clear. The aim of this study was to compare the content of cytoskeletal proteins that provide cell motility in endometrial biopsy specimens obtained intrasurgically with genital and extragenital forms of endometriosis. Subjects were informed of the purpose of the study and all of them provided written consent to participate. The study was complied with the guidelines of the Declaration of Helsinki and approved by the Biomedical Ethics Commission of the SSC RF - IBMP RAS (Minutes No. 523 of September 26, 2019). The following research groups were formed: control, adenomyosis, genital and extragenital endometriosis. Protein content was determined by western blotting using specific antibodies to actin isoforms and actin-binding proteins, as well as tubulin and tubulin-binding proteins. It was shown that the content of alpha- and beta-tubulin was at the same level in all study groups. Some tendency toward a decrease of the content of CKAP protein was noted in the extragenital endometriosis group. In addition, in this group, as well as in the adenomyosis group, there was a decrease of the content of the actin-binding protein alpha-actinin, which can contribute to an increase in cell movement. The results can be of value in the analysis of the mechanisms of development of various types of endometriosis and, further, lead to the development of new approaches to treatment strategies. This work was financially supported by the program for fundamental research SSC RF – IBMP RAS 65.4 and Strategic Academic Leadership Program.

P-07.3-17
Proteomic analysis of *Mycoplasma hominis* cells forming colonies of a previously unknown morphological type.

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Mycoplasma hominis belongs to the class of Mollicutes and cause acute and chronic infections of the urogenital tract. The main features of this bacterium are the absence of the cell wall and the reduced size of the genome (559 genes). Earlier we isolated colonies of *Mycoplasma hominis* of unknown morphological type, called a micro-colonies (MCs) from the serum of the patients with inflammatory diseases of the urogenital tract. MCs were also obtained *in vitro* from typical colonies (TCs) after treatment with hyperimmune serum. MCs differ from TCs by colonial morphology, size (diameter of TCs is 100–300 microns, MCs - 7–9 microns), a significantly slow growth and extraordinary resistance to various adverse factors. HPLC-MS comparative proteomic analysis of TCs and MCs colonies revealed significant differences in the protein distribution between these morphological types. We found a significant decrease in the abundance of proteins involved in the processes of replication, translation, cell division and synthesis of ATP in the MCs comparing to TCs. Considering the very slow growth of the MCs the data obtained may indicate the inhibition of the main cellular processes and the transition of the cells to a state of energy deficiency. In addition to that, we discovered the decreased level of glycolysis and arginine-dehydrolase pathway enzymes and the increased level of thymidine phosphorylase (deoA) and deoxyribose-phosphate aldolase (deoC) enzymes, involved in nucleoside catabolism, which might point to the utilization by MCs of phosphorylated pentoses, which are formed as a result of nucleoside catabolism, rather than products of degradation of simple sugars and arginine. To prove this

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assumption, we obtained a mutant with overexpression of the deoA gene. The deoA mutant, demonstrated antibiotic resistance- and has a proteomic profile similar to MCs. This work was supported by the Russian Science Foundation grant No. 19-75-10124

P-07.3-18
4-Nonylphenol induces ER-stress-related apoptosis and mitochondrial dysfunction in human hepatic cells

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4-Nonylphenol (4-NP) is an emerging environmental pollutant widely diffused in waters and sediments, mainly deriving from the degradation of alkyl phenol ethoxylates, compounds commonly employed in several industrial applications. Humans are constantly exposed to 4-NP by ingestion of contaminated food and water. After ingestion, 4-NP tends to accumulate in body fluids and tissues. One of the main organs where 4-NP and its metabolites are concentrated is the liver. Here, 4-NP causes oxidative stress and apoptosis, even at low doses. Our aim has been to analyze the effects of 4-NP on a human hepatic cell line (HepG2) in order to deepen the knowledge on its cytotoxic mechanism. We studied cytotoxicity by MTT assay, S-phase entry assay and caspase 3 detection. We also investigated the level of ER-stress and mitochondrial markers by Western blot and PCR. We found that 4-NP significantly reduced cell viability, partially caused by a block of proliferation and partially by an increase of apoptosis. Moreover, 4-NP induced-apoptosis seemed to involve both an ER-stress response, with the appearance of high level of GRP78, CHOP and the spliced XBP1, and a dysregulation of mitochondrial physiology, characterized by an over-expression of main markers of mitochondrial dynamics, MFN2 and DRP1. These findings support the hypothesis that prolonged exposure to 4-NP through the diet may lead to a local damage at the level of hepatic cells, with potential negative consequences for liver homeostasis and physiology. *The authors marked with an asterisk equally contributed to the work.

P-07.3-19
Evaluation of protein diversity in hemocytes of Baikal endemic amphipod *Eulimnogammarus verrucosus*

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Immune response aimed at protecting against the effects of pathogens is an important function in any organism. A key component of crustacean immunity are hemocytes, which circulate in the hemolymph. These cells are capable of encapsulating foreign objects and express immune compounds to the hemolymph. However, the molecular processes underlying the functioning of hemocytes in Baikal endemic amphipods remain understudied. This work is devoted to study of protein composition in hemocytes of a Baikal amphipod *Eulimnogammarus verrucosus*. The amphipods were collected in the littoral zone of Lake Baikal near the Listvyanka bay and acclimated for 4 days at the temperature of 6 °C in well-aerated aquaria. Hemolymph was collected, followed by purification of hemocytes and protein isolation. The hemocyte proteome was evaluated using liquid chromatography/tandem mass spectrometry. Proteomic analysis was performed at the Advanced Mass Spectrometry Core Facility (Skoltech, Moskow). Data analysis was carried out using SearchGUI v3.3.17 and Peptide Shaker v1.16.44 software based on the assembly of the *E. verrucosus* GHHK01 transcriptome. 1152 proteins were found. Annotation of these sequences using the Panther database showed that proteins of the larval storage protein/phenoloxidase family are most abundant in the hemocyte proteome. Proteins of the C-type lectin family, engaged in recognition of pathogen-associated molecular patterns, were found in large quantities. Also, we found integrins, superoxide dismutase, scavenger receptors and antimicrobial peptides, involved in the immune response. Thus, we characterized the first hemocyte proteome of an amphipod and identified proteins that are directly or indirectly involved in the immune response. The reported study was funded by RFBR and the Government of the Irkutsk Region, project number 20-44-383007, and Ministry of Science and Higher Education of the Russian Federation FZZE-2020-0026.

P-07.3-20
Evaluation of cellular response to fipronil sulfone on SerW3 Sertoli cell line

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Human exposure to chemicals increases each passing day. Pesticides are known to affect human health adversely. Fipronil is an insecticide belonging to the phenyl pyrazole family and used for agriculture of corn, wheat, and sunflower which are the main products of the human diet globally. Fipronil is known to affect male fertility by DNA damage and apoptosis in spermatozoa. Fipronil sulfone is the main metabolite of fipronil and its toxicity was reported to be more toxic than the main compound fipronil. Due to lack of information about fiproniland its metabolite on Sertoli cells in male reproductive system, this study was designed to exert the effects of fipronil sulfone on SerW3 cells as an *in vitro* Sertoli cell model. In the present study, SerW3 cells were exposed with fipronil sulfone (0–40 μM) for 24 hours and cell viability was performed by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl-2H-tetrazolium bromide (MTT) assay. Furtherly, SerW3 cells were examined morphologically by hematoxylin and eosin staining and transmission electron microscopy (TEM) for detailed morphological evaluations. According to results of the study, fipronil sulfone as a main metabolite of fipronil decreased cell viability and had adverse effects on cell morphology in a concentration dependent manner. Keywords: Fipronil sulfone, SerW3 cells, cell viability, cell morphology, TEM. Acknowledgement: This work was supported by Scientific and Technical Research Council of Turkey (Project No: 219S895).

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P-07.3-21
The role of human SFPQ protein in HIV1 replication

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One of the developing HIV1 cure approaches is inhibition of interactions between viral proteins and their cellular partners. Consequently, the search for new cellular participants of HIV1 replication is still relevant. SFPQ protein (splicing factor, proline and glutaminerich) is considered as one of HIV1 integrase partners but its role in the viral replication is obscure. To determine the step in the virus life cycle that is affected by SFPQ, we transduced HEK 293T cells with normal, increased and reduced SFPQ levels with an HIV-based lentiviral vector and analyzed the efficiency of early replication stages using qPCR approaches. We showed that changes in the SFPQ level did not affect reverse transcription whereas influenced both integration and postintegrational DNA repair. Using pulldown assay we studied binding of recombinant SFPQ protein with HIV1 reverse transcriptase and integrase as well as a cellular integrase partner Ku70, which is involved in the postintegrational repair [Anisenko AN et al. (2017) Sci Rep, 7:5649]. SFPQ was shown to interact with both integrase and Ku70 but not with reverse transcriptase. In addition, we established dissociation constant of integrase and SFPQ. Thus, we demonstrated for the first time the functional role of SFPQ in HIV1 integration and postintegrational repair, as well as characterized its interactions with viral proteins. This work is supported by Russian Foundation for Basic Research, grant 200400437.

P-07.3-22
Interplay between DTS calcium and mitochondrial potential governs platelet necrosis but not platelet apoptosis

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Non-nuclear blood cells platelets after strong activation tend to differentiate into 2 subpopulations, that either become pro-aggregatory and form thrombi or pro-coagulant that accelerate membrane-dependent reactions of blood plasma coagulation. It was shown that procoagulant platelets experience a cell death of necrotic type. However, platelets can experience a caspase-dependent cell death (apoptosis) either with age or after an anti-cancer treatment. The exact signaling pathways of platelet cell death are yet to be established. Here we aimed to define the chain of events that lead to either platelet necrosis or platelet apoptosis. Blood of healthy donors was collected into tubes with hirudin. Continuous Flow Cytometry Assessment was performed on platelets, activated by agonists of GPVI (CRP) and PAR1 (TRAP) receptors or ABT-737. Ca^{2+} level in the dense tubular system (DTS) was measured by FLUO5N fluorescence, and the level of cytosol Ca^{2+} was measured by FuraRed. The formation of procoagulant platelets was detected using annexinV and the mitochondrial potential (MP) was estimated by DiOC6 fluorescence. Platelet activation with CRP and TRAP (strong activation) lead to a rapid and prolonged elevation of cytosolic Ca^{2+} alongside with the DTS depletion. This resulted in formation of 2 subpopulations. The first one retained MP and became pro-aggregatory (binding fibrinogen). The second one rapidly lost MP after store depletion and then a further elevation of cytosolic Ca^{2+} was observed. The loss of ability to bind fibrinogen happened the last. Platelets treated with ABT-737 had a fraction with low MP but that was not caused by store depletion. The initial elevation of cytosolic Ca^{2+} was not observed, while a moderate elevation to micromolar concentrations was observed after the loss of MP. Therefore this work shows different chains of events that lead to platelet cell death via 2 distinct pathways. The study was supported by Russian Science Foundation (Grant 21-74-20087).

Molecular interactions of plants with the environment

P-07.4-01
Effect of increased temperature on seed germination and DNA methylation in *Arabidopsis thaliana, Brassica rapa* and *Solanum lycopersicum*

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Temperatures vary geographically and are predicted to rise with global warming. For dormant seeds, temperature and light are two main cues required for germination. Therefore, appropriate germination timing and response to temperature cues are necessary for plant development and later growth and reproduction. Plants have a very rich epigenetic machinery that enables them to readily adapt to environmental conditions. Understanding the epigenetic basis of sensitivity to temperature is therefore required for understanding interactions between plants and environment. Here, we will examine how environmental temperature impacts germination and DNA methylation of three plant species, model plant Arabidopsis thaliana and two globally important crop species *Brassica rapa* and *Solanum lycopersicum*. For germination tests, seeds were exposed to a set of rising temperatures ranging from 28 to 40°C and global DNA methylation level was assessed by immunospecific detection. DNA methylation in plants was shown to be temperature dependent.

P-07.4-02
Monitoring phytoplasma infection in tomato during two growing seasons

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Phytoplasma is a wall-less worldwide pathogenic microorganism affecting a large number of economically important crops. The aim of the study was to identify '*Candidatus* Phytoplasma solani' in tomatoes during the growing season. The study was carried out on four genotypes (varieties) of tomato over two years (2019 and 2020). Molecular diagnosis revealed a significant difference in plant infection rates between two years of research. In whole, the infection spread was significantly lower in 2020 than in 2019. The total proportion of plants infected with phytoplasma in July of 2019 was 37.5%; it reached 69.6% at the end of the growing

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season (mid-September). In July of 2020, '*Ca*. P. solani' infection was absent in the tomato field. In September of 2020, the percentage of infected plants was no more than 14.5%. Such contrasting results obtained over two years can be explained by the influence of different climatic conditions of these years in combination with specificity of agricultural techniques. In addition to different levels of infection during the years of research, it was found that the sensitivity of tomato plants to phytoplasma infection varied depending on the genotype. Thus, the tomato variety Mary Gratefully showed a highest sensitivity to '*Ca*. P. solani' in both growing seasons (92% of infected plants in September of 2019 and 33% in 2020). The tomato varieties Elvira and Desteptarea had intermediate indicators of susceptibility to phytoplasma infection in both years. On the contrary, the Cerasus variety can be characterized as the most resistant to '*Ca*. P. solani' compared with three other studied varieties. Namely, in July and August of 2019, the percentage of infected plants of Cerasus was 25% and reached 58% only at the end of the growing season. In 2020 this variety demonstrated complete immunity to phytoplasma during the growing season. Obtained results can be used in the breeding and creating tomato varieties resistant to phytoplasma infection.

P-07.4-03
Thionins from blackseed (*Nigella sativa* L.) with multiple activity

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Antimicrobial peptides in the thionin family are known to have inhibitory activity against a wide range of microorganisms and tumor cells. Here, we represent the results of a study on thionins that were isolated from black cumin (*Nigella sativa* L.). *N. sativa* is a common species that is used as a medicinal plant. Ten novel thionins (NsW 1–10) were isolated from *N. sativa* seeds using acetic acid extraction followed by liquid chromatographic fractionating. The molecules obtained were assigned to the thionin family according to their primary structure. Four of the molecules were tested in the functional study. Activity against three clinical *Aspergillus* species was detected for three of the tested peptides. Activity against *Candida albicans* and Gram-positive bacteria was also shown. *C. albicans* cell death occurred by disruption of cell membrane integrity. This mode of action on the yeast cells was confirmed by studying the effect of peptides on the tumor cells *in vitro*. Upon incubation of the peptides with four different cancer cell lines (AsPC-1, Colo357, RD, Jukart), a dose-dependent cytotoxic effect was found. The recombinant peptide NsW2 was obtained using heterologous expression in a prokaryotic model of the *Escherichia coli* Origami strain. The target peptide was obtained in association using a chimerical construction with the original *E. coli* protein thioredoxin, which provides normal peptide molecule folding. Insecticidal action of NsW 1-2 toward a mealworm (*Tenebrio molitor*) and a flesh fly (*Sarcophaga carnaria*) via direct injection to the hemolymph was detected. There was no cytotoxic effect toward the Sf9 insect cell line. Thus, there is likely another molecular mechanism of insectotoxicity that may be associated with neuromuscular transmission blocking or have specific activity on ion channels. This work was supported by the Russian Science Foundation (grant № 18-74-10073).

P-07.4-04
Novel hevein-like defense peptides from wild cereals

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Defense peptides are known to provide an important role in innate plant immunity to biotic environmental stress factors, including fungal diseases. Hevein-like peptides (six and ten cysteine-containing molecules) were preliminary characterized by the presence of a chitin-binding domain, which is needed to initially act on plant pathogenic filamentous fungi followed by disruption of fungal cell wall biosynthesis. Already published data indicate that these peptides can effectively inhibit non-chitin microbes, such as oomycetes. One alternative molecular mechanism of action is associated with blocking of zinc-containing metalloproteinase (fungalysin) secreted by *Fusarium* spp. and cleavage by plant chitinases. In our studies, we isolated and characterized two hevein-like peptides with ten-cysteine motifs each (called EAMP and LAMP), which belong to the WAMP subfamily, from barnyard grass (*Echinochloa crusgalli*) kernels and lyme grass (*Leymus arenarius*) ears, respectively. All of these molecules are homologous and contain a single amino acid substitution based on the model WAMP-1a peptide. Their recombinant analogues have been obtained by heterologous expression in an *Escherichia coli* system, a spectrum of antifungal activity against some plant pathogenic and opportunistic fungal species has been determined, and it has been shown that their ability to recover total protease and chitinase activity in vitro after incubation with concentrated culture liquids of *F. oxysrorum, F. solani*, and *F. sporotrichoides*. These results suggest that these peptides can be considered antimicrobials and novel fungal protease inhibitors. This work was supported by the Russian Science Foundation (grant № 18-74-10073).

P-07.4-05
Identification of high light-induced Hli protein in pigment-protein complexes of thylakoid membranes of cyanobacteria *Arthrospira platensis*

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Cyanobacteria contain a family of stress Hli (high light inducible) proteins, which are considered as evolutionary precursors of chlorophyll a/b binding proteins of plants and algae. Most of the Hlips studies were performed on *Synechocystis* sp. It was shown that Hlips are necessary for the survival of cells in conditions of intense light, participate in the regulation of chlorophyll biosynthesis, transport and binding of free chlorophyll molecules, quenching of singlet oxygen, assembly and repair of photosystem II, and non-photochemical dissipation of absorbed light energy. Nonetheless complete picture of Hlip functioning and distribution are not fully understood and data for other cyanobacteria is missing. In current study Hlips of multicellular *Arthrospira platensis* were investigated. According to the NCBI database,

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there are three Hli genes in *A. platensis* genome that encode proteins of 47, 64, and 69 aa long. The *A. platensis* cells were exposed to light stress (500 μmol photons/m^2·s, 1 h). Then two-dimensional electrophoresis and mass spectrometry were used to determine the association of Hli proteins with pigment-protein complexes of thylakoid membranes. MALDI-TOF mass spectrometry analysis revealed presence of Hli 47 aa long only. This data allows us to conclude that the identified Hlip is associated with photosystem II and is a homologue of HliC *Synechocystis* sp. Bioinformatics analysis of the amino acid sequence of the identified Hli protein of *A. platensis* revealed a high degree of homology with the amino acid sequences of proteins of a number of other multicellular cyanobacteria and a lesser degree with the Hli amino acid sequence of single-celled cyanobacteria. This work was partially supported by the Russian Foundation for Basic Research (Grant No. 19-04-00798).

P-07.4-06
Cold stress affects leek plastid gene expression pattern

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Crop productivity depends on the photosynthesis intensity in chloroplasts, since the formed simple carbohydrates are energy source for plant growth and development. Expression of plastid genes is crucial for the photosynthesis; about half of the photosystem I and II subunits, as well as subunits of cytochrome-b6f-complex are encoded in the plastid genome. The abiotic and biotic stresses affect the level of plastid gene transcription and, therefore, photosynthesis intensity and productivity. In this study, we determined the expression pattern of genes of the photosystem I (psaA, psaB) and II (psbA, psbB, psbE) main subunits in the leek *Allium porrum* leaves before and after exposure to cold stress. For this, 60-day-old leek plants (cv. Premiere) grown in a greenhouse were transferred to a climatic chamber under controlled conditions (day/night – 16 h/ 8 h at + 22°C/ +16°C). Two weeks later, the plants were exposed to cold stress (+4°C) for 2, 4, 6 and 24 h. In leek leaves, after 2, 4, and 6 h at +4°C, the psaA, psaB, psbA expression level was significantly upregulated (3–10-fold). The transcript number of the other two genes psbB and psbE was growing after 2 and 4 h of cold treatment, but after 6 h fall down to (psbB) or below (psbE) the control level. After 24 h cold stress, in leek leaves, the psaB, psbB, psbE expression levels were decreased to the control level, but the psaA and psbA expression remained 2–3 times higher than the control. It may be suggested that increasing the expression level of the analyzed photosystem genes in response to cold contributes to the simple carb accumulation, thereby enhancing leek resistance to low temperatures. This work was supported by grant MK-3350.2019.11.

P-07.4-07
Transcriptome analysis of the mechanisms of cucumber–green mottle mosaic virus interactions

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Cucumber (*Cucumis sativus* L.) is an important cucurbit crop grown in Russia and worldwide. Cucumber green mottle mosaic virus (CGMMV) is an RNA-containing virus of the Tobamovirus genus. It is an economically important pathogen that causes significant reduction of both yield and quality of Cucurbitaceae crops. The aim of this work was to study transcriptional changes in a highly inbred cucumber line 229 sensitive to CGMMV caused by infection with the virus at two time points, 3 dpi and 20 dpi. At the early stage of infection (3dpi), no disease symptoms on the cucumber plants were observed, while at the late stage (20 dpi), the symptoms of the viral disease were clearly pronounced. Differentially expressed genes (DEGs) were those with an expression fold change ≥2 (up-regulation) or ≤0.5 (down-regulation). A total of 5766 DEGs were identified in the cucumber transcriptome 3 dpi (2536 up- and 3230 down-regulated). 4388 DEGs were revealed in the cucumber transcriptome 20 dpi (2022 up- and 2366 down-regulated). 4248 transcripts were differently expressed in both transcriptomes. Among differentially expressed genes were transcription factors, histones, small heat shock proteins, stress response proteins, transcripts involved in photosynthesis and plant hormone signal transduction. In addition, 9 DEGs involved in the RNA-silencing processes were identified. Most transcripts (4 RNA-dependent RNA polymerases, 2 argonaut proteins, 1 DSL-protein) show a 2–4-fold decrease in expression level induced by CGMMV infection. We also identified 4 DEGs with significantly increased expression in response to CGMMV and which functions were uncharacterized before. The obtained results provide a basis for further studies of the molecular mechanisms underlying CGMMV infection in cucumber. This work was supported by the Russian Science Foundation (grant no. 16-16-00032).

P-07.4-08
Effects of joint and separate uses of the Quercus cortex extract and antibiotics on the microbiome of the broiler intestine

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Efforts to find alternatives to feed antibiotics have gained a lot of interest in poultry farming in recent years; medicinal plants are considered among such alternatives. Therefore, it seems interesting to assess the effects of these plants and antibiotics on the structure of the intestine microbiome. The effects of Quercus cortex extract (QC) containing "quorum sensing" inhibitors and the antibiotic chlortetracycline (Ch) on the microbiocenosis of the small and cecal intestine of broilers were evaluated (4 groups, n = 30, 42 days). The control group received the main ration (MR); the first experimental group was given MR+QC; the second one – MR+Ch; experimental group III – MR+Ch+QC. Microbiota of bacteria was analyzed using high throughput

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sequencing (Illumina). The bioinformatical analysis of the results was made with the application PEAR. There was a significant decrease in representatives of the Firmicutes phylum (by 14.4%) in the small intestine of group III. At the same time, classes were found to vary within one phylum depending on the factor under study. Thus, the Bacilli class in groups II and III decreased by 21.2 ($P \leq 0.05$) and 11.9% (due to the Lactobacillaceae families) while the Clostridia class increased by 25.1 ($P \leq 0.05$) and 12% (due to the Clostridiaceae families). In the cecum, representatives of the Firmicutes phylum tended to decline by 10.9 to 53.8% ($P \leq 0.05$) against the background of MR+Ch and MR+QC+Ch (due to the Clostridia class and the Ruminococcaceae family) whilst the number of microorganisms of the Bacteroidetes phylum increased by 9.4 and 52.6% ($P \leq 0.05$) (due to the Bacteroidia class and the Rikenellaceae family). Thus, the use of MR+Ch and MR+QC+Ch in diets for broiler led to an increase in the same representatives of phyla in the small intestine against the background of their decline in the cecum with a considerable variation of representatives of the Clostridia class. Research was performed in the framework of project 0761-2019-0005.

P-07.4-09
Metagenomic analysis of the broiler intestine microbiome against the background of plant extracts and probiotics

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Propagation of microorganisms' resistance to the action of various antibiotics has led to an active search for substances that can weaken or eliminate this effect in cultivation and fattening. Plant substances, probiotics, and their synergistic effect are of particular interest in this regard. The effects of probiotics *Bifidobacterium adolescentis, Lactobacillus acidophilus* (Pr), and Quercus cortex extract (QC) containing "quorum sensing" inhibitors on the microbiome of the small and cecal intestine of broilers were evaluated (4 groups, n = 30, 42 days). The control group received the main ration (MR); the first experimental group was given MR+QC; the second one – MR+Pr; experimental group III – MR+Pr +QC. Microbiota of bacteria was analyzed using high throughput sequencing (Illumina). The bioinformatical analysis of the results was made with the application PEAR. In the small intestine of groups I and II, there was an increase in the Bacilli class (by 54.2–27%) due to the Lactobacillaceae family (by 26.1–54.2%) against the background of a decline of the Clostridia class (by 27–50.5% ($P \leq 0.05$)) in comparison with the control. Group III revealed that Bacilli representatives decreased by 23.1 % and Clostridia increased by 27.8%. The cecal intestine in the first and third groups was characterized by an increase in representatives of the Firmicutes phylum (by 9.2–19.9%) due to the Clostridia class and the Ruminococcaceae, Lachnospiraceae, and Clostridiaceae families against the background of a decline in representatives of the Bacteroidetes phylum. In the second group, there was a 55.1% decrease in Firmicutes phylum due to the Clostridia class ($P \leq 0.05$). Thus, we evidenced a sharp change in the number of individual bacteria (Clostridia) in the intestine against the background of Pr and QC whilst the combined use of the preparations negates their quantitative fluctuations. This research was performed with financial support from the Russian Science Foundation (16-16-10048).

P-07.4-10
The synergistic action of plant extracts and enzymes on the cecal microbiome of broilers

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The use of enzyme-based drugs is accompanied by the active growth of the intestine microflora and the loss of some digested substances. Some suppression of the microflora can significantly increase the productive action of enzymes. As a promising approach, there were evaluated direct and joint actions of an enzyme containing glucoamylase and cellulolytic enzymes (En) and a purified extract of Quercus cortex (QC) containing natural "quorum sensing" inhibitors on the microbiome of broilers' cecum (*Gallus gallus*, 4 groups, n = 30, 42 days). The control group received the main ration (MR); the first experimental group was given MR+QC (2.5 ml per kg of live weight); the second one – MR+QC+En (5 g/10 kg of feed); experimental group III – MR+En. Microbiota of bacteria was analyzed using high throughput sequencing (Illumina). The bioinformatical analysis of the results was made with the application PEAR. The dominant phyla in the groups were Bacteroidetes and Firmicutes, the dominant classes – Bacteroidia, Bacilli, and Clostridia. The introduction of QC to the diet increased the number of Bacteroidetes by 24.6–19.9% and the Bacteroidia class increased by 24.4–19.8%. At the same time, there was a significant advantage of the Rikenellaceae family. The combined use of QC+En in the diet led to the active development of microorganisms of the Bacilli class as well as members of the Lactobacillaceae family (by 6–8%) and the Lactobacillaceae genus (by 9–11%). The experimental groups showed a decrease in bacteria of Clostridia, at that the decrease in groups II and III was less expressed (by 14.6–16.6%). The joint use of plant extracts and exogenous enzymes can be a promising diet scheme for broilers due to the positive effect on the development of Bacilli-class bacteria and the inhibition of Clostridia-class bacteria. This research was performed with financial support from the Russian Science Foundation (16-16-10048) and in the framework of project 0761-2019-0005.

P-07.4-11
Influence of Quercus cortex molecules on biofilm formation

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Bacterial populations mainly exist in the form of cell associations – biofilms. In this form, microorganisms show a higher level of resistance to external influences. Natural plant compounds can have not only a direct bactericide effect but also disrupt the intercellular interaction, which contributes to the disintegration of biofilm. The aim of the research was to study the effect of molecules derived from oak bark on the formation of biofilms. *Staphylococcus aureus* (SA) and *Pseudomonas aeruginosa* (PA)

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strains were used as model systems. The formation of biofilms was evaluated according to the O'Tolle method. Bacterial cells were cultivated in nutrient broth (HiMedia, India); the measurement of optical density was carried out with the photometer Stat-Fax 303+ (Awareness, the USA). Propylresorcinol, the effect of which has a two-phase character, showed the greatest activity among the compounds under analysis. For SA, the density of biofilm increases by 2.2 times without changing the free cell population in the concentration range of 10 to 100 micrograms/ml. With a higher concentration of propylresorcin, there was registered a 62 and 28% decrease in the number of free cells and biofilm formation, respectively. Speaking of PA, the effect was noticed only on the number of cells formed in culture but not on the formation of biofilm. On the other hand, the influence of scopoletin on both cultures was characterized by the positive dynamics of cell culture and the intensity of biofilm formation. The development of this area of research will allow identifying the most promising compounds that disengage the communication signals of bacteria and subsequently violate the formation of biofilm, which will lead to more efficient use of the antibacterial drugs. This research was performed with financial support from the Russian Science Foundation (16-16-10048).

P-07.4-12
Changes in the molecular profile of compounds released from plant lignin during the process of its fungal decomposition

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Forests are major ecological units existing on our planet. Every year, wood contributes an enormous pool of organic matter to the environment. While carbohydrate part of the wood can be readily utilized by many microorganisms, its lignin portion is highly recalcitrant. Being a complex polymeric molecule composed of aromatic units, lignin represents an indispensable source of many different phenolic compounds. Although phenolics are believed to be decomposed slowly in soils, recently, it was shown that some groups of phenolic compounds can seriously affect nutrient dynamics of forest ecosystems – they affect the cycling of key nutrients to plants and soil microorganisms. In nature, the release of phenolic compounds from lignin is primary performed by different species of wood rotting fungi, mainly white-rots. However, the exact processes by which fungi can release different phenolic compounds as well as the full spectrum of compounds that can be released are still purely understood. In this work, negative-ion electrospray (ESI) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) was used to determine a full spectrum of compounds released during the cultivation of white-rot fungus *Trametes hirsuta* on the lignin-contained medium. The collection of samples and corresponding controls (without fungus) was performed on the third, six, tenth and fourteenth days of cultivation. As a result of data processing, the dynamical changes in the composition of released compounds was described and several groups of compounds were identified: (1) compounds that were present during all cultivation time; (2) the compounds that were released at some stage of cultivation and persisted during all cultivation time; (3) the compounds that completely disappeared at some cultivation stage; (4) and the compounds that were uniquely presented at some stage of cultivation. The reported study was partially funded by RFBR according to the research project No. 19-04-01183.

P-07.4-13
Parasite-host interaction site – profiling of enzymes and proteoglycans, involved in *Cuscuta campestris* parasitism

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Cuscuta campestris Yunck. is among the most widely distributed parasitic flowering plants, causing significant crop plants damages worldwide. The critical step in Cuscuta parasitism is the formation of haustoria, a complex process which involves multiple cell wall modifying enzymes and proteoglycans from both host and parasitic plant. In the present study, we profiled the protease and pectinmethyl esterase isoforms, as well as hydroxyproline-rich proteins (extensions and arabinogalactan proteins) with differing carbohydrate epitopes as dependent on: 1) the stage of parasite development; 2) the host species – incompatible or compatible; and 3) under different salinity treatment. We identified both parasite and salinity induced protease isoforms in the host, as well as host-specific and salinity induced ones in the parasite, while pectinmethyl esterases were mostly altered by salinity. Most importantly, we identified several groups of arabinogalactan proteins that might be involved in the incompatibility to Cuscuta infection of certain plants (tomato, *Solanum lycopersicum* and maize, *Zea mays*) and others, that are key to the *Arabidopsis thaliana* susceptibility. This research was financially supported by grant KP-06-N31/10 of the National Science Fund, Ministry of Education and Science, Bulgaria. *The authors marked with an asterisk equally contributed to the work.

P-07.4-14
Stress-induced Kunitz protease inhibitor-like protein functions as a positive regulator of plasmodesmata conductivity

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Plant cells form a multicellular symplast in which neighbouring cells are connected via plasmodesmata (Pd), the unique nanopores piercing cell walls. Through these structures, diffusion of low molecular weight metabolites and active transport of macromolecules occur, contributing to the functional integrity of plants. Recently, we proposed a mechanistic model of mature leaf Pd regulation according to which Pd-associated proteins (PdAPs) are classified as negative and positive regulators (published in: Dorokhov YL et al. (2019) Plants 8, 595). Our studies of the Kunitz protease inhibitor-like protein (KPILP), which is encoded by the "matryoshka" gene, showed that KPILP is not detected in intact leaves, but is synthesized in cells only after

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exposure to abiotic and biotic stress (published in: Sheshukova EV et al. (2017) Front Plant Sci 8, 2137). During secretion via Golgi apparatus KPILP is N-glycosylated. However, KPILP final destination is plasmodesmata-associated puncta but not the apoplast. This phenomenon could be explained by KPILP retrograde trafficking, i.e. the internalization to the cytoplasm via clathrin-mediated endocytosis mechanism and targeting to Pd. Indeed, upon suppression of endocytosis by overproduction of the C-terminal fragment of the clathrin heavy chain, KPILP is diffusely distributed in the apoplast. The stress-induced increase in KPILP production leads to the activation of the intercellular transport of macromolecules and the presence of all KPILP N-glycosylation sites is indispensable for the manifestation of this function. To explain the mechanism of KPILP action, we proposed a hypothetical model in which this glycoprotein can "switch off" or "displace" the factors responsible for the suppression of Pd permeability under stressful conditions. This study was performed with the financial support of the Russian Science Foundation (project No. 19-74-20031).

P-07.4-15
Glutathione S-transferase genes as enhancers of the plant response to stress

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Glutathione S-transferases (GSTs) are enzymes that catalyze the conjugation of glutathione with toxic molecules. Forty seven genes of GSTs are identified in *Arabidopsis thaliana*. The expression of some of them is induced by stress. Each gene could perform a specific role in the regulation of growth under changing environmental conditions. We studied the abundance of transcripts of AtGSTF11 gene under different types of stress. Its expression increased by 3.3 times under salt treatment and 2 times in the conditions of drought. Low positive temperatures (8°C) had no significant effect on AtGSTF11 gene expression, however under heat stress (42°C) it decreased dramatically. So AtGSTF11 is a gene of interest for plant genetic engineering. We used it for *Agrobacterium tumefaciens* and *A. rhizogenes*-mediated transformation of Nicotiana tabacum and *A. tumefaciens*-mediated transformation of *Brassica napus*. Tobacco with constitutive expression of the transgene had longer stems and increased biomass. It demonstrated enhanced resistance to drought, salinity and cold stress. Transgenic hairy roots of *N. tabacum* demonstrated better growth parameters than control hairy root cultures in normal conditions, under heat and salinity stress and in the presence of heavy metals. Transgenic *B. napus* demonstrated increased resistance to cold stress, most probably provided by a 3-fold increase in mRNA content of target gene. Drought and heat stress, on the contrary, had no effect on the fitness of the plants and resulted in a reduction of the expression level of AtGSTF11 gene. Transgenic *B. napus* was also less susceptible to powdery mildew *Erysiphe cruciferatum*. So, the expression of the same transgene AtGSTF11 contributed to different changes in stress response in various tissues and plant species. Positive effect was most probably achieved due to the weakening of the negative impact of oxidative stress. Research was supported by grant of President of Russian Federation MK-1146.2020.11. *The authors marked with an asterisk equally contributed to the work.

P-07.4-16
Effect of surfactants, iron and copper components and changes in pH-value on the production of algae *Chlorella vulgaris*

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Chlorella vulgaris (*C. vulgaris*) is a strain of unicellular green eukaryotic microalgae with small spherical cells that can thrive in both aquatic and terrestrial environments. *C. vulgaris* is used for several purposes: as food additive, as raw material for the production of biodiesel, in medicine and in the treatment of waste water. The goal of this research was to observe the influence of some pollutants such as iron-, copper-, and sulphur-based reagents on the algal growth. Effects of a few surfactants (Tween 80, Span 80, Q-naturale R200V) and the change of pH-value were assessed as well. The *C. vulgaris* was grown in Bold's Basal medium in the respirometer, at 22°C, with air ventilation of 0.3 m^3/s, at 12-hour day/night illumination cycle (light intensity: 150 μmol/m^2s). The experiments of substance exposure were performed on cultures of *C. vulgaris* in autotrophic state and in the exponential phase of growth, as determined from fluctuations of O_2 and CO_2 in the culture air. The test cultures were added the selected substance and grown for five days in the incubator at same conditions as the starting culture (22°C, 150 μmol/m^2s, 12-hour day/night illumination cycle). The concentration of cells in each treated sample culture was assessed every 24 hours by light microscopy. Inhibitory effects were noted for Span® 80, FeCl3, Cu(OPiv)2 and $FeSO_4$. *The authors marked with an asterisk equally contributed to the work.

P-07.4-17
The ethylene response factor StERF49 increases susceptibility of potato to potato virus Y

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Potato is the world's most widely grown tuber crop and potato virus Y (PVY) is one of the major potato pathogens causing severe crop losses worldwide. It is thus important to improve our understanding of potato-PVY interaction for more efficient crop breeding strategies. The analysis of transcriptomics data previously obtained in our group suggested that the ethylene response factor StERF49 is an important player in Ny-mediated hypersensitive response to PVY. Studies with stable transgenic potato lines in which StERF49 was knockdown revealed that the gene has a positive role in viral multiplication and symptom development and, consequently, can be considered as a susceptible factor in PVY infection. We performed RNAseq experiments on PVY

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infected and mock inoculated leaves of StERF49-knockdown potato plants. Several gens involved in the protein degradation pathway seem to be regulated by StERF49 after PVY infection. On the other hand, the StERF49 is strongly accumulated in cell nucleus only after PVY infection. Considering these results we hypothesis that PVY might interfere in the host mechanisms for its own benefit by preventing degradation of StERF49. We are further studying the complex network of plant signalling pathways involved in this response using Y2H and Y1H assays. We identified two transcription factors, involved in the ethylene signalling pathway, that activate the expression of StERF49 gene.

P-07.4-18
Induction of oxidative stress in tobacco seedlings treated with differently coated silver nanoparticles

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Among various nanomaterials, silver nanoparticles (AgNPs) stand out due to their enhanced antimicrobial effects that have been exploited in many industrial sectors and daily life. Increase in AgNP consumption has led to greater potential for their release into the environment where they can be absorbed by plants and enter the food chain, posing a threat to human health. The main mechanism of AgNP toxicity lies in excessive ROS formation and subsequent oxidative stress induction, but the degree of oxidative damage depends on the intrinsic properties of AgNPs (size, shape and coating) which determine their stability against aggregation and dissolution in the environment. This study compared the effects of two differently coated AgNPs [polyvinylpyrrolidone (PVP) and cetyltrimethylammonium bromide (CTAB)] and ionic silver ($AgNO_3$), applied in 100 mM concentration, on oxidative stress parameters in tobacco (*Nicotiana tabacum* L.) seedlings. In situ detection of ROS was performed using dihydroethidium for superoxide radical ($O2^{\cdot-}$) and 2',7'-dichlorodihydrofluorescein diacetate for H_2O_2. Activity and changes in superoxide dismutase (SOD), catalase (CAT), ascorbate peroxidase (APX) and pyrogallol peroxidase (PPX) were analysed spectrophotometrically and in gel, and their expression was determined with immunoblotting. Obtained results showed enhanced $O2^{\cdot-}$ and H_2O_2 production in all treated plants. Overall SOD, APX and PPX activities did not show significant changes in the treated plants; however, isoenzyme patterns revealed differences in activity of certain isoforms upon exposure to AgNP-CTAB and $AgNO_3$. Activity of CAT was significantly elevated in AgNP-CTAB treated seedlings, which was additionally confirmed in gel. Western blots showed higher abundance of aforementioned enzymes only in $AgNO_3$ treated seedlings. These results show that the coating used for AgNP stabilisation plays an important role in AgNP toxicity, which cannot be ascribed only to the release of Ag^+ ions.

P-07.4-19
Validation of insecticidal action of double-stranded RNA targeting Colorado potato beetle mesh gene in spray-induced gene silencing assays

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Colorado potato beetle (CPB) is an agricultural pest of Solanaceous crops, notorious for its rapid resistance development to chemical pesticides. Foliar spraying of dsRNA formulations is a promising innovative technology providing a highly specific and environmentally acceptable CPB control strategy. We designed a dsRNA to specifically silence the CPB mesh gene (dsMESH) and performed laboratory spray-induced gene silencing (SIGS) feeding trials to assess dsMESH's impacts on beetle survival and development. We produced dsMESH *in-vivo* in *Escherichia coli* production system and compared its effectiveness with *in-vitro* produced dsMESH in a series of laboratory experiments. Additionally, we performed field trials to compare the efficacy of dsMESH SIGS management strategy with a spinosad-based insecticide treatment. We showed that dsMESH ingestion consistently and significantly impaired larval growth and decreased larval survival in laboratory feeding experiments. *In-vivo* produced dsRNA performed similarly as *in-vitro* synthesised dsRNA in laboratory settings. In the field trials, dsMESH showed significantly reduced foliar damage by CPB, albeit less prominent compared to spinosad, presumably due to its mode of action.

P-07.4-20
Phytohormone dynamics induced by a local stimulation in wheat plants

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Plants respond to environmental factors by developing the complex systemic reaction including generation of electrical signals, changes in the photosynthesis activity and transpiration and changes in the content of phytohormones. The impact of external factors can be either systemic or local. Local damaging stimuli causes the propagation of variation potential (VP) – an electrical signal, which is a transient depolarization of the membrane. Local stimuli also suppress photosynthesis activity and transpiration. The photosynthetic response develops in two phases: the first phase is fast and is caused by changes in ionic concentrations during the VP propagation; the second phase is slow, which is probably due to a change in the phytohormones content. Thus, the aim of the present work was to analyze the dynamics of the content of plant stress hormones, specifically abscisic and jasmonic acids. The content of phytohormones was determined in 17–19 days old wheat plants (*Triticum aestivum* L.) in a fragment of an adult leaf distant from the site of local stimulation, which was exerted by burn or heating of a leaf tip. At certain time points after stimulation, untreated leaf fragments were homogenized, followed by extraction of the hormones. The phytohormones content in the plant extracts was determined by liquid

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chromatography-mass spectrometry. Both burn and heating of the leaf tip caused changes in the content of abscisic and jasmonic acids in distant areas of the wheat leaf; the peculiar features of these changes depend on the type of stimulus. The dynamics of the jasmonic acid content is characterized by rapid rise with a maximum concentration at 10 min; and the characteristic time is consistent with the times of the slow phase of the photosynthetic response, which may indicate the role of the studied phytohormone as an inducer of the changes in the photosynthesis activity. This work was supported by the Russian Foundation for Basic Research (project No. 19-34-90179).

P-07.4-21
Metabolic alterations in pea leaves and roots during arbuscular mycorrhiza development

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For the proper use of arbuscular mycorrhizal (AM) symbiosis in agriculture, a detailed understanding of the molecular basis of the plant developmental response to mycorrhization is needed. Information for pea (*Pisum sativum* L.) is scarce and its biochemical aspects need further study. Using GC-MS, we analyzed metabolic alterations in pea leaves (previously published in: Shtark OY et al. (2019) PeerJ 7, e7495) and roots (unpublished) associated with root colonization by *Rhizophagus irregularis*. Plants were analyzed at three time points, which corresponded to key developmental stages of the pea – I: first leaf with two pairs of leaflets and a complex tendril; II: the first open flower; and III: when the pod is filled with green seeds. Both the leaf and root metabolic profiles showed a strong correlation with plant age, and, to a lesser extent, was influenced by mycorrhization. Metabolic shifts influenced the levels of sugars, amino acids and other intermediates of nitrogen and phosphorus metabolism, and lipophilic compounds. Significant differences were revealed between the metabolic profiles of roots and leaves, as well as between those of individual organs at distinct time points. Particularly, in the roots of AM plants at stage II (characterized by the most intensive AM development) higher levels of fatty acids in comparison to roots of both the control plants and the AM plants at other stages were observed. At stages II and III, both the leaf and root metabolic profiles of AM plants shifted towards the profiles of the control plants at earlier developmental stages. Thus, mycorrhization led to the retardation of plant development, which was also associated with an extended vegetation period. This effect promises to be beneficial for agriculture, especially for the green pea cultivars which are harvested before seed maturation. This work is supported by the grants of RSF (20-16-00107) and RFBR (20-04-01136). *The authors marked with an asterisk equally contributed to the work.

P-07.4-22
Transcriptomic response of pea (*Pisum sativum* L.) plants to inoculation with nodule bacteria, arbuscular-mycorrhizal fungi and PGPB

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The garden pea (*Pisum sativum* L.) can form mutualistic symbioses with different beneficial microorganisms, namely nodule bacteria (rhizobia), arbuscular-mycorrhizal (AM) fungi, and plant growth-promoting bacteria (PGPB). In order to study the differential response of pea to inoculation with either single rhizobia or combinations of rhizobia with other microsymbionts, plants of cv. Frisson were grown in quartz sand and inoculated with rhizobia (*Rhizobium leguminosarum* bv. viciae RCAM1026, AM fungi (*Rhizophagus irregularis* BEG144) and PGPB (a component of a microbiological preparation 'Myzorin') so that 4 variants of treatment were studied. After 2 and 4 weeks of growth, the total gene expression was assessed in plant roots and in shoots using a modified RNAseq technique named 3'-MACE (the application of the method to the pea is described in Zhernakov AI et al. (2019) PeerJ 7, e6662), and transcriptomic markers of successful inoculation were determined. For AM inoculation, the most stable up-regulated markers were PS_0021302 (encoding Glutathione S-transferase) and well-known mycorrhiza-specific gene RAM2. For PGPB inoculation, the gene encoding putative LRR protein (PS_0029785) was found as a stable down-regulated marker. Also, we studied pea mutants in Sym28 and Sym29 genes with impaired autoregulation of nodulation and mycorrhization in the same inoculation conditions. The mutants were more responsive to inoculation than the wild-type plants of cv. Frisson, and the strongest reaction to mycorrhization was described for P64 (sym28), for which the activation of plastid metabolism in roots and modulation of plant immune reactions in shoots was detected. Together these data form the basis for comprehensive description of the molecular-genetic processes occurring in pea plants under the use of complex inoculants. The work was supported by the Russian Science Foundation grant # 17-76-30016.

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P-07.4-23
Analysis of defense reaction in two pea (*Pisum sativum* L.) lines, contrasting in tolerance to cadmium

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Mechanisms of plants tolerance to environmental factors are actively studied, but still represent an intriguing problem. Heavy metals, including cadmium, affect plant development and present a serious challenge, as they can be transferred through the food chains. Particularly significant components of plant defense system can be identified using the analysis of the results of the mutation process. In this work we present analysis of defense reaction caused by cadmium chloride in two pea (*Pisum sativum* L.) lines, contrasting in reaction to this heavy metal: initial line SGE and mutant SGECd^t, characterized by an increased tolerance to cadmium. The region of localization of the cdt locus was identified using genetic analysis, but the search for a specific gene of interest is still in process. Analysis of genes, related to processes of reaction oxygen species deactivation revealed activation of expression of genes encoding catalase and chitinase in the line SGE and chitinase and gluthatione reductase in the line SGECd^t (previously published in: Kulaeva et al. (2018) Ecological genetics 16(4), 75–84). As a part of this work 55 unique sequences encoding defensins (related to antimicrobial peptides) were identified in the transcriptome assembly of pea (previously published in: Zorin et al. (2019) Ecological genetics 17(3), 39–46). Analysis of expression of some of these genes revealed that cadmium increased their expression, especially in line SGECd^t. It is important to note, that the expression of several defensins encoding genes were enhanced in the mutant compared to the wild-type line even in control conditions. So, mutation in the cdt locus caused an alteration in response to cadmium at the molecular level. Enhanced expression of some defensin genes may indicate increased ability of cells of mutant SGECd^t to resist the destructive effects of cadmium. Analysis of defensins was supported by the Russian Science Foundation (http://www.rscf.ru/en) (Grant 17-76-30016).

P-07.4-24
Breeding glutinous varieties of rice and millet in Kazakhstan

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According to the type of starch, cereals like rice (*Oryza sativa* L.), millet (*Panicum miliaceum* L.), barley (*Hordeum vulgare* L.), sorghum (*Sorghum bicolor* L.) and corn (*Zea mays* L.) are divided into two groups: common and waxy. The common type of starch contains about 20% amylose and 80% amylopectin, while the waxy type of endosperm consists almost entirely of amylopectin. Products of waxy (wx) varieties of grain crops with high dietary properties and production of amylopectin for industry. The state register of breeding achievements approved for use on the territory of the Republic of Kazakhstan includes 35 varieties of rice, 21 varieties of millet. Among them no varieties with low amylose activity are noted. For the strategic development of agriculture in the Republic of Kazakhstan needed varieties that have a valuable trait for the breeding process of creating varieties with different content of glutinous rice and millet with useful economically valuable traits, adapted to local soil and climatic conditions. The results of biochemical screening of the collection of rice and millet by the content of amylose in grain facilitate identification of valuable samples.Glutinous rice varieties include samples from foreign breeding: Violetta, Viola, HeTiang (China), Black rice, Kuromochi, Lugovoi, while millet samples PI 436626 (Lung Shu 18), PI 436625 (Lung Shu 16) and Ma Zha Yan with low amylose content.Selected foreign glutinous samples of rice and millet were used as a genetic source to create domestic low-amylose forms of rice and millet by integrating the "waxy" gene into the local varieties.As a result of the breeding works, perspective hybrids of late generations for further selection were characterized by useful economically valuable traits. Presentor author is Mynbayeva Dana, PhD student of Al-Farabi Kazakh National University

P-07.4-25
The phytotoxic effect of herbicide diuron on Arabidopsis cell culture is accompanied by increasing of mitochondrial membrane potential and intensifying of ROS production

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In this work, the phytotoxic effects of herbicides diuron and fluorodifen have been studied using heterotrophic plant cell culture. It is known that the negative effect of these chemical agents on plants is mainly due to inhibition of photosynthesis and the associated oxidative stress however heterotrophic plant cells lack the main targets for their action. A 7-day culture of *Arabidopsis thaliana* L. cells were treated by herbicides of various concentrations. To assess the effect of herbicides on cell metabolism, fluorescent probes were used. At the first stage, the phytotoxic effect of various concentrations of diuron and fluorodifene was analyzed, treating the cells for 24 hours at 26°C. At the control condition, the number of living cells ranged from 70 to 80%. Diuron caused a significant decrease in cell viability only in the case of incubation of cells with concentration 200 μM. Fluorodifene at a concentration to 200 μM led to death 45% of the cells. It should be noted the phytotoxic effect of diuron was accompanied by an increase in the ROS level and a short-term increase in the electrochemical transmembrane potential on the inner mitochondrial membrane ($\Delta\mu H^+$) in the cells. As showed by us previously the same link between these parameters was observed under heat shock conditions in plant and yeast cell cultures. At the same time, the phytotoxic effect of fluorodifen was not accompanied by changes of ROS and $\Delta\mu H^+$ levels. The results obtained indicate that the phytotoxic effect of herbicides may be associated with the development of oxidative stress in cells and depends on level of $\Delta\mu H^+$. Acknowledgements The research was done using the collections of The Core Facilities Center "Bioresource Center" and the equipment of The Core Facilities Center "Bioanalitika" at The Siberian Institute of Plant Physiology and Biochemistry SB RAS (Irkutsk, Russia).

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P-07.4-26
StSNRK2, a novel regulator of potato immunity identified by plant immune signalling network analysis

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The interaction between the plant and its pathogen initiates a complex signalling network, resulting in massive changes of the gene activity and extensive reprogramming of the cell metabolism. Potato is the fourth most important crop, but, due to sensitivity to environmental factors, its average yields still do not reach the physiological potential. Therefore, to provide means for novel crop breeding strategies and agricultural practices, it is crucial to understand the mechanisms underlying the interaction between plants and their pathogens. Here we employed a systems biology approach to describe complex biological processes and dynamical mechanisms involved in potato response and to identify novel regulators of potato immunity. Network modelling of previously constructed Plant Immune Signalling model (PIS, Ramšak et al, 2018, doi: 10.1104/pp.18.00450) and experimental data of time series transcriptomics response in tolerant interaction of potato cv. Desiree to potato virus Y (PVY, Stare et al., 2015; doi:10.1186/s12864-015-1925-2) identified a potential novel regulator, a group III SNF1-related protein kinase 2 (StSNRK2), harbouring an abscisic acid (ABA)-binding domain. To better understand its role, we searched for potential interactors of StSNRK2 using yeast two-hybrid and co-immunoprecipitation assays and discovered that our target protein interacts with components of salicylic acid and ethylene (ET) signalling pathways. Moreover, its gene expression is activated by ABA and ET but repressed by jasmonic acid treatments. StSNRK2-silenced plants accumulate more virus than non-trangenic plants, confirming the role of StSNRK2 in defence response to viruses. Moreover, StSNRK2-silenced plants also show different growth and response to salt stress than non-transgenic plants. These results indicate the role of StSNRK2 as a node of convergence between biotic and abiotic stress signalling.

P-07.4-27
The antioxidant status of the shoot peas

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The antioxidant status of the shoot mass of peas (*Pisum sativum*) was studied in the research work. The size of the leaf surface is one of the main factors that determines the potential of the genotype in the formation of economic productivity, and the adaptive reserves of plants in stressful environmental conditions depend on it. For a comparative analysis, 7 varieties of peas with different leaf shapes were taken – leafy (Viola, Barin, 15.5.19) and mustachioed – "afila" (Darunok, Triumph, 89.4.19 and 365.19). It was established that the accumulation of dry matter in the shoot organs of pea plants under various environmental conditions is due to the varietal specificity and does not depend on the leaf shape. Analysis of the total content of water-soluble antioxidants showed that in leafy varieties, their values in the leaf and stipule increase under stress. Under optimal conditions, in varieties "afila" their content is in the same range as leafy, and under stress, a decrease was noted, which depended on the variety specificity. In the stem, a decrease was observed for all varieties by 1.25–1.73 times, regardless of the leaf shape. Triumph (afila) was an exception where the indicators increased by 9%. The ratio of the content of water-soluble antioxidants (leaf/stipule) under optimal conditions was in the range of 0.9–1.0 for all varieties. The correlation coefficient calculated by us revealed a high relationship (R = 0.91) between the content of polyphenols and the amount of antioxidants in the organs of vegetable pea plants. Analyzes carried out on vegetable peas showed significant differences in the content of antioxidants in plant organs. Variety specificity is noted, which will allow further selection in this direction and selection for resistance to stress.

P-07.4-28
The auxin amino acid conjugate, IAA-aspartate modulates thiol redox status in pea during salt stress

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Auxins are the earliest discovered group of phytohormones that play a key role in plant growth and development. Thanks to the processes of synthesis, degradation, transport and hormone conjugation, it is possible to control auxins concentration and therefore regulate developmental and environmental plants responses. The main, inactive auxin in plants is a conjugated form, where phytohormone is linked to alcohol/ sugar molecule (ester conjugate) or to amino acid/peptide/protein molecule (amide conjugate). In pea, the pivotal auxin, indole 3-acetic acid (IAA) can occurs as IAA-aspartate (IAA-Asp) conjugate by ATP-dependent two-step reaction. This one, unlike others auxin-amino acid conjugates which are responsible for increasing pool of free auxin, is involved in the catabolism of this phytohormone. However, recent studies suggest a different role for IAA-Asp conjugate like modifying the response to salt stress through changing molecular markers of oxidative stress (catalase, ascorbate peroxidase, guaiacol peroxidase activity and H_2O_2 level). The aim of the study was verification of the hypothesis that IAA-Asp modulates pea responses to salt stress via regulation of status redox of –SH thiol groups. To verify this hypothesis 7-days-old pea seedlings were treated with: a) water (control), b) 200 μM IAA-Asp, c) 150 mM NaCl, d) 200 μM IAA-Asp + 150 mM NaCl for 3 hours at 22–25°C. After that, enzymatic activity of glutathione peroxidase (GPX), glutathione reductase (GR) and GSH/GSSG ratio was determined. Depending on the environmental conditions, IAA-Asp indicates a different effect on redox status of thiol groups. IAA-Asp alone increased activity of GR and decreased of GPX in comparison to control. However, the ratio of GSH/GSSG was slightly affected in seedlings treated with the conjugate. Interestingly, IAA-Asp weakly potentiated the inhibitory effect of NaCl on GR activity and slightly diminished activatory effect of salt on GPX activity. IAA-Asp in combination with salt decreased also GSH/GSSG ratio. Moreover, S-glutathionylation, the post-translational modification of protein which is associated with maintenance of the redox status, has also been studied using SDS-PAGE/Western blot.

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P-07.4-29
The role of small RNAs in tolerance and resistance responses of potato to PVY infection

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In the past decades, plant small RNAs (sRNAs) have been shown to have an important role in gene expression reprogramming and fine-tuning of defence responses against various pathogens, including viruses. Our study aimed to identify key players in sRNA-regulatory networks controlling the establishment of tolerant and hypersensitive response (HR)-conferred resistance responses of potato to potato virus Y (PVY), one of the most damaging viruses infecting potato. Employing high-throughput sequencing technology, we compared expression of sRNAs among two tolerant (cv. Désirée, Pentland-Squire), a resistant (cv. Rywal) and two sensitive potato genotypes, impaired in accumulation of salicylic acid (NahG-Désirée, NahG-Rywal). This information was linked with expression profiles of their target transcripts and used for sRNA regulatory networks construction. Besides the already described regulation of transcripts encoding immune receptors, we have discovered an interesting novel sRNAs-gibberellin regulatory circuit being activated only in tolerant plants. Moreover, we discovered that sRNA regulation in tolerant interactions resembled the one required for the establishment of a mutualistic symbiosis. We for the first time employed spatially resolved non-targeted approach to get detailed insight into the expression and spatial distribution of sRNAs in response to PVY in HR-conferred resistance. More than 30 sRNAs were found showing different expression gradients around the site of viral entry. Few sRNAs exhibited opposite expression pattern in response to PVY infection in tolerant and resistant plants, suggesting that some common modules of the sRNA-regulatory network are activated but the behavior of its component is different. Certain sRNAs linked to tolerance and resistance response identified within the study are now further studied due to their high potential of being used for genetic manipulation to improve potato's immune response to PVY.

P-07.4-30
Pepper cultivar-specific response to potato spindle tuber viroid infection is mediated by hormone-signaling genes

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Potato spindle tuber viroid (PSTVd) is a long non-coding RNA with a size of 359 nt, which is infectious to various plant species. While some members of Solanaceae remain asymptomatic upon infection, most develop symptoms of varying severity. PSTVd pathogenicity is not yet fully understood but is seen as a complex process mediated by host species-specific and viroid strain-specific interactions in a dynamic environment. Recently, we observed that microRNA expression dynamics reshape the cultivar-specific response of pepper (*Capsicum annuum* L.) to PSTVd infection (Apostolova et al., 2021). To further characterized this response, Illumina HiSeq RNA analysis was performed for the same Bulgarian cultivars. Leaf samples were collected at 43 days after inoculation with PSTVd KF440-2. Among the differentially expressed genes, several showed contrasting expression profiles in response to PSTVd infection in the two cultivars. Some of these genes are involved in hormone-signaling pathways (auxin-repressed 12.5 kDa protein-like, abscisic acid receptor PYL8-like, ethylene responsive element binding protein C3) and were selected for validation by real-time qPCR. On this basis, we can assume that the hormone-mediated effectiveness of disease control is one of the mechanisms for the pepper cultivar-specific response and protection against the viroid pathogen. Acknowledgments: This work was supported by National Science Fund Grant DN06/6 Apostolova, E.; Hadjieva, N.; Ivanova, D. P.; Yahubyan, G.; Baev, V.; Gozmanova, M. MicroRNA expression dynamics reshape the cultivar-specific response of pepper (Capsicum annuum L.) to potato spindle tuber viroid (PSTVd) infection. Sci. Hortic. (Amsterdam). 2021, 278, 109845, doi: https://doi.org/10.1016/j.scienta.2020.109845. *The authors marked with an asterisk equally contributed to the work.

P-07.4-31
Anthocyanin-related transcription factor expression is correlated with that of pathogenesis-related genes

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Rye is a cereal crop with high resistance to unfavorable environmental factors which is a valuable trait due to the climate change and increased agricultural risks. Resistance to environmental factors and the synthesis of secondary metabolites is correlated. The cotton, mango and other species having higher content of anthocyanins and flavonoids generally also demonstrate higher resistance to pathogens and cold. Specific mechanisms of this phenomena are not yet fully understood. We studied four forms of rye, differing in the composition of anthocyanins and flavonoids in the grain, which is due to the action of different alleles of the same Vs (violet seed) gene: a strongly colored violet-grain form (VsVs), a weakly colored form (week alleles Vs) and 2 uncolored forms (vsvs). Transcriptome analysis of the upper layers of the grain allowed us to pinpoint a gene homologous to the maize R-S gene transcription factor, the expression of which strongly correlates with the intensity of the grain color. This suggests that the Vs gene is in fact the R-S gene. A correlation between the transcript expression level and different seed color was revealed for 44 more genes. Ten of them were involved in the synthesis of anthocyanins and flavonoids, eleven were involved in plant development, and the most numerous were fifteen pathogenesis-related genes (PR-genes). We analyzed contaminating species composition of the raw data and found a number of fungal and bacterial plant pathogens with more reads in anthocyanless forms than in strongly colored. Our data suggests that Vs gene responsible for violet color of rye grains is homologous to the maize R-S gene and there is a possibility of interaction between R-S transcription factor and the regulation of expression of some PR-genes, either directly or by having a common regulatory transcription factor. This study was supported by the state budget (project "Genetics and breeding of rye on the base of natural hereditary diversity"). *The authors marked with an asterisk equally contributed to the work.

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P-07.4-32
Strategy for development of jasmonic acid signaling pathway biosensor for following plant immune response in potato (*Solanum tuberosum* L.)

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In stressful conditions, there is an uprising immune response in affected plant cells. Its spatial and temporal dimensions determine resistant, tolerant or susceptive phenotype. The main three hormones that are involved into the spread of the immune response are salicylic acid, ethylene and jasmonic acid. We could follow them with biosensors such as transcriptional reporters, which are based on a promoter sequence that contains analyte-responding cis-elements and drives transcription of the reporter gene. However, due to the intertwined pathways of these three hormones, the specific reporters have not yet been developed. We are developing a transcriptional reporter of jasmonic acid signaling pathway in potato. Transcriptomic data led to the choice of a family of JA-responsive genes. Their promoter regions were amplified from genomic DNA of potato cultivars Rywal and Désirée according to 3 homologue gene promoters in Phureja cultivar genome. We identified 11 unique promoter sequences, of which only one is identical to one of the reference Phureja promoters, while all others, 2 from Rywal and 8 from Désirée, show a different degree of similarity to one or two reference promoters. We compared the frequencies of the known cis-elements involved in biotic stress response and jasmonic acid signaling pathway among all known cis-elements in the identified sequences, and thus chose the most promising candidates for the biosensor. Four candidates of different lengths, 300bp, 500bp and two of 1kbp, were fused with firefly luciferase coding sequence and transiently expressed in *Nicotiana benthamiana*. The luciferase assay results matched with results of the cis-elements analysis, confirming that a 500 bp long candidate has the highest strength and inducibility to jasmonic acid in a repeatable manner. The work now continues with testing specificity of the promoter to jasmonic acid triggered response and preparation of biosensoric stable transformants of potato.

P-07.4-33
Transcriptomic analysis of symbiotic pea (*Pisum sativum* L.) nodules using laser microdissection

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The development of the symbiotic nodule of legumes is accompanied by both differentiation of rhizobia into bacteroids and differentiation of the cells infected with rhizobia. The aim of this work was to study changes in transcriptomic profiles associated with the differentiation of the infected cells. Using laser capture microdissection, cells from the early infection zone, the late infection zone and the nitrogen fixation zone were isolated from 11 days old pea wild-type SGE nodule samples. RNA from these samples were sequenced using Illumina HiSeq 2500 platform. Obtained reads were filtered and mapped to the pea reference genome. Differential gene expression analysis (P-value < 0.01; LFC > -1-) and functional analysis were carried out for three comparisons: (1) cells of the late infection zone / cells of the early infection zone; (2) cells of the nitrogen fixation zone / cells of the early infection zone; (3) cells of the nitrogen fixation zone / cells of the late infection zone. For these comparisons, significant differences were found in the expression of genes associated with key biological processes in the plant cell, such as control of cell cycle, response to phytohormones, and polysaccharide metabolism. The authors are very grateful to Alexey Afonin for providing access to the pea reference genome and assistance in conducting the study. The research was made with support of the Ministry of Science and Higher Education of the Russian Federation in accordance with agreement № 075-15-2020-920 date November 16, 2020 on providing a grant in the form of subsidies from the Federal budget of Russian Federation. The grant was provided for state support for the creation and development of a World-class Scientific Center "Agrotechnologies for the Future".

P-07.4-34
General patterns and species-specific differences in the organization of the tubulin cytoskeleton in indeterminate nodules of three legumes

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Tubulin cytoskeleton take a significant part in establishing legume-rhizobial symbiosis at all stages of its development. Earlier, tubulin cytoskeleton organization was studied in detail in indeterminate nodules of two legume species i.e. *Pisum sativum* and *Medicago truncatula* and general patterns, as well as species-specific ones, were revealed. To get a more precise understanding of the formation of general and species-specific microtubule patterns in indeterminate nodules, the organization of the tubulin cytoskeleton was studied in three legume species (*Vicia sativa, Galega orientalis*, and *Cicer arietinum*). In this work, we've shown that these species differ in the shape and size of bacteroids. *V. sativa* and *G. orientalis* had E morphotype bacteroids, and bacteroids of *C. arietinum* belong to S morphotype. The unity of organization of cortical and endoplasmic microtubules in the meristematic cells, infected cells of the infection zone, and uninfected cells in nodules of analyzed species was revealed by immunolocalization of microtubules. The difference in the organization of endoplasmic microtubules in nitrogen-fixing cells between studied species was revealed and confirmed by quantitative analysis. It seems that the disclosed difference is linked to bacteroid morphology (both with the shape and size of bacteroids).

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P-07.4-35
Suppression of mutation in the pea (*Pisum sativum* L.) symbiotic gene Sym40 by *Rhizobium leguminosarum* bv. viciae strain NaPi

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Legumes are able to fix atmospheric nitrogen in symbiotic associations with rhizobia that involved in the formation of a new plant organ – symbiotic nodule. Establishing of successful symbiosis requires both partners to be compatible with each other throughout the development process. However, violations due to bacterial or plant mutations lead to abnormal nodule formation or to inability of nitrogen fixation. It was shown that some strains of rhizobia are able to suppress mutations in plant symbiotic genes and form effective nodules. During inoculation experiments of pea mutant line SGEFix$^-$-1 (sym40-1) with *R. leguminosarum* bv. viciae (RLV) strains, an isolate (NaPi) was found capable to form large pink nodules on the roots of this genotype in contrast to small white nodules characteristic to sym40-1 phenotype. Histological and ethylene reduction analysis of symbiotic nodules formed on NaPi-inoculated sym40-1 plants revealed partial suppression of sym40-1 nodule phenotype. The genomes of NaPi and the parent strain RLV 3841 were sequenced using NGS (Illumina HiSeq and MinION) and compared with the reference RLV 3841 genome from GenBank (ASM926v1). As a result, 5 single-nucleotide polymorphisms (SNPs) and 3 insertions/deletions were detected in various genes including Na^+/H^+ antiporter, phosphatase, microcin B-17 transporter, regulatory noncoding RNA and LacI transcriptional regulator family member. Also it was shown that transposon region from RLV 3841 genome was absent in NaPi genome. The research was made with the support of the Ministry of Science and Higher Education of the Russian Federation in accordance with the agreement № 075-15-2020-920 dated November 16, 2020 on providing a grant in the form of subsidies from the Federal budget of Russian Federation. The grant was provided for state support for the creation and development of a World-class Scientific Center "Agrotechnologies for the Future".

Life on the edge – extremophilic/extremotolerant organisms

P-07.5-01
Optimisation of the production of bacterioruberin in wild-type *Haloferax mediterranei* R-4

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Haloarchaea are extremophilic microorganisms that inhabit hypersaline environments since they require high salt concentrations for their survival. Therefore, halophilic archaea are frequently exposed to oxidative stress (high sun radiation, high ionic strength, etc.) and have developed different strategies to cope with these conditions, which have derived in particular features, such as the synthesis of antioxidant pigments. Haloarchaea synthesise mainly the rare carotenoid bacterioruberin (BR) and its derivatives. BR is characterised by a high number of carbon units (C50) and a high number of double bonds, which make this carotenoid a superior radical scavenger than C40 carotenoids. The remarkable antioxidant activity of bacterioruberin could be very interesting for the biomedical and biotechnological industries due to the potential beneficial effects of carotenoids on human health. Several factors are involved in the regulation of carotenoid biosynthesis in haloarchaea, as for example salinity, temperature and C/N ratio. In this study, *Hfx. mediteranei* was grown under aerobic conditions in a medium containing inorganic salts (10–25% w/v) in combination with different carbon sources (glucose (0.5–2.5% w/v) or starch (0.5–2.5% w/v)). The higher levels of BR were observed with 12.5% of inorganic salts and high concentrations of carbon sources. In parallel, the response of *Hfx. mediterranei* to an oxidative stress such as the sudden exposure to as H_2O_2 was also analysed. *Hfx. mediterranei* successfully tolerated until 20 mM H_2O_2, leading to a higher production of the pigment when compared to the control. Therefore, BR was very efficient scavenging the strong oxidizer H_2O_2. In conclusion, BR synthesis could easily be enhanced modifying environmental conditions which could be useful to produce it at a higher scale. Currently, the antioxidant effect of this compound is being evaluated preliminarily in human cell lines.

P-07.5-02
The peculiarities of three-dimensional structure of recombinant purine nucleoside phosphorylase from the thermophilic bacterium *Thermus thermophilus* HB27

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Purine nucleoside phosphorylases (PNPs) catalyze the reversible phosphorolysis of purine nucleosides and are key enzymes involved in purine metabolism. Apart from phosphorolysis they catalyze transfer of a carbohydrate moiety from one purine base to another and used widely in biotechnology for chemical–enzymatic synthesis of analogous of natural nucleosides with antiviral and anticancer activity. According to amino acid sequence and oligomeric structure PNPs are divided into trimeric and hexameric enzymes which differ in substrate specificity. Both types of PNPs are found in the thermophilic microorganisms. Recombinant purine nucleoside phosphorylase from thermophilic bacterium *Thermus thermophilus* HB27 (ThthHB27) coded by gene TT_C0194 was isolated and purified. The enzyme has highest amino acid sequence identity with trimeric human PNP but contains Asp and Ala as key residues defining its substrate specificity instead Asn and Glu and uses adenosine as a substrate. Its molecular weight determined by analytical gel filtration is equal to 185 kDa and corresponds to hexameric quaternary structure, but its sequence identity with hexameric PNP from Thth HB8 is 17% only. The 3D structure of ThthPNP HB27 was solved at 2.5 Å resolution by molecular replacement method using crystals grown in microgravity from protein solution containing phosphate buffer. It was found that six enzyme subunits in the asymmetric part of the unit cell form two trimers bound by pseudo

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two fold axis into hexamer with 32 point symmetry. Revealed type of PNP hexamer is different from usual hexameric molecules of PNPs which have disc-like shape. The inter-subunit contacts in ThthPNP trimers and hexamer are determined. The peculiarities of polypeptide folding in the protein subunit, the nearest surrounding of phosphate ion located in the active site and positions of amino acid residues responsible for purine base recognition are described.

P-07.5-03
Investigation of the role of the electron bifurcating hydrogenase Hnd in the metabolism of an anaerobic, sulfate-reducing bacterium using NMR-based metabolomics techniques

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The genome of the anaerobic, sulfate-reducing bacterium *Desulfovibrio fructosovorans* encodes for 6 different hydrogenases. The most recently characterized is Hnd, a cytoplasmic heterotetrameric enzyme (HndABCD) that carries out flavin-based electron bifurcation, a newly described mechanism of free energy conservation in anaerobic microorganisms. In the presence of H_2, Hnd couples the exergonic reduction of NAD^+ to the endergonic reduction of a ferredoxin, previously published in Kpebe A et al. (2018) BBA Bioenergetics 1859, 1302–1312. We have failed to establish the specific metabolic role of Hnd in *D. fructosovorans*, due to a probable compensation mechanism between the other hydrogenases. Deletion mutants of hndC and hndD are non-lethal, and the overall yield during respiratory growth is unaffected. We found that the hnd deletion mutant, when grown on pyruvate with limited sulfate, produces more H_2 than the WT strain, suggesting that Hnd functions as an H_2-consuming hydrogenase in these conditions. We determined that a metabolomics approach would provide a comprehensive view into the metabolic modulations caused by Hnd. The hnd deletion mutant and WT strain were grown in pyruvate/sulfate medium with diminishing concentrations of sulfate, corresponding to respiration, mixed (limited sulfate) and fermentation conditions. Metabolic profiling was carried out using ^{1}H NMR spectroscopy, and a discrimination between the metabolic profiles of the hnd mutant and the WT strains was obtained across the different growth conditions. Surprisingly, we found that during fermentative growth, the hnd mutant accumulates acetone instead of ethanol in the culture medium, indicating a the presence of a metabolic shunt arising from the deletion. These data demonstrate Hnd's global importance to the energetic metabolism of the cell, but the implicated metabolic pathways still need to be fully elucidated.

P-07.5-04
Co-expression networks and gene expression analysis: different energy supply strategies of Baikal and Holarctic amphipods under temperature stress

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Temperature is one of the most important environmental factors as it exerts a direct effect on all biological processes, from molecular to behavioral. The aim of this study was to assess interspecific differences in energy metabolism in two Baikal endemic amphipods and one species widely distributed in the Holarctic exposed to gradually increasing temperature (0.8°C per day). Thus, the expression of genes involved in key metabolic processes genes was analyzed. All investigated amphipod species are characterized by different energy-supply strategies under temperature increase and we found interspecific differences at the level of co-expression of the studied genes. Gradual temperature increase strongly affected energy metabolism and stress response in the thermosensitive Baikal endemic amphipod *E. verrucosus*; moreover correlation network analysis showed clear disorganization of gene correlation networks. In the more thermotolerant Baikal endemic amphipod *E. cyaneus* there was a switch to anaerobic energy production, insertion of glycolytic transcripts and extinction of ATP turnover-related transcripts in/out correlation network when exposed to increasing temperature. Temperature increase led to an increase in aerobic capacity in *G. lacustris* (ubiquitous Holarctic amphipod) mirrored by rising activity of aerobic capacity indicators. Baikal endemic amphipods showed more pronounced gene expression changes under gradual temperature increase than the Holarctic ubiquitously spread amphipod *G. lacustris*. The study was carried out with the financial support of the Russian Science Foundation and Helmholtz Association grant № 18-44-06201.

P-07.5-05
Effects of vanadate on antioxidant systems in mycelium of fungus *Phycomyces blakesleeanus*

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Fungi represent the main route of vanadium's entrance in ecosystem and are known to tolerate high concentrations of this transition metal. Despite it, its effects on antioxidant systems of fungi are not well-known. The aim of this research was to examine the effects of vanadate on antioxidant enzymes and non-enzymatic antioxidants of filamentous fungus *P. blakesleeanus*. Mycelium in early exponential (20h) and stationary (56h) phase of growth was treated with relatively high concentrations of V^{+5} (1, 5 and 10 mM) for 1h or 5h. Activities of peroxidase (POD), glutathione reductase (GR), glutathione transferase (GST), glutathione peroxidase (GPx), concentrations of total phenols and total

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glutathione were examined. In the 20h old mycelia, V^{5+} in all concentrations, after 1h treatments, induced decrease in total phenols with the largest decrease (25 ± 4%) after 10mM V^{5+} treatment, while in 56h old mycelia decrease in total phenols was noticed only after 1 mM V^{+5} treatment (11 ± 3%). Decrease of total glutathione, in 56h old mycelia, was noticed for all concentrations after 1h treatments, and the largest 38 ± 7% was induced by 5 mM V^{5+}. The same effect was noticed in 20h old mycelia treated for 5h, with the largest decrease of 29 ± 7% after 5 mM V^{5+} treatment. In 56h old mycelia, after 1h treatments with 10 mM V^{+5}, activities of GPx and POD increased, while after 5h treatments, concentration dependent increase in activities of GST, GPx and GR was noticed, with the largest increase of 112 ± 30% for GPx, 74 ± 39% for GST and 68 ± 26% for GR. Increase in the activities of GPx and GST was noticed in 20h old mycelia treated for 5h, with 5 mM and 10 mM V^{+5} and also increase of POD in 56h old mycelia, was noticed in same treatments, but the only statistically significant increase was after treatment with 10 mMV^{+5} in 56h old mycelia (43 ± 10%). The results shown indicate that after 1h treatments, V^{5+} influenced primarily on non-enzymatic antioxidants and after 5h treatments on enzymes.

P-07.5-06
Specialized DNA polymerases of extremophile bacteria of the *Deinoccoccus phylum*

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Extremophile bacteria of the Deinococcus phylum show remarkable resistance to UV and gamma radiation. The molecular pathways ensuring genome stability in these bacteria under stress conditions are only partially understood. Two Deinococcus species, the model bacterium *D. radiodurans* and recently characterized *D. gobiensis*, encode specialized DNA polymerases of the X-family (both species) and Y-family (D. gobiensis). These polymerases can potentially participate in the replication of damaged DNA containing various lesions, caused by different types of radiation. In this study, we investigated the expression levels of these polymerases in both species under DNA damaging conditions in vivo. Utilizing recombinant proteins, we showed *in vitro* that the X-family polymerases from both species surprisingly do not possess the DNA polymerase activity, due to substitutions of key residues in the polymerase active site, but exhibit 3'-5' exonuclease and AP(apurinic site)-endonuclease activities in the presence of Mn^{2+} ions. We localized the active site responsible for these activities in the so-called PHP domain of polymerases. Both of these activities may potentially play a role in DNA repair pathways. Recombinant Y-family polymerase from *D. gobiensis* was also tested for the ability to pass various DNA lesions such as 8-oxoguanine, O-6-methylguanine, AP-sites, thymine dimers, and 1,N6-ethenoadenine. It was shown that this polymerase has an increased ability to replicate damaged DNA in comparison with replicative polymerases. Together, our data provide new insights into the functions of specialized DNA polymerases in DNA repair in DNA-damage resistant bacteria. This project was in part supported by the Russian Foundation for Basic Research and Russian Science Foundation (17-14-01393). *The authors marked with an asterisk equally contributed to the work.

P-07.5-07
Helichrysum italicum: crown jewel of Mediterranean phytomedicine

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Medicinal extremophiles represent an outstanding reservoir of bioactive compounds with unique biological properties. *Helichrysum italicum* (Roth) G. Don is an iconic Mediterranean plant, well adapted to this dry and saline environment, i.e. a xero- and halotolerant plant, respectively. It is better known under the name Immortal or Everlasting, due to its never-fading flowerheads that preserve their yellow colour when dry. The essential oil and organic solvent extracts of *H. italicum* have been studied extensively in the last two decades, but research on traditional extracts is rather scarce. For this purpose, we investigated aqueous whole plant extracts of *H. italicum* grown in the Slovenian coastal region for possible health-promoting properties. Dried plant material was ground and used for hydroalcoholic maceration or water infusion. The extracts were evaluated *in vitro* for their free radical scavenging activity and protective effect against induced oxidative stress. Furthermore, they were tested for cytotoxicity on mammalian enteric cell lines and subjected to gene expression analysis using Human Clariom^{TM} S GeneChip. The whole plant extracts showed high DPPH radical scavenging potential, similar to ascorbic acid. They also managed to protect cells against t-BOOH-induced oxidative stress substantially. At the same dilution, the infusion was toxic to the colorectal adenocarcinoma cell line Caco-2 and non-cytotoxic to primary enteric fibroblasts. Exploratory grouping analysis showed clear clustering into three groups depending on the treatment applied (cells treated with *H. italicum* for 6 h or 24 h and untreated cells). A detailed transcriptome analysis is currently underway. Oxidative stress is an underlying cause of several degenerative diseases that can be prevented by the use of antioxidants. In this context, *H. italicum* can be viewed as the Mediterranean panacea, and its extracts have the potential to be developed as ingredients for dietary supplements.

P-07.5-08
The recoded α-L-fucosidase from *Saccharolobus solfataricus* and its transcript regulation *in vivo*

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The decoding of genetic information is a dynamic mechanism in which, in some genes and in specific physiological conditions, the standard rules can be altered by programmed deviations of the ribosomes, a phenomenon called recoding. Translational recoding has been found in all three domains of life and has crucial roles in the regulation of gene expression (Previously published in: Atkins J.F. et al. (2018) NAR 44(15): 7007–78). Increasing evidences suggests that the flexibility of genetic decoding might have relevant implication in physiology, being a trait selected during evolution that may increase microbial fitness under certain conditions (Previously published in: Ling J. et al. (2015) Nat. Rev.

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Microbiol. 13, 707–721). This could be particularly relevant in extreme environments, which are often spots localized in places dominated by mild conditions. In these "extreme" sites, microbial communities might encounter sudden and reversible changes of the optimal growth conditions. Therefore, translational recoding could be used to maintain the expression of certain genes latent, and up- or down-regulate them under specific conditions. In Archaea, recoding was demonstrated for termination codon read-through events that regulate the incorporation of amino acids selenocysteine and pyrrolysine, and programmed frameshifting -1 for the expression of a fully functional α-L-fucosidase in the crenarchaeon *Saccharolobus solfataricus* (Previously published in: Cobucci-Ponzano B. et al. (2006) NAR. 34, 4258–4268). Here we report, for the first time, on the analysis, *in vivo*, of the transcription of this recoded archaeal α-L-fucosidase and of its full-length mutant in different growth conditions. Our results indicated that the increased level of fucA mRNA cannot be explained by transcript up-regulation alone, suggesting a possible different mechanism related to translation efficiency.

P-07.5-09
Discovery of hyperstable carbohydrate-active enzymes through metagenomics of extreme environments

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Enzymes from hyperthermophilic microorganisms populating volcanic sites represent interesting cases of protein adaptation and biotransformations under conditions where conventional enzymes quickly denature. Difficulties related to extremophiles' isolation and cultivation severely limit the discovery of new biocatalysts of industrial relevance. To circumvent this, we carried out a detailed analysis of the microbial biodiversity of the solfatara Pisciarelli, Agnano (Naples, Italy), to discover hyperthermophilic carbohydrate-active enzymes (CAZymes) and to characterize the entire set of such enzymes in this environment (CAZome). Here, we report the results of the metagenomic analysis of two mud/water pools that greatly differ in both temperature and pH (T = 85°C and pH 5.5; T = 92°C and pH 1.5, for Pool1 and Pool2, respectively). DNA deep sequencing and subsequent in silico analysis led to the annotation of 14,934 and 17,652 complete ORFs in Pool1 and Pool2, respectively. They exclusively belonged to archaeal cells and viruses with great genera variance within the phylum Crenarchaeota, which reflected the difference in temperature and pH of the two Pools. Surprisingly, 30% and 62% of all of the reads obtained from Pool1 and Pool2, respectively, had no match in nucleotide databanks. Genes associated with carbohydrate metabolism were 15% and 16% of the total in the two Pools, with 278 and 308 putative CAZymes in Pool1 and Pool2, corresponding to ~ 2.0% of all ORFs. Biochemical characterization of two CAZymes of a previously unknown archaeon revealed a novel subfamily GH5_19 β-mannanase/β-1,3-glucanase whose hemicellulose specificity correlates with the vegetation surrounding the sampling site, and a novel NAD^+-dependent GH109 [2] with a previously unreported β-N-acetylglucosaminide/β-glucoside specificity [3] [3] Strazzulli A. et al., 2020. FEBS J, 287: 1116–1137. https://doi.org/10.1111/febs.15080

P-07.5-10
The fluorescence-activating and absorption-shifting tag (FAST) protein is a powerful and versatile tool to study methanogenic Archaea

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Methanogenic Archaea are strictly anaerobic microorganisms that are capable of producing methane from various substrates, e.g. methylated C1-compounds, acetate and $H_2 + CO_2$. Methanogens have an important role in the global C cycle, completing the conversion of organic carbon into methane gas. Several Methanogens became model organisms to study unique aspects of archaeal genetics and biochemistry. In contrast to most bacterial and eukaryotic model organisms, the oxygen-sensitivity of Methanogens delays scientific progress. For example, most real-time fluorescence reporters are oxygen-dependent and cannot be used in Methanogens. To overcome this limitation, we employ a recently developed fluorescence-activating and absorption-shifting tag (FAST) protein. In association with a fluorogenic chromophore, FAST is highly fluorescent in methanogenic archaea under anaerobic conditions. Using microscopy, flow cytometry and fluorescence microplate reader, we demonstrate the usability of this reporter system to visualize *in vivo* protein localization, protein interaction and real-time gene expression in anaerobic Archaea. Thus, our work opens the door to *in vivo* fluorescent labeling of methanogenic Archaea.

P-07.5-11
The first collection of truffle mushrooms in Siberia as available source for new biomedical research

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Among the variety of bioactive compounds of microbial origin, molecules promoting neuroprotection and neurogenesis seem to be one of the most significant and promising biomedicine compounds. These metabolites' action maintains a high functional activity of the nervous system and relevant for each person. The interest in current studies is described by the increase in neurodegenerative diseases around the world. Oxidative stress plays an essential role in the development of many neurodegenerative diseases. The screening for new natural compounds, eliminating the negative effects of reactive oxygen species, and promoting nerve tissue regeneration and formation is an important scientific task.

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The use of non-model organisms as truffle-fungi as a producing organism led to the increased probability of isolating new natural products and discovering a biotechnological and genetic potential. Here we report about forming the biological bank that included unique and non-typical organisms and their biotechnological and genetic resources. There are 15th pure black truffle-fungi cultures were isolated while performing studies using modern assays of microbiology and chemical attraction principles. Samples of truffles collected in the South part of Russia were prepared for proteomic and lipidomic analysis. Also, during the current studies, the variety and biodiversity of microorganisms associated with truffles were estimated using metagenomic sequencing of ITS region. Based on the preliminary experiments and low-resolution mass-spectrometry analysis, we found that cultural liquid includes natural products essential for increasing cognitive functions and can be used as an effective biological fertilizer in agriculture. Even though these fungi' geographical origin is non-Siberian, the role of these organisms as factories for the biosynthesis of new neuroactive molecules should be noted and described. This study was supported by the Russian Science Foundation (Project ID 20-76-00001).

P-07.5-12
Alicyclobacillus mali FL18: a new polysaccharide waste-degrading extremophilic bacterium isolated from a hydrothermal hot-spring in Southern Italy

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Pisciarelli is a hot spring located within the hydrothermally active zone of the Campi Flegrei volcano near Naples, in Italy. This extreme environment is an excellent place to find microorganisms with many interesting features such as resistance to heavy metals, pH, or temperature[1]. Among these, we have recently isolated and characterized *Alicyclobacillus mali* FL18, a new arsenic-tolerant strain[2]. In this work, we explored the genome of *A. mali* FL18 to find hypothetical genes that could be involved in polysaccharide biomass degradation. An integrated physiological, biochemical, and proteomic approach was employed to identify the expressed secreted enzymes that can be used for the degradation of agri-food waste biomasses. *A. mali* FL18 was grown in the presence of synthetic cellulolytic and hemicellulolytic polysaccharides under diverse experimental conditions and the secreted enzymatic activities were analysed by assays and zymography. Moreover, to assess the possibility to employ *A. mali* FL18 as a whole-cell degrading bacterium, it was grown in a minimal medium supplemented with different agrifood wastes: under these conditions, the microorganism was able to degrade about 10% of the used biomasses. Therefore, *A. mali* FL18 and its enzymatic collection look as promising candidates for the degradation of selected agri-food biomasses. Reference: 1. Puopolo, R. et al., Int. J. Environ. Res. Public Health 17, (2020). 2. Aulitto, A et al., Frontiers in Microbiology. doi: 10.3389/fmicb.2021.643589

P-07.5-13
A comprehensive study of hydrolytic enzymes from *Bacillus coagulans* MA-13 and characterization of a GH42 β-galactosidase with transglycosylation potential

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A thermophilic lactic acid bacterium *Bacillus coagulans* MA-13 was isolated from bean processing waste and successfully employed for the sustainable production of lactic acid from lignocellulosic biomass [1,2]. In this work, *B. coagulans* MA-13 was explored as a source of enzymes with the potential to produce galacto-oligosaccharides (GOS) as well as to improve the digestibility and nutritional value of foods. To this aim, *B. coagulans* MA-13 was cultivated under diverse growth conditions and afterwards, the intra- and extra-cellular glycosyl hydrolase (GH) repertoire was analyzed using a combination of mass spectrometry analysis with conventional biochemical approaches. Interestingly, in the cytosol the highest enzymatic activity detected was on ortho-Nitrophenyl-β-D-galactopyranoside (ONP-β-gal), whereas a para-Nitrophenyl-α-D-galactopyranoside hydrolysis was detected intra- and extra-cellularly. These activities were unambiguously identified as a β- and α-galactosidases and herein, we focused on the study of the β-galactosidase (named BcGalB), since there is no evidence about the ability of β-galactosidases from other *B. coagulans* strains to produce GOS upon transglycosylation reactions. A full biochemical characterization of BcGalB revealed that the enzyme has an optimal hydrolytic activity at 60°C and pH 5.0 of 4300 U/mg and 1283 U/mg on ONP-β-gal and lactose, respectively. Furthermore, BcGalB was able to produce GOS in homo- and hetero-condensation reactions from artificial and natural substrates. In this work we have proven the ability of *B. coagulans* MA-13 to eco-friendly produce prebiotics from dairy food waste, thanks to the high efficiency of BcGalB to synthesize galacto-oligosaccharides from lactose[3]. [1]Aulitto, M. et al. (2017) Biotechnol. Biofuels, 10 (1), 1–15 https://doi.org/10.1186/s13068-017-0896-8. [2]Aulitto, M et. al. (2019) Biotechnol. Biofuels 12 (1), https://doi.org/10.1186/s13068-019-1382-2. [3]Aulitto, M et. al. (2021) Microb. Cell Fact., Accepted.

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Cancer immunology and immunotherapy

P-08.1-01
Cysteine cathepsins in tumor-immune cell interactions: immunological synapse and tumor cell–myeloid-derived suppressor cell cross-talk

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Infiltration of immunosuppressive myeloid cells, as well as cytotoxic T cell and natural killer cell unresponsiveness, are major hurdles that fuel recent research efforts in the field of cancer immunotherapy. One of the characteristic of tumor-associated myeloid cells is high protein load and activity of lysosomal cysteine peptidases. They execute numerous physiologically important biological processes. However, their increased activity exacerbates certain chronic disease states, including cancer. Cysteine cathepsins are recognized as cancer biomarkers and established targets for new anti-cancer treatments. Redundant action and limited knowledge of their effect on other cells, apart from tumor cells, are preventing use of cathepsin inhibitors in clinics. Our aim was to elucidate the mechanisms underlying interactions between cancer cells and immune cells, in particular between cancer cells and cytotoxic T cells or myeloid-derived suppressor cells (MDSC). In order to study immunosuppressive interactions, conveyed by MDSC, an *in vitro* model was generated by co-culturing MDA-MB-231 breast cancer cells with monocytes from peripheral blood of healthy donors. For immunological synapse studies, cytotoxic T cell line TALL-104 was employed, together with its target cell line K-562. Molecular dynamics of established immunological synapse was monitored by imaging flow cytometry, which benefits from capturing large quantity of events. Contribution of cysteine cathepsins, in particular cathepsin X and cathepsin L, in both models was evaluated by using their selective inhibitors. We observed marked differences in tumor cell invasive properties when cathepsin X and cathepsin L inhibitors were used alone or in combination. The effect on T cell cytotoxicity was, similarly, dependent on the type of inhibitor used. Our results indicate that evaluation of cysteine cathepsin inhibitors as potential therapeutics should also include analysis of their functional effects on immune cells.

P-08.1-02
Herpesvirus-based vectors for oncolytic biotherapy

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Herpesviruses are attractive for cancer therapy because of several characteristics including their cytolytic nature and well-characterized genome; some herpesviruses, namely C-strains of herpesvirus saimiri, are also able to immortalize T-lymphocytes. The challenge in designing a new recombinant HVS vector that maintains its specificity without being harmful to normal cells represent a huge obstacle to come over as it requires performing a homologous recombination in virus-permissive cell line that are tricky to transfect. The aim of this work is to design a new recombinant herpes virus that can replicate *in vitro* and comprise an effective way to deliver virus particles to tumor cells. Achieving this goal needs to fulfill several tasks: design the new recombinant virus, demonstrate an effective way of replication *in vitro* and developing a new method of delivery *in vivo*. Our results showed a successful recombination of herpes virus genome with transgenic cassette expressing Orange Fluorescence Protein (OFP) in infected OMK cells with forming syncytia with another type of cells. We were able to clone the cells and see the cytopathic effect of the monoclonal virus stocks prepared from single OMK cells. Infecting T-lymphocytes with the recombinant HVS-OFP resulted in immortalization of these cells with expressing functional T cells markers in comparison with control uninfected cells. These results lead to the successful preparation of recombinant HVS *in vitro* in order to experiment it *in vivo* as well. The cytopathic effect of this virus was confined to simian OMK cells which means it conserves the species specificity of the original virus. The immortalization of T-lymphocytes after infecting with HVS-OFP means that it maintains the ability to transform human lymphoid cells and utilized for cellular immunotherapy. Further application by targeting HVS-immortalized T-cells to cancer antigens and by using them as carriers to precisely deliver oncolytic viruses to tumor site

P-08.1-03
Infiltration, lysis and differentiation of glioblastomas by super-charged natural killer cells in 3D spheroid model

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Glioblastomas (GB) remain the most aggressive malignancy of the brain. GB are resistant to conventional therapeutics due to the presence of therapy-refractory glioblastoma stem cells (GSC). Natural killer cell (NK)-based therapy is a promising immunotherapeutic strategy in treatment of many aggressive cancers, including GB. Primary NK and highly-activated cytotoxic super-charged NK have been shown to preferentially recognize and kill resistant cancer stem cells. In this study, we aimed to investigate therapeutic role of super-charged NK in GB by studying interactions between primary or super-charged NK and patient-derived GSC in 2D and 3D (spheroid) models. The selected GSC tumors exhibited different cell surface marker profile, previously established to correlate with the stage of differentiation of tumors. Differential susceptibility to NK-mediated cytotoxicity, determined by chromium release assay, suggests that some of GB tumors may be enriched by GSC and represents the early progenitor states. Accordingly, primary NK were able to lyse stem-like/undifferentiated GSC NCH421k cells significantly more than a more differentiated GS25 cells. When we tested cytotoxicity of super-charged NK cells, they lysed GSC more efficiently than primary NK. High percentages of dead GSC were

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observed after addition of super-charged NK to GSC spheroids. Moreover, NK secreted high levels of pro-inflammatory cytokine IFN-ϒ and mediated differentiation of GSC, as determined by increased surface expressions of MHC class I and II in GSC. Confocal microscopy revealed that NK were able to infiltrate GSC spheroids and interacted with GSC through direct cell-cell contacts. Taken together, we demonstrated that NK mediated lysis and differentiation of GSC in spheroid models, likely modeling the *in vivo* conditions. Studies in humanized mice are planned to determine direct correlation of our spheroid model with *in vivo* model and to confirm therapeutic role of super-charged NK in GB.

P-08.1-04
Development of oncolytic viruses expressing polycistronic cassettes with immunomodulatory proteins

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Oncolytic viruses are one of the promising directions for the development of cancer therapy. These viruses can selectively kill tumor cells due to a significant difference in replication efficiency in normal and tumor tissues. In addition to direct lysis, the development of a viral infection stimulates anticancer immune responses, attracting a large number of immune cells into the tumor microenvironment. Inhibitors of the immune checkpoints, which themselves have high therapeutic efficacy, have a synergistic effect in combination with oncolytic viruses. Many DNA viruses, such as herpes simplex virus and vaccinia virus, have very high genome capacity as vectors, and are capable of being efficient expressors of extended polypetides. We developed a recombination system for the expression of a polycistronic frames containing several 2A autocatalytic sites, in which up to 6 different proteins, including secreted ones, can be expressed with different and wellpredicted efficiencies depending on the position from the start of translation. As a potential immunotherapeutic combination, the expression bacterial flagellin, as well as granulocyte-macrophage colony-stimulating factor (GM-CSF), macrophage inflammatory protein 1-alpha (CCL3) and interleukin 15 is proposed. For convenience of in vivo studies and intravital microscopy, a fluorescent protein (RFP) gene can be expressed under a separate promoter at the same vector. *The authors marked with an asterisk equally contributed to the work.

P-08.1-05
Cystatin F is expressed in glioblastoma cancer and cancer stem like cells

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Glioblastoma is the most aggressive type of brain tumors, composed of heterogenous cell populations with great invasive potential. Tumor tissue contains not only differentiated and stem-like cancer cells but also activated microglia and other immune cells. Cystatin F, member of cystatin family of endogenous inhibitors of lysosomal cysteine peptidases is generally present in immune cells. Its expression is regulated by C/EBPα. Although cystatin F can be secreted in dimeric form it is only active intracellularly. In monomeric form it inhibits activity of cathepsins L, S, H and C. In some pathological conditions, cystatin F can be upregulated, like in cancer and neurodegeneration diseases. The aim of this study is to examine the expression of cystatin F in glioblastoma. Imunohistochemical detection was used to determine cystatin F expression and localisation in formalin-fixed paraffin-embedded tissue. Gene expression analysis of cystatin F and C/EBPα was performed with glioblastoma and low grade glioma samples, as well as glioblastoma tumor cells. In trans action of cystatin F was tested using U-251 MG glioblastoma and U-937 pro-monocyte cell lines by non-reducing SDS PAGE and western blot analysis. Impact of cystatin F internalisation on cathepsin L activity was also checked. We found that cystatin F is present in glioblastoma tissue but not in healthy brains. It is localised in cancer cells, cancer stem cells and microglia cells expressing GFAP, SOX2 and Iba-1, respectively. Its expression is increasing with glioma progression and is elevated in cancer stem cells in comparison to differentiated cancer cells. The same trend is true for C/EBPα. Cystatin F was able to internalize U-251 MG cells after their exposure to U-937 cells. The internalisation affected cathepsin L activity in glioblastoma cells and the interaction was confirmed by co-immunoprecipitation of cystatin F and cathepsin L. Our future aim is to elucidate the role of cystatin F expression in glioblastoma.

P-08.1-06
IDO inhibitors increase the antitumor immunity of ICD-dying cells

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Dual strategy, when antitumor drug induces the death of cancer cells and simultaneously activates immune system by propagation of immunogenic cell death (ICD) to form a sustained antitumor immune response is highly desirable. However, to avoid immune system attack, tumors can involve various mechanisms of immune suppression. Indoleamine-2,3-dioxigenase (IDO) degrades tryptophan and facilitates tumor immunotolerance in immunosuppressive tumor microenvironment. In this context we suggested that IDO inhibitors can enhance tumor infiltration with cytotoxic T-lymphocytes and increase anticancer vaccination effects of ICD inducers. Earlier we showed that pro-apoptotic protein RL2 induced death of cancer cells with signs of ICD: ecto-calreticulin, ecto-HSP70 as well as ATP and HMGB1 release into the extracellular space. In this study we investigated whether IDO inhibitors enhance antitumor vaccination effect of RL2-treated cancer cells. To study antitumor vaccination, MX-7 mouse rhabdomyosarcoma cells were used as a model. Ethyl pyruvate and 1-methyltryptophan were chosen as IDO inhibitors. RL2-treated MX-7 cells were transplanted on C3H/He syngeneic mice and next, one week after, same mice were challenged with live MX-7 cells. Vaccination effect was evaluated by survival and

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appearance of tumor nodes. We showed that ethyl pyruvate increased the survival of vaccinated animals compared to vaccinated-only mice from 67% to 83% and percent of tumor-free mice – from 33% to 50%. Peritoneal macrophages of vaccinated mice showed efficient uptake of intact MX-7 cells that indicated antitumor immunity against MX-7 antigens. Transplantation of RL2-treated cells in secondary necrosis state was less effective against challenge with live cells. Overall, we showed that inhibitors of IDO can enhance antitumor vaccination effects of RL2-treated cancer cells. This work was supported by RFBR grant № 19-34-90134.

P-08.1-07
Obesity induces pro-tumoral programming of macrophages by a survivin-dependent mechanism

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Purpose: Recent studies point to adipose-derived stem cells (ASCs) as a link between obesity and cancer. We aimed to study whether survivin, which is highly secreted by ASCs from subjects with obesity, might drive a pro-tumoral phenotype in macrophages. Methods: The effect of ASC conditioned medium on the macrophage phenotype was assessed by expression studies. Survivin intracellular localization and internalization was examined by subcellular fractionation and immunofluorescence. Loss- and gain-of-function studies were performed with adenoviral vectors, and gene expression, migration and invasion capacity of cancer cells were examined. Heterotypic cultures of ASCs, macrophages and cancer cells were established to mimic the tumor microenvironment. Survivin-blocking experiments were used to determine the impact of survivin on macrophages and cancer cells. Immunohistochemical analysis of survivin was performed in macrophages from ascitic fluids of cancer and control patients. Results: Obese-derived ASCs induced a macrophage phenotypic switch characterized by both pro- and anti-inflammatory markers. Macrophages were found to internalize extracellular survivin, generating hybrid macrophages with a tumor-associated phenotype that included secretion of survivin. Forced expression of survivin in macrophages generated a similar phenotype and enhanced the malignant characteristics of cancer cells by a mechanism dependent on survivin phosphorylation at threonine 34. Survivin secreted by both obASCs and tumor-associated macrophages synergistically boosted malignancy of cancer cells. Importantly, survivin was mainly detected in human ascites-associated macrophages from patients with a malignant diagnosis. Conclusion: Survivin emerges as a molecular link between obesity and cancer and might be a novel marker for tumor-associated macrophages. *The authors marked with an asterisk equally contributed to the work.

P-08.1-08
Cystatin F is internalised into cytotoxic T lymphocytes and downregulates their killing efficiency

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Cystatin F is an endogenous protein inhibitor of cysteine cathepsins that is expressed predominantly in immune cells and localised intracellularly in the endosomal/lysosomal pathway as well as secreted. Secreted inactive cystatin F can be internalised into bystander cells and after internalisation becomes inhibitory active. Cytotoxic T lymphocytes, immune cells responsible for elimination of cancer cells and virally-infected cells, express cystatin F and endogenous intracellular cystatin F can affect their killing efficiency. Here we investigated if extracellular cystatin F can be internalised into cytotoxic T cells and affect their effector functions. We first treated TALL-104 cells, a model cell line for cytotoxic T lymphocytes, with cystatin F and demonstrated by western blot as well as confocal microscopy that cystatin F can be internalised. In addition, cystatin F interacted with cathepsin C, as demonstrated by immunoprecipitation, and partially localised in lytic granules, as demonstrated by its colocalisation with perforin and granzyme A. Next, we showed that activities of progranzyme convertases, cathepsins C and H are attenuated by extracellular cystatin F, and importantly, this effect is translated into decreased activities of final effector molecules responsible for target cell killing, granzymes A and B. Similarly, cathepsin L activity was also decreased, but it did not lead to decreased perforin processing. Notably, extracellular cystatin F also downregulated killing of target cells, as demonstrated by calcein-AM release assay. Our results thus indicate that cystatin F is an important mediator that can impair killing efficiency of cytotoxic T lymphocytes and designate it as a possible target in cancer immunotherapy.

P-08.1-09
In vitro modulation of protein O-GlcNAcylation and its impact on function of selected immune cells

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O-GlcNAcylation is an important metabolic process in cells, which needs to be carefully regulated. O-β-N-acetylglucosaminyl transferase (OGT) is the enzyme that catalyses transfer of O-GlcNAc from uridine diphosphate to serine and threonine residues of proteins [1]. Its dysregulation is linked to several pathologies, including cancer, inappropriate immune response and impairment of the nervous system [2]. In our research we focused on the potential effects of OGT inhibitors on the functionality of dendritic (DC) and natural killer (NK) cells. NK cells and monocytes were isolated from PBMCs of health donors. Isolated NK cells were exposed to OGT inhibitors for 24 h. The cytotoxic potential of NK cells was determined by addition of K562 labelled cells, commercially available viability dyes and flow cytometry. The 20 μM OGT inhibitor, OSMI-1, reduced NK cells activity against K562 for 43.5 ± 3.5% if compared to control. We also investigated the effect of OGT inhibitors on the differentiation process of monocytes to DCs. We studied DCs

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functionality in terms of their (i) phagocytic abilities, and by (ii) investigation of their T-cells activation potential. For the latter, mature DCs were co-cultured with allogenic labelled T cells and the extent of their proliferation was assessed by flow cytometry. In presence of OSMI-1 the differentiation of monocytes to DCs was suppressed. In comparison with non-treated cells, reduced expression of CD86, CD80 and DC-SIGN, and increased expression of CD14 and HLA-DR were observed. Also, a slightly decreased phagocytic activity could be determined. Taken together, we confirm that OGT inhibitors modulate the function of DC and NK cells in *in vitro* conditions, but further studies are needed to delineate the role of O-GlcNAcylation in these immune cells. [1] Haltiwanger RS et al. (1990) J. Biol. Chem. 265, 2563–2568. [2] de Jesus T et al. (2018) Cell Immunol. 333, 85–92.

P-08.1-10
A novel chemical compound inhibits BCAT2 in cancer cells

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Branched-chained amino acids (BCAAs) are essential for energy production and protein synthesis. Our previous studies have shown that branched-chained amino acid transferase 2 (BCAT2), which breaks down BCAAs for energy production, is overexpressed in MCF-7 breast cancer cell line. This indicates that BCAAs are of greater importance to tumor cells. The aim of our study was to find a new inhibitor for BCAT2 and evaluate the effect it has on cancer cell proliferation, viability and mitochondrial membrane potential (ΔΨm). We used data from the Protein Data Bank to determine the active site of the protein. An inhibitor named EL2 (PDBID: 5HNE) was already known for its inhibitory effect on BCAT2. So we used the most important structural component of the protein-ligand interaction - the benzimidazole ring, as a template, to find commercially available molecules. We screened the carefully selected compounds through a number of druglikeness filters and identified the best drug candidate by docking using QuickVina-W and the result was 2-{4-[2-(1-Pyrrolidinyl)ethoxy]phenyl}-1H-benzimidazole (2FBI-1). The concentration of 50 mM of compound was used in the study. Five different cell lines were tested: MCF-7 breast cancer, MCF-10A normal epithelial breast cells, BCC primary culture of breast cancer, HeLa ovarian cancer and HCT116 colon cancer cells. For the evaluation of proliferation we performed flow cytometry assay using fluorescent dyes 7-AAD for viability assesment and JC-1 for determining ΔΨm. After 24 h of the exposure we found that 2FBI-1 decreased the proliferation of MCF-7, BCC, HeLa and HCT116 by ~30%. Viability was decreased ~35% in cancer cell lines in compare to normal cells 2.25%. 2FBI-1 had no effect on ΔΨm of MCF-10A, but ΔΨm dramatically increased in malignant cell lines by at least 5 times. To conclude, the new BCAT2 inhibitor 2FBI-1 has inhibitive effect on cancer cell proliferation, viability and mitochondrial membrane potential.

P-08.1-11
In vitro evaluation of IL12 plasmid gene electrotransfer efficiency and functionality

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Gene electrotransfer (GET) is an electroporation-based method, where genes encoded in plasmid DNA are delivered into the cells and tissues. Gene electrotransfer of plasmid encoding IL-12 is already in clinical trials in USA, demonstrating positive results in treatment of melanoma patients. To comply with EU regulatory requirements for clinical application, which recommend the use of antibiotic-free plasmids, the aim of our study was to construct antibiotic-free plasmid encoding the human IL-12 (p21-hIL-12-ORT) and its analogue encoding the murine IL-12 (p21-mIL-12-ORT). To demonstrate the suitability of human IL-12 plasmid for further in vivo studies and clinical trial, biological activity of the designed plasmids, the level of transgene expression and plasmid copy number *in vitro* in human squamous cell carcinoma cell line FaDu and murine colon carcinoma cell line CT26 were determined. We have confirmed the expression of transgene IL12 mRNA in both cell lines after GET. The IL12 mRNA expression reached the maximum 24h after GET and decreased with time. Protein expression has shown increased mil12 and hIL12 levels on day 1, followed by decrease until day 7 and 9 to reach control values, respectively. Minimal amounts of IL12 in control cells were detected due to endogenous IL12. DNA plasmid copy number in the cells after GET has exponentially decreased with the time until day 4 to reach control values. The biological activity and potency of every phIL12 and pmIL12 batch has been confirmed after lipofection and GET. To conclude, GET of phIL12 and pmIL12 has proven efficient delivery method resulting in production of biologically active IL12 in electroporated FaDu and CT26 cells, respectively. Importantly, the transcript level as well as biologically active protein and amount of electroporated plasmid showed time-dependent decrease. Finally, our results demonstrate that the plasmid encoding hIL12 is suitable for further in vivo studies.

P-08.1-12
Gene electrotransfer of plasmid DNA encoding proinflammatory chemokines CCL5 and CCL17 to murine tumors modifies tumor microenvironment

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Immune cell trafficking mediated by proinflammatory chemokines in combination with immune response eliciting therapy such as irradiation presents one of potential modalities in cancer immunotherapy. Through the use of plasmid DNA encoding either CCL5 or CCL17 we studied the effects of these two proinflammatory chemokines after lipofection in vitro and gene electrotransfer (GET) *in vivo* in murine breast (4T1, E0771) and colon (CT26, MC38) cancer models. Viability of all cell lines 48 h after lipofection with plasmids encoding CCL5 or CCL17 remained above 80%. Concurrent expression analysis of 11

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cytokines 48 h after lipofection showed significantly increased expression of CCL5, CCL17 and slightly increased levels of IL-6 and CXCL10 in the surviving cells. Utilizing GET after intratumoral injection of plasmids encoding either CCL5 or CCL17 in CT26 and 4T1 murine tumor model (animal license: U34401-1/2015/43) resulted in minor tumor growth delay. Concurrent expression analysis of 7 cytokines after GET of CCL5 and CCL17 to CT26 tumors showed increased expression of both chemokines, while levels of proinflammatory cytokines IL-6, IL-12 and IFNγ were only slightly increased. Increased expression profile, both *in vitro* and *in vivo*, is typical for inflammation. GET of plasmids encoding CCL5 or CCL17 combined with irradiation in CT26 and 4T1 tumor model resulted in significant tumor growth delay. Therefore, based on changed tumor microenvironment due to the inflammation produced by our therapy future experiments will be focused towards elucidating the infiltration of immune cells into the treated tumors and determining the optimal time window to combine GET of plasmids encoding chemokines CCL5 and CCL17 with irradiation.

P-08.1-13
Stability and transfection of antibiotic-free plasmids encoding mouse, canine and human interleukin-12

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Interleukin 12 (IL-12) is a cytokine that is used as a therapeutic molecule in cancer immunotherapy. It works by strengthening the host's immune system to detect and kill cancer cells, and it needs to be released locally. This can be achieved by gene-electrotransfer (GET) with IL-12 encoded on plasmid vectors. We prepared four antibiotic free plasmids encoding mouse, canine and human interleukin-12 using ORT (Operator-Repressor Titration technology) technology. To evaluate the stability of antibiotic-free plasmids produced with this technology, we isolated DNA from bacterial cultures after five passages on agar plates and compared the yields obtained with the yields of a commercially available IL-12 plasmid. Since these plasmids are intended for GET, we additionally tested the transfection efficiency after GET in corresponding cell lines: human SK-MEL-28, mouse B16-F10 and canine CMeC-1 melanoma cell lines. In this part of our study, we investigated cytotoxicity (clonogenic assay), the number of transfected plasmids and the expression of IL-12 mRNA encoded on plasmid vectors. For the latter two analyzes, we designed a forward primer positioned in the p40 subunit of IL-12 construct and a reverse primer extending across the linker and the second (p35) subunit. For the determination of the plasmid copy number, we performed an absolute quantification qPCR method using a synthetic dsDNA gBlocks® (IDT) to generate a standard curve. The expression analysis was performed on cDNA synthesized from isolated total RNA after GET. The results show that plasmids are not lost during passage on agar plates. Expression analysis showed that transfection of IL-12 was successful because the highest IL-12 expression was detected in GET group, which correlated with the determination of the plasmid number. Our results show that plasmids without an antibiotic resistance gene are stable and have a high IL-12 expression, so they could potentially be relevant in clinical melanoma studies.

P-08.1-14
Inhibition of valine degradation pathway affects cancer cell viability

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Altered metabolism is one of the hallmarks of cancer cells. The best known alteration in cancer metabolism is Warburg effect, which states, that cancer cells tend to favor metabolism via glycolysis rather than the oxidative phosphorylation pathway, which is the preference of normal cell. Genome-scale metabolic modeling has shown that the valine degradation pathway plays a major role in the production of ATP in cancer cells (Antanaviciute et al., 2017). In our previous work we have shown the effect of BCAT2 on breast cancer cell viability and we decided to demonstrate that HIBADH reaction can be critical in suppressing valine degradation in cancer cells. In order to inhibit valine degradation pathway we used siRNA specific to HIBADH gene and antimetabolite of its protein – 2,2-hydroxymethyl propionic acid (bis-HMPA), specified by the computational analysis. We measured breast cancer (MCF-7) and normal (MCF10A) cell viability by MTT and flow cytometry assays and determined that HIBADH-specific siRNA and bis-HMPA decreased cancer cell growth but did not affected normal cell viability in both cases. Reduced levels of the target protein HIBADH after siRNA transfection and bis-HMPA treatment were evaluated by Western blotting and immunocytochemical analysis. We demonstrate, that valine degradation pathway is important to cancer cell proliferation and it could be the way to target selectively cancer cells while keeping unaffected healthy dividing cells.

P-08.1-15
Upregulation of cytosolic DNA sensors and cytokines in response to irradiation is associated with HPV16 status of pharyngeal squamous cell carcinoma

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Oropharyngeal squamous cell carcinoma (OPSCC) caused by human papillomavirus 16 (HPV16) responds better to irradiation (IR) than HPV16 negative pharyngeal squamous cell carcinoma (PSCC); however, the exact mechanisms are still unknown. Previous studies have shown that HPV16 oncoproteins E6 and E7 inhibit cytosolic DNA sensors, which are associated with innate immune system activation. Therefore, the aim of our study was to investigate the radiosensitivity and involvement of DNA sensing pathways in response to IR in OPSCC and PSCC cell lines. A metabolic viability test was used to determine the radiosensitivity of PSCC (FaDu and 2A3) and OPSCC (UM-SCC-6 and

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UPCI:SCC090) cell lines with different HPV16 statuses. Gene expression of different cytosolic DNA sensors (cGAS, STING, RIG-I, DAI, IFI16, DDX60) and cytokines (IFNβ, TNFα, IL1β) was determined after IR with different single doses (0, 4 and 8 Gy). After 24, 48 and 72 hours total RNA was isolated and reverse transcribed into cDNA. qRT-PCR was used to determine the expression of DNA sensors, cytokines and HPV16 viral load. Our results demonstrated a greater response to IR only in HPV16 positive OPSCC compared to HPV16 negative OPSCC. The upregulation of gene expression of cytosolic DNA sensors and cytokines was dose-, time- and HPV16 status-dependent. Cytosolic DNA sensors DAI and RIG-I were upregulated after IR in HPV16 negative OPSCC and PSCC but not in HPV16 positive cell lines. After IR, the expression of cytokines was significantly upregulated only in HPV16 negative cell lines. In HPV16 positive cell lines, expression of DNA sensors and cytokines was dependent on the level of expression of HPV16 oncoproteins E6 and E7. In conclusion, our results suggest that in vitro DNA sensors and cytokines are differently expressed in response to IR in OPSCC and PSCC cell lines based on HPV16 status.

P-08.1-16
Barnase–Barstar molecular pair in a system for the stepwise delivery of cytotoxins to HER2-positive tumors

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The development of targeted drugs aimed to tumor cells with a definite molecular profile and possessed minimal side toxicity is an urgent problem in modern oncology. One of the solution of this problem is to use a stepwise targeted delivery, or pre-targeting: in a first step, a primary non-toxic vector molecule (antibody, non-immunoglobulin scaffolds) binds to cancer cells and non-bound primary agent then is cleared from the blood, in a second step a cytotoxic agent (toxin, radioactive isotope) with high affinity to the recognition tag on the primary agent is added. In pretageting system developed we propose to use barnase-barstar molecular pair for fast and strong interaction between antigene-specific and cytotoxic modules. It is known that ribonuclease barnase interacts with its native inhibitor barstar with high affinity (KD 10–15 M) and thus can provide quick and highly selective interaction of two modules *in vivo*. During last ten years proteins of non-immunoglobulin scaffold (such as DARPins, affibody) are considered as a perspective targeting vectors. As an anti-HER2-targeting module we use DARPin-barnase fusion protein. We have shown that the DARPin-barnase is located on the surface of the HER2-positive cancer cell up to 20 hours before being internalized. We have shown that liposomes functionalized with barstar and loaded with a pseudomonas exotoxin A effectively kill cancer cells pretreated with the DARPin-barnase fusion protein (IC50 40 nM). In conclusion, the studied pretargeting system can efficiently delivers anti-HER2-targeting module to HER2-positive cancer cells and provides strong interaction between targeting and cytotxic module by means of barnase-barstar interaction. The work is supported by the Russian Science Foundation (project no. 19-14-00112).

P-08.1-17
Recombinant oncolytic vaccinia virus strains for treatment of malignant neoplasms

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Metastatic cancer remains an incurable disease. Oncolytic viruses infect and lyse cancer cells without any harm to normal cells, while many of them show the ability to stimulate the immune system against tumor cells. Recombinant vaccinia viruses have great prospects as an antitumor agent, due to their large capacity genome, high onco-selectivity and repeatedly proven safety of use. Also, different strains of vaccinia virus are capable to induce interferon in tumor cells on different levels. We used two platforms: Modified Vaccinia Ankara (MVA) and strain from Leningrad Institute of viral preparations (LIVP) for obtaining recombinant variants. To assess the oncolytic effects of strains, we obtained recombinant virus expressing reporter construct with red fluorescent protein (RFP) and firefly luciferase (fluc) for intravital microscopy investigation and therapeutic effectiveness evaluation on orthotopic tumor mouse models using IVIS spectrum. We used 4T1 breast cancer and B16 melanoma cell lines for these experiments. LIVP-fluc-RFP strain has demonstrated greater therapeutic efficacy on both models and less potent induction of interferon than MVA-fluc-RFP. The effectiveness of viral replication also varied significantly between these two strains. The LIVP-fluc-RFP strain replicated 10–12 times faster, which was estimated by RFP signal in tests *in vitro* and *in vivo*. Our study validated the hypothesis, that new recombinant viruses provides a superior therapeutic effect in breast cancer and melanoma mice models leading to significant tumor regression and robust immune response, inducing CD4+ and CD8+ cells.

P-08.1-18
The multi-targeted receptor tyrosine kinase inhibitor sunitinib inhibits natural killer cell-mediated antibody-dependent cellular cytotoxicity against JIMT-1 breast carcinoma cells

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Natural killer (NK) cells evolved to kill virus-infected and tumor cells. Antibody-dependent cellular cytotoxicity (ADCC) is a key mechanism in the NK cell-mediated tumor cell lysis and it also mediates NK cell-induced killing of HER2-positive JIMT-1 breast cancer cells in the presence of the anti-HER2 monoclonal antibody trastuzumab. The aim of our work was to identify molecules that enhance or inhibit ADCC of NK cells (CD16.176 V. NK-92 cell line) against trastuzumab-covered JIMT-1 breast cancer cells. First we optimized killer assay conditions such as effector to target cell ratio and incubation time using electric cell substrate sensing (ECIS). Then, we transferred the assay to a high-content screening-compatible format which is based on the detection of the surviving target cells with calcein-AM viability probe.

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After optimization of the assay conditions we screened a library of 774 FDA approved compounds and identified five drugs that reduced ADCC activity. The multitargeted receptor tyrosine kinase inhibitor sunitinib malate was chosen as the most interesting hit for follow up experiments. The ADCC inhibitory effect of sunitinib malate has been confirmed with ECIS which also indicated that the drug caused a stronger attachment of JIMT-1 cells to the plate surface. Cell morphometry and dispase assay also confirmed the adherence-inducing effect of sunitinib. Experiments are under way to confirm that the effect of sunitinib on cell adhesion plays a role in breast cancer cell resistance to NK cell cytotoxicity. Our results may have implications for the assessment of anti-tumor immune state of cancer patients receiving sunitinib treatment. Funding: This work was supported by grants from the NRDIO (GINOP-2.3.2-15-2016-00020 TUMORDNS", GINOP-2.3.2-15-2016-00048-STAYALIVE, OTKA K132193 to LV, PD 116845 to CH), from the Hungarian Academy of Sciences (BO/00468/17/8 to CH) and from the Ministry of Human Capacities (ÚNKP-19-4-DE-299 to CH).

P-08.1-19
Novel tumor cell-based vaccine with *in vivo* gene electrotransfer of plasmid DNA encoding IL-12 as adjuvant

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Cancer vaccination is a promising field of research; however, it remains largely elusive. One category of cancer vaccines are tumor cell-based vaccines that use tumor cells as a source of tumor-associated antigens. We aimed to develop a tumor cell-based vaccine from B16-F10 or CT26 tumor cells killed by irradiation with adjuvant in vivo gene electrotransfer (GET) of plasmid encoding interleukin-12 (IL-12). The vaccine contained 0.5 mg or 1 mg of proteins and 50 μg of IL-12 plasmid. We examined our vaccine's therapeutic effect in combination with tumor irradiation and its preventative effect in two immunologically different tumor models: the less immunogenic malignant melanoma B16-F10 and more immunogenic colorectal carcinoma CT26. The vaccine applied in the skin distant from the tumor was encompassed with a contact hexagonal multielectrode array and GET was performed (24 pulses, 170 V/cm, 5.64 Hz, 150 ms). Concomitantly, the tumor was irradiated with 15 Gy. In the preventative setting, vaccination was administered before tumor induction. While we observed a synergistic effect between vaccination and tumor irradiation in the B16-F10 tumor model, we did not observe any therapeutic contribution of our vaccine to tumor irradiation in the CT26 tumor model. All experimental procedures were done in accordance with the national guidelines (U34401–1/2015/16) and EU directive (2010/63/EU). In contrast, preventative vaccination with the higher dose before tumor induction was the most effective in the CT26 tumor model (up to 56% protection); while no preventative effect of vaccination was observed in the B16-F10 tumor model. To conclude, the contribution of a single dose therapeutic vaccination to local tumor irradiation was greater in the less immunogenic B16-F10 tumor model, while a single dose preventative vaccination was more effective in the more immunogenic CT26 tumor model. Previously published in: Remic et al. (2020) Vaccines 8, 111.

P-08.1-20
Silencing of melanoma cell adhesion molecule by gene electrotransfer radiosensitize B16F10 melanoma and TS/A carcinoma

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The efficacy of radiotherapy as a common treatment of cancer can be improved with adjuvant treatments, i.e. vascular targeted and immunomodulatory therapies. In the study, we evaluated the radiosensitization of murine B16F10 melanoma and TS/A carcinoma silencing by gene electrotransfer (GET) of plasmid encoding shRNA against MCAM (pMCAM), with dual action; vascular targeted and immunological effects. The antitumor effects and underlying mechanisms of combined therapy were evaluated *in vitro* by clonogenic assay and *in vivo* by tumor growth delay assay and tumor cures. In vivo, tumors were treated by triple GET and single-dose irradiation 1 day after the first GET. Proliferation, apoptosis, necrosis, vascularization, hypoxia, infiltration of immune cells, expression of IL-12 and TNFα were evaluated histologically. Melanoma was significantly more responsive to radiosensitization after the silencing of MCAM than carcinoma, resulted in 81% and 27% of tumor cures, respectively. Moreover, 59% of melanoma were resistant to secondary challenge, while none of the carcinoma. This effect cannot be ascribed to the intrinsic sensitivity of cells since in vitro the effect was reversed, higher enhancement factor of combined treatment was obtained in carcinoma cells. Silencing of MCAM combined with irradiation affected tumor vasculature, induced hypoxia, apoptosis, necrosis, and reduced tumor cell proliferation. However, the significant increase of infiltrating immune cells was observed only in the melanoma model, also when the non-target control plasmid was used, which correlated with the antitumor response, being higher in melanoma than in carcinoma. GET of plasmid silencing MCAM radiosensitizes melanoma and carcinoma, predominantly due to a direct effect on tumor vasculature and tumor cells. Furthermore, the activated immune response potentiates the antitumor effectiveness, but its contribution depends on tumor type-specific immunological status.

P-08.1-21
Cell lines B16F10, 4T1 and CT26 are comparably sensitive to electrochemotherapy with bleomycin, cisplatin and oxaliplatin

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Electroporation is a method, which leads to increased cell membrane permeability. This enables different molecules, which usually cannot cross the membrane, to enter the cell. In electrochemotherapy (ECT), electric pulses induce the uptake of cytostatics and subsequently potentiate their cytotoxic effect. There are many different types of cell death. After ECT, apoptosis and necrosis have been described. Furthermore, ECT also induces immunogenic cell death. The aim of the study was to compare the sensitivity of three immunologically different cell lines to ECT with bleomycin (BLM), cisplatin (CDDP) or oxaliplatin (OXA) *in vitro*. Additionally, the aim was to determine the concentration of cytostatic that induce death of 50% of cells (IC50) for further evaluation of different types of cell death and other immunologically important biomarkers. Three different murine tumor cell lines (B16F10, 4T1 and CT26) were treated with CDDP, BLM or OXA ECT *in vitro*. The cytotoxicity of cytostatics alone or in combination with electric pulses was compared between cell lines using a clonogenic assay. Plate electrodes and ECT electric pulse parameters were used (1300 V/cm, 100 μs, 1 Hz, 8 pulses). Electric pulses potentiated the cytotoxicity of BLM, CDDP and OXA in all three tested cell lines. 4T1 cells were more sensitive to ECT with BLM (IC50) than CT26 cells. Namely, IC50 values after ECT with BLM (B16F10: 0.37 ± 0.05 nM, 4T1; 0.26 ± 0.07 nM, CT26: 0.49 ± 0.13 nM), CDDP (B16F10: 10.17 ± 0.48 μM, 4T1: 11.38 ± 0.61 μM, CT26: 12.28 ± 0.94 μM) and OXA (B16F10: 17.02 ± 1.67 μM, 4T1: 14.31 ± 1.73 μM, CT26: 13.71 ± 1.06 μM) were comparable. To conclude, preliminary data indicated that all three cell lines are comparably sensitive to BLM, CDDP or OXA ECT in vitro. With further experiments we will detect the type of cell death and other biomarkers such as MHC-I, MHC-II, PD-L1, calreticulin, CD40, extracellular ATP and HMGB1 after IC50 ECT *in vitro*.

P-08.1-22
Development of humanized antibodies to PRAME antigen for cancer immunotherapy

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The preferentially expressed antigen of melanoma (PRAME) is a cancer-testis antigen, which is expressed only in testis, but not in normal tissues. PRAME is frequently expressed in many human malignances, such as melanoma, acute and chronic leukemias, non-small cell lung carcinomas, breast carcinomas, renal cell carcinomas, sarcomas. Thus PRAME could be considered as an attractive potential target for tumor immunotherapy. Two humanized antibodies were developed by CDR grafting method using variable domain sequences of murine monoclonal antibodies (mAb) 5D3 and 6H8 which were previously derived by immunizing BALB/c mice with recombinant PRAME. The human Ig germ line genes with closest homology to murine mAb variable regions were used as a source of human frameworks for antibody humanization. Based on in silico 3D modeling data we identified several amino acid residues from murine mAb frameworks to be retained in humanized mAb in order to prevent affinity loss. Humanized mAbs were produced in CHO cells. Affinity of humanized mAb were measured on Attana biosensor using recombinant PRAME. Calculated KD values, 1.4 nM for antibody 5D3Hu and 1.2 nM for antibody 6H8Hu appeared to be similar to chimeric versions of these mAbs. Humanized mAbs showed binding to PRAME in melanoma cell lines lysates in Western blot and to recombinant PRAME in ELISA. We performed epitope mapping using Western blot and ELISA with different fragments of PRAME fused with thioredoxin. It was shown that mAbs 5D3 and 6H8 bind to close non-overlapping fragments of the protein corresponding to 160–180 and 180–200 amino acid residues, respectively. Incubation of melP (human melanoma) cells with humanized mAbs to PRAME resulted in inhibition of tumor cell proliferation that was assessed using xCELLigence system. This work was supported by a subsidy of the Ministry of Education and Science of the Russian Federation (No. 14.604.21.0204, Project ID No. RFMEFI60418X0204).

P-08.1-23
Tumor immune status and mutational burden as predictive factors for *in situ* vaccination by TNFα and IL-12 gene electrotransfer

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In situ vaccination is a promising immunotherapeutic approach, where various local ablative therapies are used to induce an immune response against tumor antigens that are released from the therapy-killed tumor cells. We recently proposed using intratumoral gene electrotransfer for concomitant transfection of a cytotoxic cytokine tumor necrosis factor-α (TNFα), to induce *in situ* vaccination, and an immunostimulatory cytokine interleukin 12 (IL-12), to boost the primed immune response. Here, our aim was to associate tumor immune profiles with the local and systemic effectiveness of the approach in tree syngeneic mouse tumor models: B16F10 melanoma, TS/A mammary adenocarcinoma tumor models and CT26 colon carcinoma (Permission No. U34401-1/2015/43). Tumor immune profiles were characterized in 40 mm^3 tumors by determining tumor mutational burden, tumor-infiltrating CD4+ and CD8+ lymphocytes and expression of PD-L1 and MHC-I on tumor cells. Gene electrotransfer was performed on 40 mm^3 primary tumors by intratumoral injection of plasmid mixture containing 50 μg of pORF9mTNFα and 50 μg pCol-mIL-12-ORT plasmids, followed by application of electric pulses. Therapeutic effectiveness was determined by measuring tumor growth of both the treated and the untreated tumors in the dual flank tumor model. None of the tested characteristic proved predictive for local effectiveness, conversely high tumor mutational burden, immune infiltration of tumors and MHC-I expression were associated with higher abscopal effectiveness. Hence, we have confirmed that abundance and presentation of tumor antigens as well as the absence of immunosuppressive mechanisms are important for effective in situ vaccination. These findings provide important indications for future development of in situ vaccination based treatments,

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and for the selection of tumor types that will most likely benefit from it.

P-08.1-24
Early but not late ferroptotic cancer cells are immunogenic *in vitro* and *in vivo*

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Cell-based immunotherapies hold great promise for the future treatment of cancer. The success of these therapies demonstrated the power of harnessing the immune system to eradicate tumors. It is becoming clear that type of cancer cell death contributes to the efficiency of anti-cancer therapy and long-term survival of patients[1]. Since tumors often develop resistance to apoptosis and necroptosis, triggering these processes is not always the optimal strategy[2]. That is why it is crucial to find alternative ways to kill cancer cells and to select the proper immunogenic cell death type that is able to induce potent and strong anti-cancer immune responses, potentially leading to tumor eradication. Ferroptosis, an iron-dependent form of cell death leading to lipid peroxidation in cells, is currently actively studied[3]. We reported that cancer cells undergoing ferroptosis are immunogenic *in vitro* and *in vivo*[4]. Only early (not late) ferroptotic cells stimulate the phenotypic maturation of BMDCs and induce a vaccination-like effect in a tumor prophylactic vaccination model in immune-competent mice but not in immune-compromised Rag-2-/- mice. In addition, ATP and HMGB1, the best-characterized damage-associated molecular patterns are involved in immunogenic cell death, have proven to be passively released along the timeline of ferroptosis and act as immunogenic signal associated with the immunogenicity of early ferroptotic cancer cells. Altogether, these results identify early ferroptotic cancer cells as effective inducers of an adaptive immune response, and induction of ferroptosis in cancer might be another option to overcome cell death resistance and enhance the efficacy of anti-cancer therapy. 1. Galluzzi, L. et al.(2017) Nat. Rev. Immunol. 17, 97–111 2. Krysko, O. et al.(2017) Immunol. Rev. 280, 207–219 3. Friedmann Angeli, J. P. et al.(2019) Nat. Rev. Cancer 19, 405–414 4. Efimova, I. et al.(2020) J. Immunother. cancer 8, 1–15 *The authors marked with an asterisk equally contributed to the work.

P-08.1-25
Gene therapy with IL-2 and IL-12 in murine B16F10 melanoma

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Immunotherapy has nowadays become an important approach for treating cancer. One example is the use of cytokines such as interleukin 2 (IL-2) and 12 (IL-12) that directly stimulate immune cells at the tumor site. The application of the aforementioned cytokines was primarily in recombinant protein form but nowadays it is replaced by gene therapy approach. The aim of our study was to determine the effects of plasmids encoding IL-2 (pIL-2) IL-12 (p-IL-12) gene electrotransfer *in vitro* and *in vivo*. First, the cytotoxic effect of gene electrotransfer of both plasmids individually and combined was determined in vitro on B16F10 murine melanoma cell line. Two pulse protocols were tested, called EP1 (600 V/cm, 5 ms, 1 Hz, 8 pulses) and EP2 (1300 V/cm, 100 μs, 1 Hz, 8 pulses). Cell viability was measured using PrestoBlue assay and the expression profile of IL-2 and IL-12 in cells was determined by real-time PCR. We also performed *in vivo* gene electrotransfer of pIL-2 and pIL-12 using GET protocol in B16F10 murine melanoma. Tumor growth delay was measured and the IL-2 and IL-12 concentrations in tumor and serum samples using ELISA assay were determined. Histological and immunohistochemical analysis of tumor samples were also performed. In *in vitro* experiment, we demonstrated that the percentage of viable cells treated with EP2 pulses was significantly higher than that treated with EP1 pulses. In contrast, the fold expression of interleukin genes in treated cells with EP2 pulses was lower. After the administration of EP1 pulses *in vivo*, a tumor growth delay was observed in the pIL-12 and combination (pIL-12+pIL2) group and in some mice also complete tumor regression. In addition, all mice in the combination group developed immune memory, as they remained tumour free 100 days after the rechallenge. To conclude we demonstrated high antitumor effectiveness of gene therapy with IL-2 and IL-12 in murine B16F10 melanoma.

P-08.1-26
Immunogenic cell death in photodynamic-induced tumor cells based on photosens and photodithazine

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Anti-cancer therapy is more successful when it can induce an immunogenic form of cancer cell death (ICD). ICD is characterized by the emission of danger molecules (DAMPs), leading to the induction of strong anti-tumor immune responses. Therefore, when developing new treatment strategies, it is extremely important to choose such therapeutic methods that would induce ICD and thereby would induce anti-tumor immune responses leading

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to the most complete destruction of tumor cells. It has been shown the high efficacy of photodynamic therapy (PDT) in the treatment of tumors. The aim of this work was to analyze whether the clinically widely used photosensitizers, photosens (PS) and photodithazine (PD), can induce ICD when used in PDT. For this we first identified the optimal concentrations of photosensitizers and found that in the doses of 20 J/cm^2 they efficiently induce cell death in the murine glioma (GL261) and fibrosarcoma (MCA205) cell lines. Intracellular distribution of PS and PD was studied by using the laser scanning microscope. Tumor cells undergoing PS-PDT or PD-PDT induced cell death emit calreticulin, HMGB1 and ATP and they were efficiently engulfed by bone-marrow derived dendritic cells, which then matured, became activated and produced IL-6. Next we analyzed whether the immunogenicity of cancer cells can induce a protective anti-tumor immune response. Using dying cancer cells induced by PS-PDT or PD-PDT, we demonstrate the efficient vaccination potential of ICD *in vivo*. Altogether, these results identify PS and PD as novel ICD inducers that could be effectively combined with PDT in anti-cancer therapy. The study was supported by grant from RSF (project No.18-15-00279). Iuliia Efimova, a PhD student, is paid by FWO G043219N and BOF 01/O3618. The work of Dr. Elena Catanzaro was funded by FWO G051918N. The Tecan Spark® 20 M microplate reader was purchased on the projects of FWO 1507118N and FWO 1506218N.

P-08.1-27
GD2-specific CAR-T cells armored with membrane form of IL-15 efficiently target GD2 positive cancer cells *in vitro*

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Chimeric antigen receptor modified-T (CAR-T) cells rapidly became an essential tool for CD19+ hematologic cancer treatment. Genetic retargeting of a T cell with a CAR enables a new antigen-specificity through the single-chain variable fragment, which is derived from a tumor-specific antibody. CAR also consists of transmembrane, co-stimulatory, and CD3z signaling domains. This construct allows T cells to recognize cancer-specific antigens in an MHC-independent manner, facilitates activation and release of cytotoxic cytokines when the target is recognized. There are several limitations to effectively exploit CAR-T cells for solid tumor treatment, including the suppressive activity of tumor stroma and poor migration of effectors to tissue. The aim of the project is to improve GD2, and L1CAM targeted CAR-T cells by co-expression with membrane-anchored cytokines. At this point, the lentiviral transduction efficiency reached approximately 60% with the anti-GD2 CAR construct, while CAR+ cells were almost equally represented by CD4+ and CD8+ populations. Bicistronic expression of surface cytokine IL15/IL15Ra downstream P2A and anti-GD2 CAR led to a significant decrease in transduction efficiency (15% CAR+; 5% IL15+; 5% IL15Ra+) and remains to be optimized. Although the main advantages of IL15/IL15Ra should be observed *in vivo*, its expression correlates with better survival of T cells *in vitro*. Both IL15/IL15Ra positive and negative anti-GD2 CAR-T cells proved to specifically kill GD2+ target cells of neuroblastoma and glioma origin (IMR-32, T98G) at 5:1–20:1 E:T ratio. However, degranulation analyzed by CD107a staining was much lower than that of anti-CD19 CAR-T. Thus, GD2 targeted CAR-T cells armored with membrane form of IL15 specifically kill GD2+ cancer cells *in vitro* and may serve as a prospective tool for neuroblastoma, ganglioneuroblastoma and glioma immunotherapy application.

P-08.1-28
OXPHOS genetic complexity in cancer cell biology and immune response

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Animal models with identical nuclear genomes (nDNA) but with different mtDNA haplotypes generate functionally different oxidative phosphorylation (OXPHOS) systems that shape the organismal metabolism1, supporting the conclusion that different mtDNA variants are phenotypically relevant. MtDNA haplotype profoundly influences reactive oxygen species generation, energy homeostasis metabolism and ageing parameters, resulting in different healthy longevities of conplastic strains1. Adaptation of immune cells metabolism to microenvironment generated by cancer cells was investigated in conplastic mice. To analyze the metabolic crosstalk within nucleus and mitochondria, we engineered transmitochondrial cybrids from B16F10 melanoma cells (identical nDNA, different mtDNA variants). This tool allowed us to modulate the mitochondrial metabolism and the mitoproteome of cancer cells to examine whether the metabolic changes can qualitatively and quantitatively alter epitopes and whether of these changes can be used to identify more effective tumor specific epitopes for targeting by the immune system. We characterized the modifications in the peptidome and found differences in the efficiency of the T cell immune response that is primed by such tumors. Our results show that substitution of different mtDNA variants is sufficient to promote differences in mitochondrial function, metabolic exhaustion in Tumor-infiltrating lymphocytes (TILs) and a concomitant cellular adaptive response. The existence of intrinsic mismatch between mtDNA and nDNA reveals differences in immune response and aggressiveness of malignant cancer cells. This scenario connects mitohormesis with tumor progression, mitochondrial ROS generation, metabolic demands and antitumor immunity and establishes a temporal connection between them. 1 Latorre-Pellicer, A. et al. Mitochondrial and nuclear DNA matching shapes metabolism and healthy ageing. Nature 535, 561–565 (2016).

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P-08.1-29
Tumor stem cells could promote glioblastoma sensitivity to oncolytic virus

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Virotherapy is one of the most actively developing approaches for cancer treatment. We have developed double recombinant vaccinia virus VV-GMCSF-Lact with insertion of human GM-CSF and oncotoxic protein lactaptin genes which enhance virus oncolytic activity. VV-GMCSF-Lact has been shown to have high cytotoxic activity against a number of human tumor cell lines and antitumor efficacy against human breast cancer. In this work we studied VV-GMCSF-Lact cytotoxic activity against human glioblastoma cells U87 MG and U343 MG, while U343 MG cells were significantly more sensitive to virus action compared with U87 MG cells. For this reason, we assessed the presence of glioma stem cell markers on investigated cells. Using Flow Cytometry assay we have shown U343 MG cells to be characterized by a greater presence of CD133 and CD15 markers. Also U343 MG cells have higher SOX-2 and c-Myc levels analyzed by RT-PCR assay and Western blot analysis. Furthermore, we investigated the level of proteins that could mediate the virus penetration into cells. The vaccinia virus enters the host cell through macropinocytosis and mimicry under apoptotic bodies. One of the possible mechanisms is activation of PI3K/Akt pathway with further PAK1 activation. Using Western blot analysis, we have demonstrated higher level of p110α in U343 MG cells compared with U87 MG cells, which increases the number of formed p85α-p110α heterodimers and the subsequent amplification of the downstream signals, as well as a higher activation rate of PAK1 and Akt1 after the virus influence, which confirms more efficient pathway activation in U343-MG cells. The data obtained suggest that the higher sensitivity of U343 MG cells to VV-GMCSF-Lact action is due to the high number of glioma stem cells which are believed to be characterized by upregulation of signaling pathways. Funding: The reported study was founded by RFBR, project number 20-34-90041.

P-08.1-30
DNaseI based approach for enhanced CAR-T action against solid cancers

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Adoptive immunotherapy by chimeric antigen receptor-modified T cells (CAR-T cells) is a superior approach for oncohematological malignancies, but not so effective against solid tumors. Recent studies revealed a pivotal role of the tumor microenvironment (TME) in impairing immune cells' (ICs) cytotoxic activity under solid cancer development. Some host ICs, including neutrophils, are a part of TME. Last investigations demonstrate direct influence of the neutrophil extracellular traps (NETs) on cancer development and metastasis. Neutrophil DNA fibers – major NETs component – obstacle cytotoxic host ICs to kill tumor cells. NETs are released during netosis – a specific neutrophil death – as an answer to tumor stimuli. Previous studies demonstrated efficacy of the deoxyribonuclease I (DNaseI) for NETs destroying. Administration of recombinant DNaseI provides significant supression of tumor metastasis and extend animals lifespan. In our research we combine adoptive immunotherapy approach together with hyperactive mutant DNaseI (mDNaseI). We compared three different ways of DNaseI administration: i.v., adeno-associated viruses-based vectors, and secretion by CAR-T cells. For this purpose, firstly, we constructed a bicistronic lentiviral vector including mDNaseI and chimeric antigen receptor against human epidermal growth factor receptor 2 (HER2-CAR) genes and transduced reporter Jurkat T-cells. DNaseI activity assay and flow cytometry analysis confirmed that generated mDNaseI HER2-CAR-T cells produce active mDNaseI and have functional HER2-CAR. Such CAR-T cells provide destruction of NETs by the enzymatic activity of mDNaseI and show antitumor activity against $HER2^+$ tumor cell lines. This study was supported by the Ministry of Education and Science of the Russian Federation project №. 075-15-2020-773.

P-08.1-31
Establishment and *in vitro* characterization of murine model of HPV-positive oral squamous cell carcinoma

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Head and neck squamous cell carcinomas (HNSCC) are one of the most common cancer types worldwide. It has been shown that infection with certain strains of human papilloma virus (HPV) correlates with the development and differential radiosensitivity of oropharyngeal HNSCC. HPV-positive mouse models of HNSCC that are necessary for in vivo evaluation of adaptive immune responses in immunocompetent mice are still scarce. Therefore, our study aims at establishing and characterizing a murine HPV-positive HNSCC cell line. Due to the lack of oropharyngeal HNSCC mouse models, we used MOC1 murine oral squamous cell carcinoma (OSCC) cell line and transduced it with LXSN16E6E7 retrovirus, encoding for the HPV type 16 E6 and E7 genes under the control of the Moloney murine leukemia virus (MoMuLV) promoter-enhancer sequences, as well as genes, encoding for resistance to G418 antibiotic under the control of SV40 promoter. HPV positive clones were selected with G418 antibiotic selection, and pure clones were established by serially diluting the surviving cells in a 96-well plate. To confirm the expression of HPV E6 and E7 on RNA and protein level, qPCR and western blot will be performed. This way we will obtain a MOC1-HPV cell line, which will be further characterized in vitro and compared to the parental MOC1 cell line in terms of proliferation kinetics and radiosensitivity. Proliferation rate will be analyzed using PrestoBlue™ metabolic cell viability assay. To assess the radiosensitivity, clonogenic assay and direct quantification of the newly synthesized DNA at various time points after irradiation with different doses by determining the incorporation of a thymidine analog 5-ethynyl-2'-deoxyuridine (EdU) will be employed. In conclusion, in the present study we will establish and characterize an HPV-positive murine HNSCC cell line, paving the way for the development of a murine HNSCC mouse model that is crucial for *in vivo* studies in immunocompetent mice.

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P-08.1-32
Binding rate analysis of antibodies interacting with cells captured in microfluidic cell traps

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The binding of antibodies to cell surface antigens is a pivotal step in the cellular immune response. The characterization of association and dissociation rates, affinity, and avidity is essential for the development of efficient immunotherapies. However, despite substantial efforts, straightforward methods to measure binding kinetics directly on cells are still not available, which leaves a "biophysical gap" in the research and development process of biological drugs, that hampers progress in immuno-oncology. Here, we present a novel method to capture isolated cells and to measure the association and dissociation kinetics of fluorescently labeled antibodies to/from cell surface antigens in real-time. To this end, flow-permeable, mesh-like cell cages were designed to accommodate and physically retain single cells in the microfluidic channel of a commercially available biochip. Suspension or adherent cells can be loaded into the cages by an automated workflow using only a few microliters of sample and are subsequently exposed to binders under continuous flow. We validated the method by investigating a number of different antibody clones against various antigens (CD3, CD1d, CD7 and CD305) expressed on the surface of T-lymphocyte cancer cells. Statistical analyses of antibody-cell interaction data are presented, and cell heterogeneity is assessed. Because the cells can be trapped for hours, slow dissociation processes are observable. Hence, we were able to discriminate the engagement of antibodies to one or two antigens (affinity vs. avidity) on the cell surface and ultimately correlate avidity with the antigen expression level. The established method enables the investigation of antibody-cell interactions under conditions that are close to in-vivo situations in a highly automated workflow and thus will be useful for the screening and characterization of new biological drugs that intervene in the cellular immune response for the treatment of cancer.

P-08.1-33
KIR-expressing NK cells expand *in vitro* more intensively than KIR-negative NK cells

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An ability of natural killer (NK) cells to exhibit cytotoxic activity against tumors and virus-infected cells makes them useful in immunotherapy. Expression of inhibitory killer-cell Ig-like receptors (KIR) is required for NK cells to obtain functional activity. HCMV is associated with a high content of $NKG2C^+$ cells and the formation of adaptive NK cell populations. In this work, we have examined a relationship of NK cell differentiation stage and NKG2C expression level with proliferative activity of KIR^+ cells during expansion *in vitro*. NK cells were isolated by negative magnetic separation from peripheral blood mononuclear cells of healthy individuals. HCMV serological status of the individuals was determined. Cell phenotype analysis was performed using flow cytometry. NK cell subsets that differ by differentiation stage and NKG2C expression level were sorted by 1 cell (cloned) or 100 cells into 96-well plates and stimulated with IL-2 and K562-mbIL21 feeder cells. NK cell cultures started from 100 $KIR2DL2/3^+$ cells, especially NK cell cultures originated from $KIR2DL2/3^+NKG2C^+$ cells, showed generally a higher expansion rates compared to KIR^- NK cell cultures. $KIR2DL2/3^+$ cells found in the fraction of less differentiated $CD56^{bright}$ NK cells showed expansion rate two times higher compared to other subsets during first 2 weeks of culturing. Further culturing revealed a higher expansion ability of the both $CD56^{bright}$ and $CD56^{dim}$ $KIR2DL2/3^+NKG2C^+$ NK cell subsets. NK cells at lower differentiation stages showed higher cloning efficiency and the cloning efficiency of NK cells from HCMV-seronegative donor was two times lower compared to HCMV-seropositive donors. Thus, the stimulation with IL-2/K562-mbIL21 *in vitro* led for preferable expansion of KIR^+ cells. Knowledge of the proliferative response of different NK cell subsets is important for the use of NK cells in cancer immunotherapy. This work was supported by Russian Science Foundation, grant #19-15-00439.

P-08.1-34
Gene editing and expression in hard-to-manipulate primary tumour cells by CRISPR/Cas9 and mRNA technology

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Ex vivo manipulation of patient-derived cancer cells represents a unique opportunity to understand biology of the disease. Gene editing and expression in non-dividing primary cells has been proved a difficult task, limiting the application of high-throughput screenings. Primary cells can rarely be efficiently manipulated by conventional methods based on plasmid/siRNA transfection or viral transduction. Typically, they should also be pre-stimulated, which may introduce unwanted bias in result interpretation. In our work, we set out to optimize screening protocols for minimally manipulated cancer cells from patients with B-cell chronic lymphocytic leukaemia (CLL). CLL cells are non-dividing and prone to undergo apoptosis when cultured ex vivo. Herein, by optimizing pulsing strategy of electroporation, we set up protocols for the introduction of mRNA and Cas9/RNA ribonucleoprotein complexes into CLL cells. If set up correctly, this procedure can preserve >90% cell viability and lead to the uptake of exogenous material by >90% cells. We show, on one hand, that mRNA electroporation allows to achieve persistent gene overexpression at a desired level. Combined with highthoughput in vitro production of mRNA, it allows to scale up eventual screening assays. Interestingly, the use of modified nucleotides for mRNA production does not always brings in benefits, suggesting cell-type specific requirements. On the other hand, we observe that CRISPR/Cas9 gene editing is predominantly heterozygous when electroporated cells are left without stimulation but gives >70% homozygous editing when primary cells are induced to proliferate. Overall, our results describe a workflow to elaborate robust protocols for gene editing and expression in primary tumour cells. Tailored cell-type protocols allow to manipulate even fragile non-dividing cells, thus promoting the study of gene function *ex vivo*.

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P-08.1-35
Retroviral modification of NK-cells with iCasp9 gene for their directed elimination

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The ability of NK cells to provide antitumor immunity, due to nonspecific recognition mechanisms, makes them useful for cancer therapy. The application of stimulated or genetically modified cells with enhanced functions may end up to unpredictable adverse effects. Thus, the safety switch of therapeutic cells is needed. A promising approach to the selective control of adaptively transferred cells is a retroviral transduction of gene construction made on the basis of caspase 9 for a rapid elimination of modified cells by CID (chemical inductor of dimerization). Hence, the aim was to optimize NK-cell modification by retroviral particles containing the iCasp9 gene for the directed cell death induction. NK cells were isolated from peripheral blood of healthy donors and stimulated for more efficient retroviral transduction with IL-2 and irradiated K562-mbIL21 cells expressing membrane-bound IL-21. Viral particles were collected in Phoenix Ampho cells transfected with pMSCV-F-del Casp9.IRES.GFP and RD114 plasmids. The transcriptional activity of the iCasp9 gene in transduced NK cells was checked by RT-PCR. The concentration of functional transduction units in the supernatant was estimated for more efficient NK-cell modification. Then, iCasp9-Raji and iCasp9-NK-cells were compared in their ability to trigger apoptosis determined by staining with fluorescently labeled Annexin V and Sytox AAD after incubation with CID. In the concentration range from 10 to 200 nM CID did not affect the viability of unmodified NK cells and induced apoptosis of iCasp9-NK cells. A concentration of 10 nM CID was sufficient to trigger the apoptosis of both iCasp9-modified Raji and NK cells. The rate of apoptosis induction also grew with an increase of CID concentration in the medium. iCasp9-Raji cells with a higher level of reporter protein fluorescence were more capable to initiate apoptosis than cells with lower MFI. The work was supported by the RSF grant: № 20-75-00129.

P-08.1-36
A new method for simultaneous clonal profiling of B- and T-cells infiltrating the tumor microenvironment

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T and B cells infiltrating the tumor are an important factors of tumor progression. Operating cooperatively, they modulate the antitumor immune response and determine treatment outcome and prognosis. T and B-cell receptor (TCR and BCR) profiling is a powerful approach to analyze clonal features of B and T cells. The most reliable method for TCR and BCR profiling is DNA-based multiplex PCR with subsequent high-throughput sequencing. However, the existing protocols allow profiling TCR and BCR only separately. It significantly limits the power of analysis. Here we overcome this limitation by presenting the first multiplex PCR-based method for profiling all immune receptor genes including TRA, TRB, TRD, TRG, IGH, IGK, IGL at the DNA level in the single assay. 7 DNA (tumor and paired normal) samples from patients with colorectal cancer were used for this study. Target enrichment for TCR+BCR genes was obtained in single multiplex PCR with primers for each V and J segment of all TCR and BCR genes. Each library was obtained in two replicates using 150 ng (~25000 genomes) input DNA and sequenced on NextSeq platforms. TCR and BCR extractions were performed with MiXCR. Post-analysis was performed using VDJtools. In total 28 TCR+BCR libraries (7 paired samples, two replicates) were obtained. Correlation of replicates showed sufficient reproducibility of the method ($R = 0.9797$, R-square = 0.8197). The sensitivity tested on serial dilutions of DNA from healthy donor blood showed a capacity of the method to profile TCR+BCR in low input samples (up to 30 lymphocytes). At the same time, the method allowed to detect 1250 clonotypes average in DNA from 25000 cells. Obtained results show sufficient sensitivity and reproducibility of the developed method. In combination with the capacity for simultaneous detection of TCR and BCR gene rearrangements in makes this method promising tool in the field of tumor-infiltrating lymphocytes analysis. This work was supported by RSF grant 20-75-10091.

Cancer initiation and progression

P-08.2-01
Evaluation of anticancer efficacy of temozolomide and resveratrol in human glioma cells

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Cancer is one of the leading causes of human death. In vitro studies are very important to understand the molecular mechanisms of tumor formation in cancer research. Besides this, glioblastoma is the most common and aggressive primary malignant brain tumor. These types of tumors are highly resistant to chemotherapy and the average life expectancy is not more than 14 months. The alkylating agent temozolomide (TMZ) is a chemotherapy drug to treat glioblastoma. Although it is widely used in patients with brain cancer, its therapeutic effect is very limited due to its resistance to glioblastoma. Resveratrol (RSV), a natural polyphenolic molecule, has anticarcinogenic potential and it is an efficacious compound for cancer prevention and treatment. Thus, RSV could enhance the sensitivity of glioblastoma cells to TMZ therapy. Heat shock proteins (Hsps) have been found to be elevated in many cancer types and they have been shown to be a biomarker. Their overexpression in brain tumors has been associated with cell proliferation, apoptosis inhibition and chemoresistance. Hsps have become significant

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anticancer targets and the development of Hsp inhibitors is important for cancer treatment. In this study, we aimed to investigate the molecular mechanisms of TMZ and RSV in U87 MG human glioma cells and we carried out in vitro experiments to evaluate the combination of these compounds. The results show that the combined therapy of RSV with TMZ suppressed Hsps and induced apoptosis in U87 MG cells. This combination exhibited an increase in DNA breaks, protein carbonyl content and intracellular reactive oxygen species. Conversely, there was no this kind of significant results in noncancerous HEK 293 human embryonic kidney cells. In conclusion, these findings indicate that this combined therapy is a promising candidate for cancer therapy and provides a viable strategy to achieve better therapeutic efficacy by avoiding possible toxicity and side effects to noncancerous cells.

P-08.2-02
Suppression of tumor progression by arginase and NO-synthase combined inhibition in 7,12- dimethylbenz(a)anthracene-induced breast cancer in rats

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Arginine is involved in different parts of tumour metabolism. High expression of arginase and NO-synthase has been found in different types of cancers like cervical, breast and central nervous system. Several observations support the preclinical evaluation of synthetic analogues of L-arginine nor-NOHA (NG-hydroxy-nor-Arginine) and L-NAME (NG-nitro-L-arginine methyl ester hydrochloride), for the treatment of different types of cancer *in vitro*. We have studied the new in vivo experimental treatment model (4 mg/kg/day nor-NOHA + 40 mg/kg/day L-NAME, administered for 8 weeks, after tumours development, every 4th day) effect on breast cancer progression induced by 7,12-DMBA. A total of 38 female Wistar rats weighing 80–120 g were randomly divided into 5 groups (Control, Saline, DMBA (10 rats), nor-NOHA+L-NAME control, DMBA+nor-NOHA+L-NAME), 7 rats per groups. A single dose injection of 20 mg/ml DMBA was given subcutaneously (2nd pair of right breasts), dissolved in 0.5ml olive oil and 0.5ml saline. Nor-NOHA and L-NAME were injected intraperitoneally in 0.25ml saline. The effect of nor-NOHA and L-NAME on tumours were determined at 13th, 16th and 24th weeks after DMBA administration. At the end of the 225 days, rats in all groups were killed under anesthesia. All tumours were fixed in 10% buffered formalin for histopathological examination. Results have shown polyamines and NO quantities downstream by nor-NOHA and L-NAME, attenuated tumor growth, numbers, cancer progression and mortality rate. Histopathological alteration in DMBA group rats has revealed the ductal papillary carcinoma (DPC, DCIS) by numerous intraductal proliferations, papillary (invasive) carcinoma and carcinosarcoma (bad prognosis). In DMBA+nor-NOHA+L-NAME group the histopathological examination has revealed precancerous lesions (good prognosis) and DCIS. Increasing knowledge of the interplay between arginase and NOS, suggests potential combination therapies that will have considerable clinical promise.

P-08.2-03
The effects of metabolic drugs on tumor behaviour in oral squamous cell carcinoma

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Oral squamous cavity cancer (OSCC), highly aggressive malignant tumor, is the sixth leading cancer and current treatment strategies for this cancer include surgical resection, radiotherapy, and/or platinum-based chemotherapy. Because of its highly aggressive biology, it is crucial to search for innovative therapeutic strategies to reposition FDA-approved drugs, and thus to develop treatment options with lower costs and fewer side effects for OSCC patients. Metformin is an anti-diabetic agent that inhibits the complex-1 of mitochondria. Dichloroacetate (DCA), used for the treatment of lactic acidosis, is a small inhibitor of pyruvate dehydrogenase kinase. In this context, Metformin and DCA have the potential to be used in cancer treatment because of their effects on tumor metabolism. The aim of this study is to determine the effects of Metformin, DCA and their combination (Met+DCA) on cell viability, cell proliferation and cell death in oral squamous cell carcinoma (UPCI-SCC-131 cell line) under normoxia/hypoxia. The IC50 values of Metformin and DCA were determined with WST-1 cell viability assay. Colony formation assay was used to assess the long term effects of these drugs on cell viability. Cell proliferation was analyzed using the BrdU assay. Cell death and cell cycle assays were examined with flow cytometry. Metformin and DCA reduced UPCI-SCC-131 cell viability and cell proliferation. Consistent with our results flow cytometry results showed that apoptosis was increased in Metformin and DCA treated cells. As regards tumor behavior, Metformin and Met+DCA decreased 3D spheroid formation in UPCI-SCC-131 cells. In conclusion, there is an urgent need for novel agents that increase the chemosensitivity, enable the use of lower doses and have a synergistic effect. Metformin and DCA may take a step forward as anti-tumorigenic agents in the treatment of patients with oral cavity cancer. This study was supported by a grant (no. 118S576) from TUBITAK.

P-08.2-04
New antitumor unsymmetrical bisacridines derivatives affect c-myc and K-Ras level leading to cell death and accelerated senescence in human lung and colon cancer cells

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Unsymmetrical bisacridines (UAs) are new antitumor derivatives patented in Europe (EP 3070078 B1) and USA (US 10202349 B2). In their structure they consist previously synthesized in our Department drugs: C-1311 and C-1748. Importantly, UAs exhibit different properties than their monomer components. They do not intercalate to dsDNA, but interacts with quadruplex DNA. G-quadruplex structures are present in promoter regions of oncogenes, such as: MYC, KIT, RAS genes, BCL2, VEGF and in

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telomeric repeats. The aim of the studies was to evaluate whether UAs can influence the expression and protein level of c-myc and K-Ras in living cells and what is the consequence for the cellular effects of UAs treatment. The action of four UAs: C-2028, C-2041, C-2045 and C-2053 were examined in human lung H460 and colon HCT116 cancer cells. All compounds exhibited high cytotoxicity against tumor cells and the IC_{90} dose ranged from 0.04 to 0.4 μM and were similar for both cell lines. In H460 cells all studied drugs at IC_{90} concentration decreased expression and completely inhibited protein level of c-myc since 72 h of incubation and slightly increased K-Ras. In HCT116 cells UAs did not cause remarkable difference in expression and protein level of c-myc, only weak increase of K-Ras. H460 and HCT116 cell exposed to UAs underwent apoptosis what was confirmed by changes in nucleus morphology, cytometric analysis of cell cycle, active caspase-3 and mitochondrial transmembrane potential. Importantly, the apoptosis was induced by UAs earlier and to a greater extent in H460 (especially in cells exposed to C-2045 and C-2053) compared to HCT116 cells (except C-2041). Furthermore, accelerated senescence was induced by UAs only in H460 cells. Concluding, strong c-myc inhibition by UAs in H460 cells seems to contribute to induction of apoptosis and accelerated senescence in these cells. These studies were supported by the National Science Center, Poland, No. UMO-2016/23/B/NZ7/03324.

P-08.2-05
Involvement of P2X7 receptor in glioma cell growth and spreading

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The glioma tumor microenvironment is characterized by an abundant amount of ATP, released from stressed or dying cells as a result of chemotherapy or radiotherapy. A high concentration of extracellular ATP leads to the stimulation of P2X7 nucleotide receptor which acts as an non-selective ion channel. P2X7 regulates many crucial functions like inflammasome formation, ATP release, immune response and cell death in somatic and cancer cells. Role of P2X7 in glioma tumors, receptor is however still poorly characterized and requires a more detailed study. The microarray analysis data demonstrate that P2X7 is upregulated in human low grade gliomas. Interestingly, P2X7 expression was significantly lower in IV grade glioma samples compare to low grade gliomas and healthy brain tissue. Additionally, P2X7 receptor expression is significantly lower in glioma cell lines compared to primary astrocytes in vitro. P2X7 activation did not induce cell death in studied glioma cells. On the contrary, stimulated glioma cells proliferation and adhesion to ECM components. The convergent results were obtained after P2X7 inhibition in glioma xenograft tumors in vivo. Administration of selective P2X7 inhibitor, brilliant blue G (BBG), leads to significant tumor mass reduction and decreases tumor cells spreading. Namely, we observed a decrease in matrix metalloproteinase 2 activity in BBG treated tumor mass. We observed also a decrease of β-catenin expression and total reduction of N-cadherin and vimentin levels in treated tumors compared to control tumors. Summarizing, our results suggest that activation of P2X7 receptor via extracellular ATP can be engaged in shaping glioma tumor microenvironment and promotion of glioma tumor growth. Acknowledgements: This work has been supported by National Science Centre research grant no. 2015/17/B/NZ3/03771

P-08.2-06
Challenges in translating miRNA into clinics

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Prostate cancer (PCa) represents a most diagnosed neoplasm among men, with incidence rates steadily rising. There is a need for more sophisticated biomarkers for diagnosis, prognosis and management. MicroRNAs (miRNAs) in liquid biopsies and tissue emerged as potential biomarkers for PCa that could help better distinguish aggressive vs. non-aggressive tumors, and malignant vs. non-malignant conditions. However, conflicting data was reported for certain miRNAs and published papers often lack certain information which is necessary to contextualize the results. With growing body of evidence, questions are being raised regarding diverse (pre)analytical factors influencing miRNA analysis and hinder translation of miRNA into clinical practice. In order to analyze and address current problems in miRNA clinical research on PCa, a PubMed-based literature search was conducted with the last update in May 2019. Diverse (pre)analytical factors influencing miRNA analysis were studied and compared across the studies. By examining published papers, we conclude that studies widely differ in study design parameters: control groups, serum and plasma comparison, sample storage, quality control steps, data normalization. There is an immense lack of data on methodology framing parameters used in published studies. There is a lack of consistency and reproducibility, largely due to a missing consensus on preferred sample collection, sample handling, RNA extraction and miRNA analysis. Other aspects such as different control groups and sample storage also influence variable results obtained in miRNA research. Of outmost importance seems to be reaching a consensus on guidelines and widely accepted protocols for sample processing and quality control. After identifying critical variations in designs and protocols that undermined clear-cut evidence acquisition, we propose specific guidelines for critical steps that should be considered in future research of miRNA as biomarkers especially in PCa.

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P-08.2-07
Role of cancer stem cell genes in Temozolomide resistance in GBM

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GBM (Glioblastoma Multiforme) is the most aggressive brain tumor with poor prognosis and short patient survival (12–15 months). The standard treatment of GBM includes TMZ, a DNA alkylating agent, concomitant with surgical resection and/or irradiation. The emergence of resistance to Temozolomide (TMZ) poses a major clinical challenge to treat this deadly disease. Understanding the mechanisms of TMZ resistance might help to identify novel drug targets and more effective therapies. In this study, we investigated the potential biomarker genes related to Temozolomide resistance using PCR arrays in GBM. To examine the alteration in the expression of genes, we treated U118 GBM cells with IC50 dose of TZM (175.1 ± 22.56 μM) for 48 hours and then performed Cancer Stem Cell, Adhesion, Integrin, Apoptosis, Cell Surface and Cytokine/Chemokine PCR arrays (Bio-Rad). According to our results, the expression levels of most of the genes involved in apoptosis pathway were expectedly increased after TMZ treatment such as Bax and BIRC3, whereas expression levels of adhesion genes and integrins were decreased. There was a significant elevation in the expression of cancer stem cells which are thought to be responsible for chemoresistance including CD24 and CD38. The list of the genes whose expression are significantly changed with TMZ treatment will be presented on the poster. This research has been supported by The Scientific and Technological Research Council of Turkey (No:114S189).

P-08.2-08
Targeting various death pathway by resveratrol and docetaxel induces synergistic cytotoxicity of prostate carcinoma cells

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The current study was performed to investigate the efficacy of resveratrol in combination with anticancer drug docetaxel in inducing cell death on prostate carcinoma LNCaP cells, including factors related to the detailed cell death mechanisms. Using 2D monolayer and 3D spheroid culture systems, the effects of resveratrol and docetaxel on cell viability, ROS levels, mitochondrial function, apoptosis and necroptosis were examined by MTT, flow cytometry, and Western blotting assay. At concentrations not toxic to normal prostate epithelial HPrEC cells, resveratrol significantly inhibited the proliferation of LNCaP cells in a dose- and time-dependent manner. The combination treatment of resveratrol and docetaxel exhibited synergistic inhibitory effects on cell growth, demonstrated by an increase in sub-G0/G1 peak, annexin V-PE positive cells, ROS levels, loss of mitochondrial membrane potential, and DNA damage as well as upregulation of mediators for apoptosis and necroptosis, including Bax, cleaved caspase-3, cleaved PARP, p-RIPK3, and p-MLKL proteins. In conclusion, we report resveratrol as an adjunct drug to improve the outcome of treatment in docetaxel therapy. Although the underlying mechanisms of necroptosis in cancer therapy should be investigated comprehensively, simultaneously targeting apoptosis and necroptosis in cancer might provide very promising opportunities for new drug development.

P-08.2-09
Lower protein levels of ABCG2 and OSTb and up-regulation of SLCO1B3 expression in endometrial cancer

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Endometrial cancer (EC) is an estrogen-dependent disease. Locally, estrogens can be formed from steroid precursor estrone-sulfate (E1-S) after cellular uptake by organic anion-transporting polypeptides (OATP) or organic anion transporters (OAT). Efflux of E1-S is enabled by ATP Binding Cassette transporters (ABC) and organic solute transporter (OST) αβ. Currently, 19 proteins are known to transport E1-S but their roles in EC have not yet been examined. We analyzed the levels of E1-S transporters in model EC cell lines, Ishikawa and HEC-1-A, control cell line HIEEC, and in paired samples of EC and adjacent control tissue, examined E1-S uptake and metabolism and assessed association between transporters and histopathological and clinical data. In cell lines and tissue samples 15 genes were differentially expressed where the highest difference in expression was seen for SLCO1B3 (28930.7-fold up-regulation) and ABCG2 (30.2-fold down-regulation) in HEC-1-A compared to HIEEC. Imunocytochemistry revealed significantly higher levels of OATP1B3 (SLCO1B3) (6.3-fold) in HEC-1-A compared to Ishikawa and low concentrations of ABCG2 in both EC cell lines. Additionally, increased E1-S metabolism and different E1-S transport profiles were observed in HEC-1-A versus Ishikawa. In EC tissue significant changes were seen for ABCG2 and SLC51B which were significantly (3.2-fold and 2.1-fold, respectively) down-regulated and ABCC1 which was significantly (1.6-fold) up-regulated in EC tissue compared to adjacent control tissue. In patients without lymphovascular invasion gene SLCO1B3 was 15.6-fold up-regulated. Tumor grade had significant effects on expression of SLC51B, with lower levels seen in high grade cancers. Immunohistochemistry revealed significantly lower levels of ABCG2, OSTβ (SLC51B) and OATP1B3 (SLCO1B3) in EC compared to control tissue. Our results suggest that OATP, OAT and ABC transporters have important roles in pathophysiology of EC.

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P-08.2-10
Inhibitory effects of the human amniotic membrane on the adhesion, migration, and invasion of human bladder cancer urothelial cells

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Bladder cancer is a complex, heterogeneous disease that is characterized by high morbidity and mortality rates. Therefore, there is an impending need for the development of novel anticancer agents that would focus on preventing its progression and recurrence. The human amniotic membrane (hAM) is the innermost layer of the placenta, which has been shown to display several beneficial properties, such as low immunogenicity, anti-inflammatory, antifibrotic, antimicrobial and anticancer activity. In the present study, we evaluated the anticancer effect of hAM homogenate and extract on the adhesion, growth, migration, and invasion of cancer urothelial cells. We observed gradual detachment of urothelial cancer cells from the culture plate surface after the treatment with hAM preparations, suggesting that hAM may impair the cell attachment. Furthermore, we showed that hAM homogenate preparations significantly decreased the migration and invasion of cancer urothelial cells. Taken together, the present findings demonstrated that hAM preparations exerted a plethora of anticancer effects against urothelial cancer cells, which further strengthens the idea of potential clinical application of hAM in the treatment of bladder cancer.

P-08.2-11
Selective targeting of glioblastoma stem cells with camelid α–FREM2 nanobody

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Cancers are leading cause of death worldwide, and brain cancers account for only 1.35% of diagnosed cases. Despite its rare incidence of 3.19 cases per 100,000 people in the USA, glioblastoma is considered the most aggressive and lethal brain malignancy. With current standard of care patients succumb to the disease in less than 2 years after diagnosis. In our previous research (Vidak M. et al., 2018), we identified two genes, FRAS1 related extracellular matrix 2 (FREM2) and sprouty RTK signaling antagonist 1 (SPRY1), with differential expression in glioblastoma stem cells. Using various proteomic and transcriptomic approaches, we further examined the relationship between FREM2 and SPRY1 and glioblastoma (Jovčevska I. et al, 2019). First, we analyzed expression of FREM2 and SPRY1 genes and their corresponding proteins in glioblastoma patient samples. We then expanded our study with in silico analysis of large datasets available from The Cancer Genome Atlas database. Our analysis showed a correlation between high FREM2 gene expression and prolonged survival of patients with IDH-wt glioblastomas. Then, using an existing nanobody library, we developed an α–FREM2 nanobody. We propose a model for the binding of this nanobody to its antigen, and also show specific staining on the surface of glioblastoma cells using this nanobody. We also examined potential cytotoxicity and inhibitory effect of the α–FREM2 nanobody on different glioblastoma and reference cell lines. The nanobody showed cytotoxic effect on glioblastoma stem cells, while there was no change in the growth of the reference cell lines. Based on our findings, we propose the selected α–FREM2 nanobody as a molecule with selective speficity towards glioblastoma stem cells. At last, we reason FREM2 plays a role in gliomagenesis and its function should be more thoroughly investigated.

P-08.2-12
PLK2 and ATM single nucleotide variants are associated with clinical-histopathological features of gastric cancer patients

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Gastric cancer remains the fourth most common cause of cancer-related death globally. Cell cycle kinases are crucial for cell cycle progression and correct cell division. Variations in these genes lead to genetic instability and aneuploidy, which in turn contribute to carcinogenesis. The aim of our study was to investigate the association between selected single nucleotide variants (SNVs) in candidate cell cycle genes CDC20, PLK2, PLK3 and ATM and gastric cancer risk. A group of 620 gastric adenocarcinoma patients and healthy controls was included in the study. SNV genotyping was performed using TaqMan allelic discrimination assays and the results were analysed using generalized linear regression. We found significant association between the PLK2 rs963615 (c.-150C>T) and the absence of vascular invasion. Carriers of heterozygote genotype had significantly lower odds for vascular invasion, compared to carriers of homozygote genotypes (OR = 0.39, 95% CI = 0.19–0.83, P = 0.013). We also found significant associations between ATM rs228589 (c.37+19T>A), rs189037 (c.-111G>A), rs4585 (c.*3393G>T) and perineural invasion. Interestingly, carriers of at least one polymorphic allele of either SNV had significantly reduced probability for perineural invasion (OR = 0.39, 95% CI = 0.16–0.95, P = 0.034). In silico functional analysis of polymorphic sites revealed differential transcription factor and miRNA binding motifs associated with specific allele: PLK2 rs963615 (T: TBP), ATM rs228589 (A: YY1, PEA3, T: GR-α, STAT4, c-Ets-1, Elk-1), rs189037 (G: E2F-1) and rs4585 (T: XBP-1, TFIID, miR-2964a-5p). In conclusion, the study revealed significant associations between PLK2 rs228589, ATM rs228589, rs189037 as well as rs4585 and clinical-histopathological features of gastric cancer patients. These SNVs could be used in clinical setting as an aid in prognosis and possibly in determination of the most suitable treatment options for patients.

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P-08.2-13
Setting up novel type of glioblastoma organoid model that recapitulates microenvironmental conditions *in vivo*

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Glioblastoma remains the most lethal of brain cancers, which holds a median survival of approximately 15 months with the standard therapy of maximal surgical resection followed by temozolomide chemotherapy and radiation. A major obstacle for effective treatment is the extensive heterogeneity at the genetic and cellular level, associated with differences in the tumour microenvironment. As tumour organoids have been developed for various types of cancers, we believe that in vitro glioblastoma organoid model is becoming a major technological breakthrough in research and drug testing. Organoid recapitulates the complexity of its microenvironment, multiple stromal and tumour cell interactions, including the immune cells. In the present study we established organoid model from fresh patient tumour samples obtained at surgical intervention that preserve the histological features, gene expression and cellular complexity, by the method of Jacob F et al. (2020) Cell 180, Issue1, P188-204. To characterize cellular existence, we performed immunohistochemical analyses for the markers specific for astrocytes (GFAP), glioblastoma stem cells (CD133, SOX2, CD9), mesenchymal stem cells (CD105), immune cells (CD68, CD56, CD16) and other potential markers. Based on gene expression patterns, glioblastoma is classified into three subtypes: proneural, mesenchymal, and classical that differ significantly regarding patients' survival rate and tumour chemo- and radio-resistance. Clinical observations clearly show that tumours of the same histopathological diagnoses often respond different to a defined therapy. For that reason, we are specifying if glioblastoma tissue subtypes correlate with organoids' subtypes from the same tissue. These organoids are used as a platform to uncover the mechanisms underlying the tumour response to the standard therapy.

P-08.2-14
Chemokine CCL5 and its receptor CCR5 as potential therapeutic targets in glioblastoma

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The chemokine CCL5 has a role in inflammatory diseases and in cancer progression. CCL5 interacts mostly, but not exclusively with the receptor CCR5, promoting intra- and inter-cellular interactions of tumor cells within the tumor microenvironment. In this study we explored CCL5/CCR5 as possible therapeutic targets in treatment of glioblastoma (GB). We analysed CCL5/CCR5 mRNA and protein expression in glioma tissues, GB cells and GB stem cells (GSCs), derived from patients. Higher mRNA expression of CCL5/CCR5 was detected in recurrent GBs as compared to primary GBs and less-malignant low-grade gliomas. Low grade gliomas rely on stromal chemokine stimulation, whereas high grade GB, establish an autocrine chemokine stimulation, becoming independent of stroma, leading to higher tumor malignancy and lower survival rate of GB patients. CCL5 and CCR5 proteins were heterogeneously expressed among GB tissues and GB cells, present in some samples, but absent in others. Interestingly, only CCR5 was detected in GSCs. Both were expressed in stromal cells, such as mesenchymal stem cells (MSCs) and macrophages.There are four genetically defined GB subtypes: the proneural (PN), mesenchymal (MES), neural (N), and classical (CL), which differ significantly in survival rate, being the shortest in MES subtype. We have found Cl subtype exhibited the highest levels of both CCL5 and CCR5 expressions, while MES expressed the lowest. We demonstrated the invasion of patients-derived GB cells was highly dependent on CCL5/CCR5 signaling, as it was strongly inhibited by the synthetic CCR5 antagonist maraviroc. The invasion of GB cells when co-cultured with MSCs was also inhibited by maraviroc, suggesting the release of CCR5 antagonists, e.g. CCL5 from MSCs upon co-culturing. In conclusion, as GB and stromal cells in the tumor microenvironment induce expression of CCR5 and CCL5, functionally governing invasion, the CCR5/CCL5 axis may be a potential therapeutic target for this deadly disease.

P-08.2-15
Identification of the phosphatase for S6K2 may sensitize breast cancer cells to apoptosis

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Recent studies have shown that in both ER-positive and triple negative breast cancer cells, S6K2 was displayed to promote cancer cell survival. Depletion (S6K2) and restoration (Akt) studies uncovered that S6K2, unlike S6K1, exerted its pro-survival effect by positive feedback regulation of Akt. Knockdown of S6K2 sensitized breast cancer cells to apoptotic stimuli such as TNF and TRAIL [1,2]. Therefore, unraveling deactivation mechanisms of S6K2 may render breast cancer cells susceptible to apoptotic stimuli. After the discovery of S6K2, earlier studies had discovered the activation mechanisms of S6K2. Both PI3K/Akt/mTOR and Raf/MEK/ERK pathways are involved in the activation of S6K2 by unmasking a repressor domain in S6K2 by phosphorylation. Crosstalk between these pathways play an important role in S6K2 activation [3]. On the other hand, the mechanisms that attenuates S6K2 signaling are currently unknown. One such mechanism to be uncovered is the phosphatase which dephosphorylates S6K2 to inactivate it and stop signaling. Also, the mechanism from which S6K2 escapes inactivation and sustains its pro-survival effect in breast cancer remains to be elucidated. We aim to search for the phosphatase that dephosphorylates S6K2 by epitope-tagged immunoprecipitation of S6K2 followed by mass spectrometry. Based on the mass spectrometry results, we will perform depletion and overexpression studies in order to identify the phosphatase for S6K2. References: 1. Sridharan, S., Basu, A.

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P-08.2-16
Prospects for studying the E-cadherin level in tumors of patients having ovarian cancer of III-IV stages

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After the neoadjuvant polychemotherapy (NPChT) it becomes possible to make an operation to delete the original tumor in patients having ovarian cancer (OC) at the late (III-IV) stages. Complex of biochemical, clinical and ultrasound criteria [Knyazyeva M., Prokopyuk A., 2014; 2015] helps to find optimal number of NPChT courses for every patient and increase effectiveness of treatment. However, despite the effectiveness of the influence of NPChT on the treatment of late stage OC, the threat of relapse of the OC due to the formation of tumor stem cells (TSC) and the threat of epithelial-mesenchymal transformation (EMT) remains in the patient's body. Prevention of EMT can be a promising approach to the development of new methods of treating patients having OC of late stages, in which the determination of E-cadherin level in tumors can play an important role. The results of an immunohistochemical study of molecular markers of the tumor process bcl-2, cytokeratin-7, E-cadherin on paraffin sections in 12 tumor samples from patients with stage III-IV cancer with serous carcinomas and 15 patient samples after NPChT (3 or more courses) showed the probability of a poor prognosis further course of ovarian cancer in 37% of patients. Tumors of these patients were characterized by increased expression of bcl-2 and decreased expression of cytokeratin-7 and E-cadherin. In accordance with the theory of TSC, one of the factors of EMT, cell de-differentiation, and the transition of cells to the mesenchymal phenotype is the breaking of intercellular bonds with a decrease in the expression of E-cadherin. Such cells have an aggressive malignant potential that affects survival. Therefore, the prevention of the loss of E-cadherin may be a promising approach to the development of new methods for treating late stages of OC.

P-08.2-17
Determining the pathogenicity of variant in the SDHD gene in vagal paragangliomas using *in silico* modeling

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Vagal paragangliomas (VPGLs) are rare neuroendocrine tumors that amount to 13% of all head and neck paragangliomas. These tumors can occur both as sporadic and hereditary forms. Genetics and molecular mechanisms of the tumor initiation and progression are still unclear. Base on exome sequencing data of a set of VPGLs derived from the Russian patients, we found a germline variant in the SDHD gene with highly pathogenic potential, NM_003002: c.A305G, p.H102R (chr11: 111959726, rs104894302). We performed molecular modeling of the mutation to assess its effect on the protein structure and function. We constructed the structure of the SDH apoenzyme using homology modeling with the Modeller program. Porcine SDH structure (PDB ID:1ZOY) was used as a template. Human reference sequence and target amino acid sequence were aligned on the PDB 3D model to create corresponding SDH homologous structures. The CHARMM-GUI package was applied for the building of a full atomic system consisting of the membrane and integrated protein that is required for state-of-the-art biomolecular simulation. Data analysis was performed in Python with the NumPy package. Matplotlib and PyMol programs were used for data visualization. Molecular modeling experiments were carried out using the equipment of the shared research facilities of HPC computing resources at M.V. Lomonosov Moscow State University supported by the project RFMEFI62117X0011.We analyzed the structure of the mutant SDHD subunit and compared with those of wild-type protein. We revealed that the change histidine to arginine in this position leading to the destabilization of the system. This change can affect protein folding resulting in unstable SDH complex. Thus, the studied variant in the SDHD gene significantly affects the protein structure that can alter the stability of succinate dehydrogenase and disrupt its function. This work was financially supported by the Russian Science Foundation, grant no. 19-15-00419.

P-08.2-18
Myosin 1C isoform A as a novel diagnostic marker of prostate cancer

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Heterogeneity of morphological features, molecular pathogenesis and clinical aspects put the obstacles in diagnostics of prostate cancer (PCa) and implies the need in the novel diagnostic markers together with less invasive diagnostic assays. Isoform A of myosin 1C was previously described as specific marker of prostate cancer cell lines (Previously published in: Saidova AA et al.

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(2018) PeerJ 20; 6:e5970). We evaluated the expression of isoform A myosin 1C on the clinical dataset of 29 prostate cancer (PCa) and 8 benign prostate hyperplasia (BPH) specimens and performed multicolor flow cytometry analysis of surface phenotype for cells with high and low isoform A expression. Median normalized expression of isoform A in PCa was three orders of magnitude higher than in BPH specimens. Upregulation of isoform A was also demonstrated on protein level. The difference in isoform A expression was statistically significant between BPH and all PCa samples (Kruskal-Wallis, $P = 0.0002$). All BPH samples demonstrated a universal staining pattern. Basic phenotype of BPH cells was CD133-/CD57-/CD90- with a small CD44+/CD24- subpopulation and CD24+ subpopulation with or without partial CD44 co-expression. Compared to BPH, PCa specimens had reduced percentages of CD24+ cells ($P = 0.029$) and/or CD44+ cells. The extended phenotype of PCa specimens was CD10-/CD13-/CD38+/CD166- with the heterogeneous expression CD54, CD38 and CD29. Correlation analysis revealed strong positive correlation between isoform A of percentage of CD54 (ICAM-1)-positive cells in BPH samples (Spearman correlation R = 0.83) and percentage of CD29 (integrin beta 1)-positive cells in PCa samples with most aggressive clinical stage 3 (R = 1). We demonstrate the utility of isoform A myosin 1C evaluation for the diagnostics of PCa. Our data also indicate that cells with low and high isoform A expression have different surface adhesion patterns. This work was supported by RFBR grant # 17-54-33009.

P-08.2-19
Study of the role human NEIL3 DNA glycosylase in the repair of cisplatin DNA damage in HeLa S3 cells

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Chemo- and radiotherapies induce complex DNA damage (CDD): bulky DNA adducts, interstrand DNA crosslinks (ICLs) and clustered lesions (including double-strand breaks, DSB) that have complex character/structure as compared to singular lesions (Deans AJ et al. (2011) Nat Rev Cancer 11, 467–480). Importantly, acquired cross-resistance to different DNA cross-linking agents of tumor cells after exposure to just one agent suggest that cells up-regulate an alternative DNA repair mechanism to counteract ICLs in DNA (Panasci L et al. (2002) Anticancer Drugs 13, 211–220). Study of the molecular mechanisms of DNA damage recognition by the Endonuclease VIII (Nei)-like 3 (Neil3) DNA glycosylase will provide insight into the mechanisms of cellular DNA damage signaling and repair coordination. The project aims to address whether new non-elucidated repair activities of Neil3 DNA glycosylase are present in cancer cells. Here we developed plasmid constructs with Neil3 and its truncated variants (Neil3 C-terminal, Neil3 catalytic and Neil3 N-terminal domains). For this, we generated pOZ-FH-C by sub-cloning the coding sequence of the protein of interest (Neil3, Neil3 C-term, Neil3 N-term) at NotI and XhoI sites, resulting in the fusion of C-terminus of Neil3 proteins with two FLAG and HA epitopes. The pOZ-FH-C-Neil3 vectors were transfected into packaging cell line (Phoenix cells) to generate transduction-competent retroviruses. HeLa S3 cells were infected with the constructed retroviruses and the replication-deficient ecotropic retroviruses (Nakatani Y et al. (2003) Methods Enzymol 370: 430–444). This approach allowed us to establish HeLa S3 cell line overexpressing Flap/HA epitope tagged Neil3 protein and its truncated variants. Generated HeLa S3 cell lines expressing Flag-HA-tagged DNA repair proteins can be used to reveal the new protein-protein interactions and posttranslational modifications of Neil3 and other DNA repair proteins in cancer cells.

P-08.2-20
Prox1 inhibits c-Myc dependent gene regulatory network and metabolic pathway to suppress the proliferation of breast cancer cells

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Breast cancer is one of the most lethal malignancies in women worldwide. Despite the progress in tumor biology and provided therapies, breast cancer is characterized by rapid growth and low survival rates. These observations underscore the need for novel insights in the molecular mechanisms of malignant transformation and progression. To this end, it was previously reported that Prox1, a homeobox transcription regulator, is dramatically reduced in breast tumors due to epigenetic silencing. Moreover, our meta-analysis of the expression data from Oncomine (www.oncomine.org) and TCGA (The Cancer Genome Atlas, www.cancergenome.nih.gov) confirmed this correlation. These clinical data render Prox1 as a candidate gene with a critical role in suppressing malignant transformation in breast cancer. Here, we provide evidence that Prox1 strongly suppresses the proliferation and migration of human breast cancer cell lines in a non-apoptotic way. Interestingly, Prox1 induces a gene expression program characterized by down-regulation of c-Myc and c-Myc-regulated genes (including glycolysis gatekeeper PDK1 and GLUT-1) mediating the establishment of glycolytic switch. These data suggest that Prox1 may orchestrate the gene regulatory network for the establishment of Warburg effect in breast cancer cells. To further study this hypothesis, we will perform metabolic assays in breast cancer cells. We are also going to investigate the ability of Prox1 to suppress tumor growth in vivo by performing xenograft assays in SCID mice. In summary, our study suggests Prox1 as a tumor suppressor gene via regulation of metabolism and potential therapeutic target in breast cancer. *The authors marked with an asterisk equally contributed to the work.

P-08.2-21
NR5A2 as a novel tumor suppressor in nervous system malignancies

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Nervous system malignancies are characterized by rapid progression and poor survival rate. Glioblastoma multiforme is the most aggressive nervous system malignacy and despite recent advances in the provided therapy the average survival time remains low,

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between 12 to 15 months. These clinical observations underscore the need for novel therapeutic insights and pharmacological targets. Towards this direction, here we identify the orphan nuclear receptor NR5A2/LRH1 as a negative regulator of cancer cell proliferation and promising pharmacological target for nervous system-related tumors. In particular, by meta-analysing clinical data from TCGA and Oncomine databases, we find that high expression levels of NR5A2 are associated with favourable prognosis in patients with glioblastoma tumors. Here, we experimentally show that NR5A2 is sufficient to strongly suppress proliferation of both human and mouse glioblastoma (U87-MG, GL261) and neuroblastoma cells (SH-SY5Y, Neuro2A) without affecting apoptosis. The anti-proliferative effect of NR5A2 is mediated by the transcriptional induction of negative regulators of cell cycle, CDKN1B (p27kip1), CDKN1A (p21cip1) and Prox1. In contrast, down-regulation of NR5A2 induces proliferation and suppresses the previous mentioned genes. Interestingly, two well-established pharmacological agonists of NR5A2, DLPC and DUPC, are able to mimic the anti-proliferative action of NR5A2 in human glioblastoma cells via the induction of the same critical genes, encoding for p27kip1, p21cip1 and Prox1. Most importantly, treatment with DLPC inhibits glioblastoma tumor growth in vivo in a xenograft mouse model. These data indicate a tumor suppressor role of NR5A2 in nervous system and render this nuclear receptor a potential pharmacological target for the treatment of nervous tissue related tumors. *The authors marked with an asterisk equally contributed to the work.

P-08.2-22
Polyoxotungstates inhibit aquaporin-3 and impair melanoma cell migration

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The biological significance of polyoxometalates (POMs) is notorious owing to their anticancer properties. Several studies have described aquaporins (AQPs), in particular the AQP3 isoform, to be aberrantly expressed in different human cancer cells and tissues, frequently correlated with tumor stage and aggressiveness. In this study, we report for the first time the ability of polyoxotungstates (POTs) of Wells-Dawson P_2W_{12} and P_2W_{18} structures to affect AQP3 function and delay melanoma cells migration. The tested POTs showed to inhibit AQP3 activity in a different impact, being P_2W_{12}, P_2W_{18} and P_5W_{30} the most potent (IC_{50} = 0.8; 2.8 and 3.2 μM) whereas P_2W_{15} is the weakest (IC_{50} > 100 μM). AQP3-selective effect of P_2W_{18} was confirmed in a yeast heterologous expression system. The effect of P_2W_{12} and P_2W_{18} on melanoma cells, confirmed to highly express AQP3, revealed impaired cell migration ≈ 60 % after 24 h, suggesting that the anticancer properties of these POMs may be, at least in part, due to the blockage of AQP3. Overall, our data indicates that P_2W_{12} and P_2W_{18} are inhibitors of AQP3 function and cancer cell migration, unveiling their promising value as anticancer therapeutics against AQP3-overexpressed tumors. *The authors marked with an asterisk equally contributed to the work.

P-08.2-23
Potential leukemogenic role of dysregulated electron transport chain complex II activity in myeloid cells over-expressing GATA-1S, the short isoform of the transcription factor GATA-1

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GATA-1 is a key regulator of hematopoiesis-related genes. A balanced expression of its two isoforms, the full-length GATA-1_{FL} and the shorter variant GATA-1_S, contributes to control normal hematopoiesis whereas their dysregulation alters the differentiation/proliferation potential of hematopoietic precursors with aberrant expression of GATA-1_S being defined as a poor prognostic factor in myeloid leukemias. Recently, we reported that GATA-1_S over-expression correlates with high levels of the succinate dehydrogenase subunit C (SDHC) (Riccio et al, 2019 J Cell Physiol). Two alternative splicing variants (ASVs) of the SDHC transcript, Δ3 and Δ5 ASVs lacking succinate-CoQ redox activity and heme binding region, respectively, are known to act as potent dominant-negative inhibitors of the full-length isoform (Satoh et al, 2015 Oncol Lett). This prompted us to investigate the levels of these isoforms in myeloid leukemia K562 cells over-expressing either GATA-1_{FL} or GATA-1_S. Interestingly, over-expression of both Δ3 and Δ5 ASVs accompained by decreased SDH complex II activity and increased mitochondrial levels of $O_2^{\cdot-}$, a byproduct of impaired complex II activity, was found only in GATA-1_S cells. Mitochondrial metabolism was then analyzed with the Seahorse XF Cell system (Agilent) revealing increased rates of proton leak, basal respiration and ATP production in GATA-1_S cells as compared to GATA-1_{FL} cells, thus further supporting results on aberrant SDHC ASVs levels, SDH activity, and $O_2^{\cdot-}$ generation triggered by GATA-1_S. Our study sheds new light on the role played by GATA-1_S in regulating SDHC expression and complex II activity in leukemia cells. Also, given the tumor suppressor role played by SDH and the relevant effects of ROS and oxidative phosphorylation in promoting leukemogenesis (Trombetti et al, 2021 Int J Mol Sci), a better understanding of these mechanisms can eventually contribute to identify novel promising therapeutic targets in myeloid leukemias. *The authors marked with an asterisk equally contributed to the work.

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P-08.2-24
Impact of ABCA1 activity on plasma membrane lateral organization and motility processes in melanoma cells.

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Melanoma arises from an accumulation of genetic mutations in melanocytes and is considered as the most aggressive and the deadliest form of skin malignancy. It has been demonstrated that cholesterol content in melanoma cells is higher than in normal cells, and this accumulation of cholesterol is directed explicitly into the lipid rafts. It suggests therefore a direct link between tumor development, the plasma membrane (PM) cholesterol level and the PM lateral organization. ABCA1, a member of the ATP binding cassette (ABC) transporters family, is essential for cellular cholesterol redistribution and efflux, and controls the lateral organization of the PM of mammalian cells. We have evidence that in several human melanoma cell lines the ABCA1 expression is high and negatively correlates with the cellular cholesterol content. Here, by using a combination of biophysical techniques: the spot variation Fluorescence Correlation Spectroscopy (svFCS) and the Fluorescent Lifetime Imaging Microscopy (FLIM) to monitor PM lateral order, we show that high ABCA1 level in melanoma cells affected the distribution of cholesterol and lipids within the PM. That was reversed by ABCA1 knockout via CRISPR-Cas9 system and also by using an ABCA1-specific inhibitor, probucol. Moreover, we show that melanoma cells without active ABCA1 had impaired migration machinery, degraded extracellular matrix to a smaller extent, and were less invasive. We therefore propose that ABCA1 activity could alter the PM lateral organization in melanoma cells thus leading to changes in processes crucial for metastasis. This work is supported by research grant No. 2019/35/N/NZ3/00633 and grant No. 2016/21/B/NZ3/00343 from the Polish National Science Center (NCN).

P-08.2-25
Molecular effects of multiple irradiation on normal mouse brain tissue *ex vivo* and *in vivo*

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Adjuvant radiochemotherapy is a conventional strategy for glioblastoma treatment. The efficacy of the treatment remains poor with most patients experiencing the disease recurrence in 6–9 months. Both chemotherapy and ionizing radiation affect not only tumour cells but also the surrounding normal brain tissue. It is known that temozolomide used for glioblastoma chemotherapy significantly changes the proteoglycans (PGs) composition of the normal brain extracellular matrix (ECM) *in vivo*, which can influence tumour cells proliferation and invasion. Here, we investigate the effects of radiotherapy on the normal brain ECM in organotypic culture ex vivo and experimental animals *in vivo*. Brain of 2-month-old C57BL/6 mice was irradiated 3 times in a dose of 7 Gy/day, and expression of main PGs was determined using real-time RT-PCR and immunohistochemical analysis. Multiple irradiation did not affect histological structure of normal mouse brain tissue but resulted in significant changes in PGs expression. In cerebral cortex expression of brevican, Cd44 and decorin was upregulated (2-fold, 4-fold and 4-fold respectively, $P < 0.05$) compared with control animals while in subcortical structures expression of decorin and brevican was downregulated (2-fold and 3-fold respectively, $P < 0.05$). To study if irradiation of the normal brain can affect the tumour growth, the *ex vivo* hippocampal organotypic slices were co-cultured with U87 glioblastoma cells. U87 cells demonstrated increased proliferation and invasion into the irradiated brain slices compared with control. Taken together obtained results demonstrate deterioration of PGs composition of normal brain ECM under the ionizing radiation which potentially can promote tumour cells proliferation and glioblastoma relapse. This work has been supported by Russian Foundation for Basic Research (RFBR grant 18-29-01036). Tsidulko A.Y. was supported by scholarship of Russian Federation President for young scientists (SP-5435.2018.4). *The authors marked with an asterisk equally contributed to the work.

P-08.2-26
Effects of switching to the tobacco heating system (THS) compared with continued smoking

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Cigarette smoke (CS) is causally linked to the development of cardiovascular diseases (CVD). Tobacco harm reduction, by virtue of substituting cigarettes with less harmful products, is a complementary approach to current strategies for smokers who would otherwise continue to smoke. The Tobacco Heating System (THS) 2.2 is a novel tobacco product that heats tobacco instead of burning it, never allowing the temperature to exceed 350°C, thereby preventing the combustion process from occurring and producing substantially lower levels of toxicants than CS. Our assessment program aims to demonstrate that switching to THS has the potential to reduce the risk of smoking-related diseases compared with continued smoking. The assessment program includes *in vitro*/*in vivo* toxicology testing methods that follow OECD guidelines, Good Laboratory Practice, and a systems toxicology approach and randomized, controlled clinical studies that follow the principles of Good Clinical Practice. The effects of THS aerosol on the adhesion of monocytic cells to human coronary endothelial cells in vitro are significantly reduced. Switching to THS halted the progression of CS-induced atherosclerotic changes in ApoE-/- mice *in vivo*. Biomarkers linked to the development of smoking-related diseases were analyzed following a 6-month randomized, controlled clinical study with THS, which demonstrated a consistent improvement of biomarkers in different pathophysiologic pathways leading to atherosclerosis. The evidence available to date indicates that switching to THS has the potential to reduce the risk of smoking-related diseases such as CVDs

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P-08.2-27
Effects of unsymmetrical bisacridines on the spherical culture system of HCT116 colon and H460 lung cancer cell lines

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3D-spheroid cultures are receiving increasing attention in cancer research, as they serve as a link between simplified 2D monolayer culture structure and the highly complex nature of tumors *in vivo*. Therefore, spherical cultures are a good model for the effective testing of anticancer drugs. Unsymmetrical bisacridines (UAs) are new antitumor compounds, patented in Europe and in the USA. Here, two cancer cell lines: colon HCT116 and lung H460, selected depending on their high sensitivity to UAs, were tested for their ability to form spheroids. Furthermore, the effects of four UAs: C-2028, C-2041, C-2045, C-2053 on the morphology, size and viability of HCT116 and H460 spheroids were investigated. We showed that both studied cell lines formed spheroids with different morphometric features. The HCT116-spheres were more condensed and had smooth, even periphery. The H460-spheres were bigger and presented more irregular shape. Treatment of 3D-spheroid cultures with UAs resulted in significant inhibition of their growth, however to a much lesser extent by C-2041 than others. Cell viability in spheroids exposed to UAs for 7 days was evaluated using the 7-AAD dye. Both HCT116- and H460-spheres presented significant fraction of non-viable cells reaching even 98% of the cellular population. The percentage of 7-AAD$^+$ cells in 2D cultures after 72-hour incubation with UAs reached 29 to 54% in HCT116 cells and 78 to 93% in H460 cells. Differences in the ratio of live/dead cells in 2D and 3D cultures may result from distinct penetration of UAs through spherical cultures than in monolayer. Some compounds require prolonged exposure times to observe an effect on cell viability in vitro. Therefore, 3D-spheroid cultures appear to better reflect the in vivo tumor growth than adherent model and are a good tool for evaluation of antitumor properties of our promising bisacridines compounds. These studies were supported by the National Science Center, Poland, No. UMO-2016/23/B/NZ7/03324.

P-08.2-28
The role of EpCAM's ectodomain cis-oligomerization in RIP initiation

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Regulated intramembrane proteolysis (RIP) is a key step in Epithelial cell adhesion molecule (EpCAM) mediated signaling that results in cancer progression. The process is catalyzed by a cascade of sheddase cleavages that result in release of intracellular domain (EpIC). EpIC interacts with proteins of ß-catenin/Wnt-signaling pathway to facilitate mitogen expression and cell cycle propagation. Because EpCAM is overexpressed in several carcinomas, detailed structural characterization of RIP cleavages would help us better understand the mechanisms of cancer progression and metastasis. The first cleavage in RIP is executed by A Disintegrin And Metaloprotease protein 17 (ADAM17, also known as TACE). Cleavage sites are located at the interface of two subunits in a cis-dimer, suggesting that the dimer must at least temporarily dissociate for cleavage to take place. However, we have recently shown that EpCAM predominately exists in the cis-dimeric form, both *in vitro* and *in vivo*. The dimerization is also relatively strong – the estimated dissociation constant for soluble ectodomains is in nanomolar range. To get a better understanding on the mechanism of TACE's cleavage in relation to EpCAM's oligomeric state we designed monomeric EpCAM ectodomains and disulfide-stabilized dimeric ectodomains that are unable to dissociate. Our results show that monomeric EpCAM is indeed more prone to TACE-mediated cleavage both in vitro and in vivo and provide the basis for further structural and functional characterization of TACE mediated EpCAM cleavage.

P-08.2-29
The interplay between Pirh2, HuR and c-Myc in lung cancer cells

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Lung cancer ranks first in terms of mortality from malignant tumors among men and women in both developed and developing countries. Despite decades of research aimed at understanding the exact tumor transformation mechanisms, the study of signaling pathways initiating the cancer progression and potential approaches for their targeted regulation remains extremely relevant especially for lung cancer. This study is focused on RING-finger E3 ubiquitin ligase Pirh2 that targets p53 and other proteins playing roles in cell cycle regulation, DNA-damage response and tumor transformation. Moreover Pirh2 expression was shown to be increased in 84% human lung neoplasm specimens. We suggested that Pirh2 can make a significant contribution to cancer-associated signaling. Despite the frequent mention of this protein in the context of p53 ubiquitination and degradation, other targets and functions of this ligase are not well investigated. In order to fill this gap we used p53-negative human non-small cell lung cancer cell line H1299 to study p53-independent Pirh2 functions. First, we found that Pirh2 acts as an oncogene in p53-negative non-small cell lung cancer cells H1299. Thus, Pirh2 overexpression leads to increased proliferation, migration and resistance to genotoxic stress, while Pirh2 knockdown caused the opposite effect. We also found that Pirh2 upregulates c-Myc expression both in mRNA and protein levels which may explain the oncogenic role of this protein in lung cancer cells. To reveal the mechanism of Pirh2-mediated c-Myc regulation we performed Pirh2 interactome identification. As a result, we revealed more than 200 previously unknown Pirh2-binding partners. Among them RNA-binding protein HuR which is known to be a negative regulator of c-Myc expression was identified. We demonstrate that Pirh2 ubiquitinates HuR and directs it to proteasome degradation, which in turn leads to a change in the expression of HuR-dependent genes. Supported by RSF Project No19-75-10059.

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P-08.2-30
Increased NF-kappaB level affects proliferation of lung cancer cells H1299

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The evolutionary conserved transcription factor NF-kappaB is constantly activated in many types of cancer, which leads to expression of various target genes and, as a result, to malignant transformation, abnormal cell proliferation, metastasis and chemoresistance of tumor cells. Several studies have shown the predictive value of its expression level for outcomes in patients with non-small cell lung cancer (NSCLC). To model constitutive activation of NF-kappaB in NSCLC cells, we created two stable H1299 cell lines with increased levels of the major activating NF-kappaB subunit RelA. Expression of the RELA gene in H1299/RelA cells was increased by about 2.5 and 5 times, respectively, compared to the control cell line. Increased expression of the NF-kappaB target genes IKBA and ICAM1 demonstrated functional activity of the exogenous RelA. We found that cell proliferation rate and the ability to form colonies substantially decreased acording to the RelA level increase. However, no differences in cell cycle, apoptosis rate, or autophagy level were observed between the cell lines. At the same time, evaluation of the EdU intercalation showed that the elevated RelA increases the percentage of EdU-positive cells, which might suggest enhanced DNA replication rate. Additionally, positive staining for the mitotic marker phospho(Ser10) histone H3 was found to correlate directly with constant RelA activation. These data indicate that increased RelaA level promotes cell replication and division, despite the decreased cell proliferation rate we observe. Further immunofluorescence analysis showed the lower beta-tubulin signal as well as the decreased number of correctly organized metaphases and anaphases in H1299/RelA cells. Our ongoing studies should clarify whether RelA activation may affect assembly of the microtubules and mitotic spindle. This work was supported by the grant from the Russian Government Program 14.W03.31.0029.

P-08.2-31
Stuctural insight into evolutionary divergence of tumor markers EpCAM and Trop2

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Trop2 is a cell-surface transmembrane glycoprotein involved in cell proliferation-enhancing signaling in normal and carcinoma cells. This 36 kDa protein and its paralogue EpCAM (GA733 protein family) are recognized as important carcinoma markers. Both are subject of regulated proteolysis of their large extracellular (EX) and transmembrane regions resulting in the release of mitogen transcription-activating cytosolic tail. The crystal structure of EpEX solved in our group gave valuable insights at the structure-function level of EpCAM, however due to heterogeneous glycosylation Trop2EX resisted crystallization and structure determination making detailed comparison of these evolutionarily related molecules impossible. Here we report the crystal structure of Trop2EX and structural comparison with EpEX, the analogous region of EpCAM. By selectively switching off two of the four N-glycosylation sites we prepared a soluble and more homogenously glycosylated protein sample that yielded crystals diffracting to a resolution of 2.8 Å. As expected from the 50% sequence identity the overall structure of Trop2EX much resembles that of EpEX. Both molecules crystallized as a cis-dimer which most likely represents a functionally relevant oligomeric state. However, there are considerable differences. First, the membrane-distal small N-terminal domain with a unique disulfide-rich core adopts a significantly different relative orientation with regard to the other part of the molecule. This small domain is the most immunogenic and is in both EpCAM and Trop2 targeted by many monoclonal antibodies. Next, the contacts between the subunits as well as their relative orientations differ. Trop2 dimer is tighter in the sense that the membrane-distal cleft is narrower. The structural differences highlight the divergent evolutionary path of the two genes/proteins leading to similar yet different functional aspects and pave the way to their specific structure-based utilization in medicine.

P-08.2-32
The brain glioma patients lymphocytes excrete polyamine oxidase after phytohemagglutinin stimulation

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Tumour-associated inflammation (TAI) promotes growth and progression of malignant brain gliomas, but the mechanisms of these processes are still unclear. Peripheral blood mononuclear cells (PBMC) play an important role in TAI. The polyamines (PA) – putrescine, spermidine and spermine – are essential for cell proliferation. The enzymes of PA catabolism – diamine oxidase (DAO) and polyamine oxidase (PAO) – break down PA and produce substances arresting cell division which makes them important factors involved in the regulation of the cell proliferative function during inflammation. Transmembrane potential (TP) is considered to contribute to the regulation of proliferation. The degree of cell aggregation and erythrocyte aggregation in particular may serve an indirect indicator of the TP change. We performed mitogenic stimulation of PBMC from glioma patients with phytohemagglutinin (PHA) and explored the DAO and PAO activities and the red blood cells (RBC) aggregation. The blood samples were taken from patients with various grade brain malignancy and from healthy donors as a control group. Patients with spinal hernia was included into the study as a comparison group with normal (not tumor-associated) inflammation. Cells were cultured according to standard technique. To evaluate the degree of RBC aggregation, the cellular fraction of heparinized venous blood was used to determine the shift in the minimum of the surface plasmon resonance (SPR) curve in degrees, representing the grade of blood cells aggregation. We found that the DAO and PAO activities were increased in cultural liquid after

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PHA stimulation of lymphocytes in all groups except the group of spinal hernias. The PHA concentration-dependent decrease in proliferative activity of PMBC was mainly associated with decrease of SPR indices. The dynamics of the mentioned characteristics differed in various groups of patients. The study was supported by the "RUDN University Strategic Academic Leadership Program".

P-08.2-33
Urinary profiles of free amino acids and metal (loid)s in testicular cancer

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Testicular germ cell tumours (TGCT) are the most common malignancy in men aged 15–35 years and its incidence is increasing worldwide. Tumour markers commonly used in the diagnosis and prognosis of TGCT have limited sensitivity and specificity and additional, minimally invasive biomarkers for disease management are needed. Metabolomic-based profiling of amino acids in urine may identify changed metabolites as potential biomarkers of testicular cancer. Impaired metal(loid) homeostasis may increase susceptibility to cancer development. We compared a quantitative profile of 30 amino acids and 18 metal(loid)s in urine samples between 86 men with newly diagnosed TGCT and 68 control subjects, aged 18 to 55 years. The concentration of free amino acids was determined by GC-MS using Phenomenex EZ:faast kit for sample preparation, multi-element analysis was performed by ICP-MS, and all results were corrected for creatinine levels. Testicular cancer patients had significantly higher levels of aspartic acid (Asp), manganese (Mn) and zinc (Zn), and significantly lower levels of threonine (Thr), serine (Ser), histidine (His) and cobalt (Co) when compared to control subjects. Linear discriminant analysis using all 30 amino acids successfully separated subjects with TGCT from control subjects and the discrimination rate was 86%. Metabolic pathway analysis (MetaboAnalyst 4.0) showed that Asp, Thr, Ser, and His were related to alterations in 12 metabolic pathways and those with the highest impact were metabolism of alanine, aspartate and glutamate (0.26) and glycine, Thr and Ser (0.23). Our results indicated that disorders in the citric acid cycle, the urea cycle and amino acid metabolism play a role in the pathogenesis of testicular cancer. The impaired urinary profile of Mn, Co and Zn may be highly relevant with regard to their role in amino acids synthesis, antioxidant defense and genetic repair processes. More research is needed to verify our findings. *The authors marked with an asterisk equally contributed to the work.

P-08.2-34
The investigation of mechanisms of secondary leukemia through etoposide exposed mesenchymal stromal cells

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Topoisomerase 2 inhibitors are generally used for treatment of solid cancers and leukemia. High dose and long term use of these agents are associated with development of secondary leukemia. Stromal cells have an important role in supporting hematopoiesis and are involved in leukemia pathogenesis. Previous studies indicated that lack of stromal factors which are associated with leukemia pathogenesis and hematopoietic stem cell (HSCs) maintenance, expressed from mesenchymal stem cells (MSCs) causes AML like changes on hematopoietic stem cells and these pathological alterations may participate in development secondary leukemia. Here we investigate the role of etoposide on bone marrow derived MSCs and drug was sequentially applied four days and four hours with different concentrations. DNA double strand break and proliferation response was assessed for drug effectiveness. Selected genes (Dicer, Cxcl12, Cyclin B1, Cyp1a1, Klf4, TGFb1) expression analyze were performed. Etoposide mediated gene expression alterations from Donors (n = 3) were compared with MSCs from Fanconi anemia patients (n = 2) that is known as a disease associated with AML predisposition. As a result, gene expression analyses showed that while Dicer and Klf4 expression correlates with increasing concentrations of etoposide, TGF-beta level did not alter between all groups. Healthy and Fanconi stromal cells show sharply decreasing of Cyclin B1 and Cxcl12 expressions. AhR pathway marker gene of Cyp1a1 shows different expression alterations between donors and fanconi patients. These results show that etoposide negatively regulates stem cell proliferation and secretory dynamics of HSCs maintenance factors. Differential expression of Cyp1a1 levels after etoposide treatment between fanconi and donors shows the variability of initial cellular status of stromal cells. These preliminary results suggest that etoposide exposure of stromal cells may have an important role in secondary leukemia pathogenesis. *The authors marked with an asterisk equally contributed to the work.

P-08.2-35
Caveolin-1-containing extracellular vesicles transport adhesion proteins which promote the development of malignant traits in recipient cells

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Breast cancer is the leading cause of cancer-related deaths in women. Caveolin-1(CAV1) is upregulated in cancer and promotes migration

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and invasion of breast cancer cells, raising the question as to how CAV1 contributes to this behavior. High levels of CAV1 are found in exosomes from cancer patients. Interestingly, a recent report from our lab shows that CAV1-containing extracellular vesicles (EVs) promote malignancy in breast cancer cell lines. Moreover, such EVs transport a subset of adhesion proteins, including Tenascin (TNC), which are not present in exosomes released from CAV1-depleted cells. Thus, an intriguing possibility is that CAV1, along with adhesion proteins like TNC, may be responsible for the enhanced malignancy tumorigenesis of recipient cells. EVs were purified with ExospinTM columns from MDA-MB-231 wild-type (WT), MDA-MB-231(shCAV1) (possessing plasmid pLKO.1 encoding a "short hairpin" against CAV1), MDA-MB-231(shTNC) (possessing plasmid pLVX encoding a "short hairpin" against TNC) and MDA-MB-231 (shControl) breast cancer cells. Particle size was determined by electron microscopy (TEM) and Nanoparticle Tracking Analysis (NTA). TNC, CAV1 and exosome markers were evaluated by Western blotting. MDA-MB-231 breast cancer cells were used to evaluate migration and transendothelial migration stimulated by CAV1- or TNC-depleted exosomes. TEM and NTA revealed exosomes of the expected size. Migration and transendothelial migration of recipient breast cancer cells increased only when stimulated with WT exosomes, but not following incubation with TNC- or CAV1-depleted EVs. Taken together, these results support the notion that CAV1 may play an active role in determining the cargo protein composition of exosomes and that such differences have functional consequences in the recipient cells. Acknowledgements: FONDECYT 1170925, 1210644 and CONICYT-FONDAP 15130011 (AFGQ), FONDECYT 1200836 (LL), Fondecyt 11150624 (MV-G)

P-08.2-36
Design and synthesis of new structural class of Kv1.3 inhibitors

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Several studies showed that the voltage gated potassium Kv1.3 channels from Shaker family have an up-regulated expression in breast, colon and lymph node cancer, in leiomyosarcoma (smooth muscle cancer), alveolar rhabdomyosarcoma (skeletal muscle cancer), chronic lymphocytic leukemia (mature neoplastic B cells) and in acute T cell leukemia. To induce the mitochondrial pathway of apoptosis, mitochondria have to express voltage gated potassium channels Kv1.3 and the necessary step is to inhibit them. The whole process is usually characterized by the combination of increased production of ROS, depolarization of the inner mitochondrial membrane and cytochrome c release. (1) Currently, three different models are proposed to explain the involvement of Kv1.3 in regulation of cell proliferation. (2) TVS compounds were identified as hits from a virtual screening, where 3D similarity searching methodology was used based on the potent Kv1.3 inhibitors from the literature. TVS-06 and TVS-12 were screened against a panel of voltage-dependent channels, TVS-06 was the most selective. TVS-12 showed to be the most potent compound of the two. (3) Our present and future work is based on the synthesis of new selective inhibitors of voltage gated potassium Kv1.3 channels. We synthesize new series of compounds based on the structure of TVS-06. We are going to analyse the SAR of new analogs. With getting results of first generation compounds, we will plan the synthesis of second generation inhibitors and try to improve their selectivity. 1. Teisseyre, A et al: Voltage-Gated Potassium Channel Kv1.3 as a Target in Therapy of Cancer Teisseyre. Front Oncol 2019, 9: 933. 2. Pérez-García, M et al: The secret life of ion channels: Kv1.3 potassium channels and proliferation. Am J Physiol Cell Physiol 2018, 314(1): C27-C42. 3. Hendrickx, L et al: Design and characterization of a novel structural class of Kv1.3 inhibitors submitted to Bioorganic Chemistry

P-08.2-37
The impact of the tumor microenvironment on transcriptome of cancer cells in conventional murine colon and pancreatic cancer models

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A malignant tumor is a heterogeneous evolving system consisting of cancer cells, tumor microenvironment cells and extracellular matrix. In this regard, the properties of the tumor are determined not only by the inherent features of cancer cells, but also arise as a result of their interaction with the tumor microenvironment. Thus, we assumed that the transcriptome of cultured cancer cells undergoes serious changes when they are grafted into mice as a result of interaction with the tumor microenvironment. The purpose of this work was to compare gene expression profile of colon and pancreatic cancer cells grown *in vitro* and same cells isolated from subcutaneous tumor grafts. Murine colon carcinoma CT26 and pancreatic ductal adenocarcinoma Pan02 were stably modified by EGFP gene. Subcutaneous grafted tumors of the same size were excised, digested by collagenase, and then GFP+ cancer cells were isolated using cell sorting. Thus obtained cancer cells "*in vivo*" and same cell line grown *in vitro* were subjected to RNA-seq. When comparing transcriptomes of CT26 cells cultured in vitro and isolated from tumors 172 and 94 genes were identified the expression of which increases and decreases by 4 or more times in the tumor environment. By a similar comparison of Pan02 transcriptome we identified 1038 genes up-regulated in the tumor environment and 461 down-regulated genes. We carried out functional annotation of identified genes using the DAVID resource. Common most significant Gene Ontology terms for genes up-regulated in CT26 and Pan02 tumors were associated with process inflammatory response, cell chemotaxis, cell adhesion. Thus, we compared gene expression profile of colon and pancreatic cancer cells grown in vitro and isolated from tumor. Findings indicate a significant impact of immune system on cancer cells and enhanced cell adhesion in both tumor models. The reported study was funded by RFBR according to the research project No 17-00-00194 (17-00-00190). *The authors marked with an asterisk equally contributed to the work.

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P-08.2-38
The influence of ectonucleotidases expression on ATP-dependent extracellular signalling

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The adenosine triphosphate is well known as an energy storage and signalling molecule. Function of ATP and its metabolites in purinergic signalling is important in many pathophysiological processes. ATP and other nucleotides may stimulate cell proliferation and increase the invasiveness of different tumour cell types [1]. Nucleoside triphosphates secreted from cells are degraded by the nucleotide metabolizing enzymes. Ectonucleotidases are the cellular surface-located enzymes, which hydrolyse extracellular nucleoside tri-, di- and monophosphates or dinucleoside polyphosphates [2]. Belonging to these class of enzymes, NPPs (Nucleotide Pyrophosphatases/ Phosphodiesterases) and NTPDases (nucleoside triphosphate diphosphohydrolases) are responsible for maintenance the proper level of extracellular nucleotides. Degradation of ATP leads to formation of metabolites, which bound to P2 or P1 (ATP and adenosine) receptors to control the cellular responses [3]. In this study, we investigated the expression of known ectoenzymes (NPP1-3 and NTPDases 1–3) on selected cell types and their impact on the cellular response to the extracellular ATP and its more stable analogues, like α-S-ATP and β,γ-methylene-ATP. Particularly the cell life parameters, such as proliferation rate, invasiveness and ability to migrate were analysed. Moreover, nucleotide-stimulating cell response after knockdown of NPP1 will be investigated. Additionally, molecular docking analysis of ATP and their analogues to NPP1-3 enzymes was investigated. Acknowledgment This project was financially supported by the grant 2017/26/D/ST5/01046 from National Science Centre in Poland. References [1] Martinez-Ramirez AS et al. (2018) Purinergic Signal. 13,1-12 [2] Zimmermann H et al. (2012) Purinergic Signal. 8,437–502 [3] Lim HM et al. (2018) Purinergic Signal. 14,157–166

P-08.2-39
Oncogenic BRAF protein as a molecular target of HDAC inhibitors in melanoma cells

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BRAF is a component of the mitogen-activated protein kinase (MAPK) signal transduction pathway and oncogenic V600E mutation affecting BRAF gene has been widely described in melanomas. Considering that pharmacological use of selective BRAF inhibitors, including Vemurafenib, is often limited by the development of resistance mechanisms, the purpose of the present study was to evaluate whether HDAC inhibitors, a well-known class of epigenetic and anti-tumor drugs, could affect BRAF oncogenic signal in melanoma cells. Here, evidence is provided that both SAHA and ITF2357, two pan HDAC inhibitors, reduce the viability of BRAF V600E-mutated SK-Mel 28 and A375 human melanoma cells and remarkably decrease the level of oncogenic BRAF protein. To investigate the involvement of BRAF signalling pathway, the MEKK inhibitor UO126 was used either alone or in combination with HDAC inhibitors. This compound was shown to strongly potentiate the antitumor effects of HDAC inhibitors and to pronounce the reducing effects on BRAF protein levels. Interestingly, we showed that BRAF has a nuclear localization in melanoma cells, which might account for its oncogenic function, and preliminary immunoprecipitation data seem to indicate that BRAF can bind to p53. Treatment with HDAC inhibitors reduced BRAF levels in both the nucleus and the cytoplasm and dramatically decreased p53 levels. These effects were particularly evident in SK-Mel 28 cells where p53 is present in a mutated form with a moderate oncogenic potential. We also found that HDAC inhibitors produce a switch from pro-survival autophagy to caspase-dependent apoptosis in melanoma cells and further studies aim to clarify the relationship between oncogenic BRAF, p53 and the autophagic process in melanoma. Taken together, our results suggest that HDAC inhibitors can target oncogenic BRAF and thus be considered good candidates in melanoma targeted therapy.

P-08.2-40
The adenosine carbocyclic analogue and its impact on human squamous carcinoma cells

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Adenosine is the well known molecule present both, extracellularly and inside the cells and it plays crucial role in cell proper functioning. Numerous data shows, that adenosine level and its interactions with receptors might be relevant in the treatment of various disorders, including psychiatric diseases, inflammation or cancers [1]. The adenosine analogues containing cyclopentane ring structure instead of ribose moiety, were shown to have antiviral and antitumor activity in different cancer cell types [2]. In current work, carbocyclic adenosine analogue was investigated for their activity in squamous carcinoma (A431) cells. The A431 cells was previously shown, to be sensitive for extracellular adenosine-dependent signalling, which makes them potentially susceptible to adenosine analogues treatment [3]. In this type of cancer cells, adenosine concentration occurred to be crucial for the proliferation properties. Furthermore, observed regulation is a result of interaction with A1 or A2 adenosine receptors. The carbocyclic analogue of adenosine was tested for their cytotoxicity and the ability to induce extracellular signalling pathways. Obtained data shown, that tested compound has a huge impact on the cell cycle progression and induce strong toxic effect in human squamous carcinoma cells. Furthermore, it was revealed that it acts through the induction of programmed cell death. Acknowledgment: This project was financially supported by the grant 2017/26/D/ST5/01046 from National Science Centre in Poland. References: [1] Stagg J, Smyth MJ (2010) Oncogene 29, 5346–5358 [2] Chandra G et al. (2015) J Med Chem 58, 5108−5120 [3] Lukasik B, et al. (2020) RSCAdv 10, 31838-31847

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P-08.2-41
HIV-Tat protein promotes B-cell activation via CD38

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Introduction of highly effective antiretroviral therapeutic regimens has greatly advanced HIV management in the recent years. Nevertheless, decreased viraemia does not reduce the risks of B-cell lymphomagenesis. B-cell lymphomas present one of the most common types of malignant neoplasia and a frequent cause of death among HIV-infected patients. Recent studies confirm that HIV-specific proteins in lymphoid tissue can promote B-cell activation and support apoptosis evasion, which, in turn, can provide the clonal advantage of tumour precursor B-cells. To reveal the effect of viral HIV-Tat protein on B-cell activation and anti-apoptotic response the lymphoblastoid B-cell line RPMI8866 and its Tat-expressing derivates were used. The original cell line is characterized by low expression levels of pan-leucocyte and pan-B-cell (B-cell receptor associated) antigens, with surface phenotype CD45low/CD5-/CD19low/CD22+/CD20-/FMC7-, as accessed by flow cytometry. Other B-cell activation molecules are moderately expressed by RPMI8866 cells, thus defining their low/moderate activation status. Tat-protein proves to enhance B-cell activated surface phenotype via the 10-fold upregulation of surface CD38 while other potential signalling molecules (e.g. B-cell receptor components, TNFR9-14 and interleukin receptors ILR2, 7, 17, 21, 27) are unchanged or decreased in the presence of Tat. Addressing this issue at effector level revealed that such BCR-independent activation results in upregulated NFkappaB (IKKalpha/beta, p65, NFkB1/2) as evidenced by RNA and protein expression. Thus CD38 may provide a shunt activation pathway in the absence of BCR, TNF and interleukin receptors with its major upregulated effector being NFkappaB. This deepens our understanding of the mechanisms of HIV-associated B-lymphomagenesis and may help in the search of effective startegies for lymphoma early detection and treatment in HIV-infected patients. The project was funded by RSF grant #18-75-00033. *The authors marked with an asterisk equally contributed to the work.

P-08.2-42
Enhanced DNA damage response through RAD50 in triple negative breast cancer resistant and cancer stem-like cells contributes to chemoresistance

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A growing body of evidence supports the notion that resistance of cancer to current treatments is driven by a small subset of cancer stem cells (CSC), responsible for tumor initiation, growth and metastasis. Some studies have pointed out that both CSC and chemoresistant cancer cells may share some common qualities that make them resistant against chemotherapeutic drugs. We have confirmed in a triple negative breast cancer model that both populations present similar stem characteristics, among them: a higher stem cell marker expression at the mRNA and protein level, a higher capacity to grow in anchorage-independent conditions, a higher colony formation capacity and resistance to chemotherapy. Moreover, through a comparative LC-MS/MS proteomic study we showed that both CSC and chemoresistant cancer cells share several upregulated proteins and pathways in comparison with their parental counterparts. We have identified several shared candidate proteins involved mainly in DNA damage response (DDR) system, ATP-binding cassette and proteasome degradation machinery. We also show that both types of cells display an enhanced and prompt DDR as compared with their corresponding parental counterparts and identified RAD50 as one of the major contributors for the resistant phenotype through the activation of homologous recombination repair pathway. We have also observed a higher expression of RAD50 protein in tumoral biopsies from triple negative breast cancer patients compared with adjacent normal breast tissue and through Kaplan-Meyer curve we show that there is a correlation between higher RAD50 expression and a worse prognosis. Finally, we provided evidence that depletion of RAD50 by siRNA or the blockage of the formation of MRE11-RAD50-NBS1 complex by the pharmacological compound Mirin re-sensitized CSC and chemoresistant TNBC cells to chemotherapeutic drugs.

P-08.2-43
Systematic identification of proliferation-relevant and compartmentalized protein kinase A substrates

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Cellular membrane receptors sense and convert the vast array of extracellular input signals and transmit information through intracellular signaling circuits. Hereby, diverse scaffolding proteins interlink receptors and intracellular effectors to coordinate the spatiotemporal activation of enzymes such as kinases. Deregulation of G protein-coupled receptor (GPCR) controlled kinase pathways contributes to the development and progression of cancer. One example for such are activating mutations in the stimulating Gαs proteins which lead to constitutive downstream activation of the cAMP-dependent protein kinase A (PKA). In order to identify central PKA-effector proteins, we set out to determine the phospho-proteomic composition of macromolecular PKA complexes [Previously published in: Bachmann, et al. (2016) PNAS 113 (28): 7786–7791] from a collection of Gαs mutated cancer cells and human glioblastoma biopsies. Using a substractive phospho-proteomic approach, we identified a multitude of proliferation-relevant PKA substrates. From those, we systematically selected druggable and cancer-implicated candidates. One such protein is the glycolytic enzyme 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase 3 (PFKFB3). PFKFB3 is a key modulator of glycolysis and has recently been implicated in maintaining cancer cell metabolism. We showed that nuclear PFKFB3 is a PKA substrate. The selective and small molecule mediated inhibition of PFKFB3 reduces proliferation of Gαs mutated colon cancer cells. Additionally, we obtained first evidence that PFKFB3 inhibition affects mitochondrial respiration. To further elucidate the correlation between PFKFB3 actity status and cancer cell metabolism, we currently perform mass spectrometry based metabolomic analyses to quantify the impact of PFKFB3 phosphorylation or inhibition on cellular glycolytic flux. *The authors marked with an asterisk equally contributed to the work.

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P-08.2-44
Impact of receptor signalling pathways on GTPase and kinase interactions and conformations

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The Mitogen-Activated-Protein-Kinase (MAPK) pathway is a major regulator of growth-factor induced proliferation. Central players of this pathway are the proteins RAS, RAF, and MEK. These signaling molecules are frequently mutated and constitutively activated in a large variety of cancers, rendering them central targets for pharmaceutical intervention. The search for bioactive small molecule inhibitors for deregulated RAS remains challenging. In contrast, for RAF and MEK several inhibitors found the way into the clinic. Nevertheless, further functional studies on the molecular details of kinase functions are needed to cope with the onset of drug resistance. We recently developed an intramolecular protein conformation reporter system, in order to track conformation changes of both the kinases RAF and MEK (=KinCon) and interactions with RAS [Enzler F et al. (2020) IUBMB life, Mayrhofer J E et al. (2020) PNAS 117(49), Röck R et al. (2019) Sci Adv. 5(8)]. We show that EGF stimulation leads to a transient conformation change of wild type RAS. Moreover, we recently showed that distinct activating mutations in RAS prevent this activation cycle. In addition, we subjected our kinase reporter platform to perturbation studies of wt and mutated MEK-KinCons. Upon drug exposure we tracked dynamic structure states of the MEK1-KinCons. More importantly, our data indicates that the MEK activation state is crucial for MEK:inhibitor binding. We extended this working hypothesis to RAF kinase dynamics. In the presence of permanently active HRASG12V we demonstrated an increase of wt BRAF activation upon BRAF inhibitor treatment. Overall, our data shows that the kinase conformation reporter system, originally established for BRAF, can be extended to the measurement of MEK1 dynamics and MEK1:drug interactions. Finally, we hope to correlate the respective enzyme activities with alternating protein-protein interactions of the full-length MEK1 and RAF proteins.

P-08.2-45
VIM, miR-124-3p and miR-9-5p are overexpressed in small extracellular vesicles of glioblastoma cells

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Glioblastoma (GBM), the most common primary brain tumor, is a complex and extremely aggressive disease. Despite recent advances in molecular biology, there is a lack of biomarkers, which would improve GBM's diagnosis, prognosis, and therapy. One of the promising sources of potential biomarkers are small extracellular vesicles (sEVs). Here, we analyzed by qPCR the expression levels of a set of miRNAs in GBM and lower-grade glioma human tissue samples and performed survival analysis in silico. We then determined the expression of same miRNAs and their selected target mRNAs in sEVs of GBM cells. We showed that the expression of miR-21-5p is significantly increased in GBM tissue compared to lower-grade glioma and reference brain tissue, while miR-124-3p and miR-138-5p are overexpressed in reference brain tissue compared to GBM. We also demonstrated that miR-9-5p and miR-124-3p are overexpressed in sEVs of GB stem cells (NCH421k and NCH644, respectively) compared to sEVs of all other cells. VIM, a target of miR-124-3p and miR-138-5p, is overexpressed in sEVs of mature GBM cells compared to sEVs of GBM stem cells (NCH644 and NCH421k) and also astrocytes. Our results suggest VIM, miR-9-5p and miR-124-3p could serve as biomarkers of sEVs of GBM cells.

P-08.2-46
Investigation of molecular mechanisms defining retroelement activity in colorectal cancer

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Retroelement (RE) transpositional activity is one of important factors of human genome instability. Somatic insertions of L1 and Alu were previously detected in several tumor types including colorectal, lung, breast and prostate cancer. Despite intensive investigation of RE activity regulation, the exact molecular mechanisms of RE activation in particular cancer types are still unclear and were not studied systematically. Here, we explore the correlation between RE insertional activity in colorectal cancer

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and factors that can define RE activity, such as regulatory genes expression, L1 transcription and methylation profiles of transposition competent L1 elements. Sequencing libraries of L1 and Alu flanking regions were prepared using genomic DNA isolated from paired normal and tumor tissue samples. Digested DNA was ligated to oligonucleotide adapters containing unique molecular identifiers (UMI) and amplified with primers, specific to young RE subfamilies (AluYa5, AluYb8 and L1HS). The same DNA samples were used for methylation profiling of transpositionally competent L1 copies promoters. In parallel we also performed whole transcriptome analysis for the same tissue samples to characterise differentially expressed genes responsible for RE repression or activation. As a result, tumor-specific insertions were identified in 23 tumors from 55 analyzed normal/tumor paired samples. In these paired samples we also identified L1HS-copies with demethylated promoters which could be the source of new L1 insertions in tumor cells. We characterized the differential expression of genes that influence RE activity and transcription activity of individual L1 copies. In this work, we conducted the first systematic study of multiple factors that can affect the transpositional activity of RE in colorectal cancer. This work was supported by GACR 19-11299S grant.

P-08.2-47

This poster has been moved a Speed Talk.

P-08.2-48
Role of ubiquitin ligase MDM2 in drug resistance of HER2-positive breast cancer

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Breast cancer is a malignant tumor that develops from the epithelial cells of ducts and lobules of the parenchyma mammary glands. One of the most important molecular markers of breast cancer for selecting further therapy is the amplification of the oncogene HER2. Our bioinformatics data revealed that expression of p53-dependent ubiquitin ligase, MDM2, is associated with poor survival patients with HER2-positive breast cancer subtype. We created cell lines of breast cancer Her2-positive subtype (SCBR3 and BT-474) with different expression status of MDM2. Treatment of doxorubicin cell lines Her2-positive subtype with over-expression of MDM2 increases the resistance compared to the control cell line. However, in the MDA-MB-231 cell line (Her2-negative) an increase in MDM2 expression led to a decrease in doxorubicin resistance. We also showed a decrease in the level of apoptotic cells in cell lines with overexpression of MDM2 after the treatment of doxorubicin. Afatinib and neratinib are two second-generation tyrosine kinase inhibitors. Using the MTT method, we also screened afatinib and neratinib for cytotoxicity in breast cancer lines with different expression states of Her2 and the E3 ligase of MDM2. Our experiments show that over-expression of MDM2 leads to the resistance of Her2-positive cell lines to afatinib and neratinib inhibitors. We assume that MDM2 contributes to the degradation of Her2, which, in turn, leads to the resistance of the cell lines with over-expression of MDM2 to Her2 inhibitors. We conducted an experiment to determine the half-life of Her2 using the inhibitor of elongation translation cycloheximide. Overexpression of MDM2 results in increase degradation of Her2. This study is important in the context of the personalized treatment regimens for HER2-positive breast cancer patients with different MDM2 expression status. This work was supported by the Russian Science Foundation Project No #18-75-10076

P-08.2-49
MDM2 ubiquitin-ligase sensitizes H1299 cancer cells to interferon-alpha

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Interferon-alpha (IFNα) is used as anti-cancer therapeutic to treat a number of malignancies. However, the application of IFNα is restricted due to its high toxicity and insufficient activity in case of many malignancies. MDM2 is the main negative regulator of tumor suppressor p53. It is ubiquitin-ligase which targets p53 for proteasome-dependent degradation. Nutlin-3a is widely studied inhibitor of MDM2-p53 protein-protein interaction. Nutlin binds MDM2 and liberates p53 which in turn transactivates genes involved in cell cycle arrest, apoptosis and so on. Interesting, that there is a growing body of evidence about Nutlin-mediated p53-independent anticancer effects. Moreover, the treatment of p53-deficient cancer cells with Nutlin increased their sensitivity to chemo- and radiotherapy. In the present work, we have demonstrated that the combinatorial treatment of IFNa resistant non-small lung cancer human p53-deficient cell line, H1299, with IFNa and MDM2 antagonist Nutlin-3a exhibited a synergistic effect on inhibition of cancer cell proliferation (CI = 0.29) and reduced the effective dose of IFNa 3.4 times. We have also shown that this depends on MDM2. The knockdown of MDM2 makes H1299 cells insensitive to both mono-treatment with IFNα or in combination with Nutlin. Moreover, the overexpression of MDM2 sensitizes cells to IFNa treatment. Both IFNa/Nutlin-3a treatment and MDM2 overexpression down-regulate cyclin D1/CDK4 and block cell cycle progression. We assume that this mechanism may be responsible, at least in part, for the antiproliferative effects on H1299 cells observed. (Shuvalov et al. 2018, BBRC, "Nutlin sensitizes lung carcinoma cells to interferon-alpha treatment in MDM2-dependent but p53-independent manner") This work was supported by RFBR grant # 18-315-20013

P-08.2-50
Nanoparticle cellular endocytosis as potential prognostic biomarker for cancer progression

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The main cause of cancer-related mortality is metastasis, thus its prediction critically affects the survival rate. Metastases are currently predicted by lymph-node status, tumor size, histopathology and genetic testing - all these methods are not infallible and getting the results may require weeks. The identification new potential prognostic markers will be an important source of risk

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information. Invasion of cancer cells is a critical step in metastases formation. The cytoskeleton machinery mechanisms utilized by cells for invasion process are similar to the involvement of actin cytoskeleton in endocytosis process. Nowadays nanoparticles are widely used for targeted drug delivery, while particles with specific coatings are encapsulated by cancer cells via endocytosis. We have used low-cost carboxylate-modified fluorescent 100 and 200 nm particles to achieve the adhesion and encapsulation efficiency of breast cancer cells with high (MDA-MB-231) and low (MCF7) metastatic potential. Using high-content fluorescence imaging microscopy, we have discovered that during short time (up to 1h) highly metastatic cells are able to adhere and encapsulate sufficiently more ($P < 0.05$) nanoparticles than lowly metastatic cells. We have created automatic image analysis algorithms to find quantitative colocalization (Pearson's and Overlap coefficients) of fluorescent nanoparticles with imaged cells. The obtained results of encapsulation and adhesion efficiency may lead to developing novel clinical tool for metastasis prediction. The method proposed here is very simple, it does require neither expensive materials and equipment nor laborious cell manipulations (serum starvation, staining etc.), it is potentially suitable for a variety of cells. Rapid (up to 1h), quantitative, patient-specific determination of cancer progression likelihood from biopsy/surgery sample, will directly affect the choice of treatment protocols for cancer patients and eventually increase their life-expectancy

P-08.2-51
Differences in the sulfatase pathway activity highlight a heterogeneity in endometrial and ovarian cancer cell lines

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Endometrial (EC) and ovarian (OC) cancers are post-menopausal pathologies with increasing incidence and mortality rate. The local estrogen synthesis and action play a vital role in the cancer initiation and progression. Estrogens can form locally from androgen and estrogen precursors, through the aromatase and sulfatase pathway, respectively. Here we investigated the contribution of the sulfatase pathway to active estrogen formation in EC and OC using cell lines. We assessed the profile of estrogen metabolites formed from 2.3, 8.5 and 85 nM estrone sulfate (E1-S), with or without 10 nM sulfatase inhibitor STX 64 by means of LC-MS/MS. We have shown that the sulfatase pathway contributes differently to the synthesis of estrone (E1) and estradiol (E2) in distinct cell lines of EC and OC. In the case of EC, this difference seems to be related with the stage of differentiation. A moderately differentiated, grade II EC cell line synthesized notably higher E1 and E2 levels comparing to a poorly differentiated, grade III EC cell line or the control line; the E1 and E2 formation was blocked by STX 64. In the case of OC, the contribution of the sulfatase pathway to E1 and E2 synthesis might be related with the tumor type from which the model line was originally established. The cell lines established from serous adenocarcinoma and undifferentiated carcinoma, both type II OC synthesized higher E1 and E2 levels than a cell line established from type I endometrioid OC or the control line; the E1 and E2 formation was blocked by STX 64. Our findings highlight the EC and OC inter-tumor heterogeneity. We imply that the sulfatase pathway is an important source of pro-proliferative estrogens in certain types of EC and OC, and is thus an auspicious therapeutic target. This work was supported by the Slovenian Research Agency. Grant number: J3-8212 and N1-0066.

P-08.2-52
Polyamines are new modulators of proteasome activity

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The cancer cells are tending to increase intracellular pH and at the same time are known to intensively produce and uptake polyamines such as spermidine and spermine, which are polycations with three and four amine groups. Almost every living cell contains polyamines in up to millimolar concentration. Polyamines are involved in important cellular processes closely linked with cell growth and differentiation. Previously it was shown that polyamines may increase activity of enzymes, such as α-chymotrypsin, and act like a chemical chaperone. On the other hand, proteins enriched with basic amino acids like arginine and lysine may directly bind proteasome and are capable for further translocation into the proteolytic chamber. In our work we investigated how alkalization and increased concentration of polyamines may modulate activity of proteasome. Special type of activation was observed in buffers based on amino acids arginine and ornithine, and natural polyamines spermine and spermidine. Activity of proteasome in these buffers was dramatically increased at pH values greater than 8.0. Addition of anionic buffers like phosphate or carbonate in opposite inhibited proteasome activity during alkalization. Importantly, simultaneous administration of spermine and carbonate counteracted carbonate-driven proteasome stalling in alkaline conditions predicting additional physiological role of polyamines in maintaining metabolism of cancer cell and its survival [1]. The reported study was supported by the Russian Science Foundation project #19-14-00262. [1] Anna A. Kudriaeva, George A. Saratov, Alena N. Kaminskaya, Vasiliy I. Vladimirov, Petro Yu Barzilovich and Alexey A. Belogurov, Jr., "Polyamines Counteract Carbonate-Driven Proteasome Stalling in Alkaline Conditions" // Biomolecules, 2020, 10 (12), 1597 *The authors marked with an asterisk equally contributed to the work.

P-08.2-53
Combining electrochemotherapy with PARP inhibitor olaparib in triple negative breast cancer *in vitro* and *in vivo*

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Electrochemotherapy (ECT) is an effective ablative therapy with different effectiveness among tumor types. In the treatment of

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breast cancer, the effectiveness is among the lowest, therefore, combined therapies are needed. One of the possibilities is combining ECT with DNA damage repair mechanism inhibitors, such as poly (adenosine diphosphate-ribose (ADP)) polymerase (PARP) inhibitor. The aim of our study was to evaluate the cytotoxicity such combined therapy in two triple negative breast cancer cell lines, different in BRCA mutation status (BRCA1 mutated HCC1937 and non-mutated HCC1143). The cytotoxic effect of ECT with bleomycin and cisplatin was determined and different olaparib concentrations were tested. Combination of EC50 dose of ECT with cisplatin or bleomycin and a non-cytotoxic concentration of olaparib was then tested in cells and HCC 1937 3D cell cultures, i.e. spheroids. Cytotoxicity of the treatment was determined and growth of spheroids was measured. Moreover, γ-H2AX histone staining of double strand break was performed to determine the extent of the non-repaired double strand breaks. Finally, the combined therapy was also tested in vivo, in HCC1937 tumor model in SCID mice. In BRCA mutated triple negative cell line HCC1937 cell viability was significantly reduced after combining olaparib with ECT with bleomycin or cisplatin. Similar effects were observed in spheroids and *in vivo* tumor model. This was further confirmed with the immunofluorescent staining showing increased number of γ-H2AX foci after combined therapy in concerned groups in HCC1937 cells and tumors. In contrary, there was no reduction in cell growth after combining ECT with bleomycin or cisplatin with olaparib in BRCA non-mutated triple negative cell line HCC1143. In conclusion, olaparib is a promising new drug that could potentiate the effectiveness of ECT in BRCA mutated triple negative breast cancer.

P-08.2-54
Endoplasmic reticulum stress dependent control of hypoxic regulation of gene expressions

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The endoplasmic reticulum stress and hypoxia are obligate component of tumor growth and are responsible for intensification of cell proliferation and down-regulation of apoptotic processes through genome reprogramming. We have studied effect of hypoxia on the expression of nuclear genes encoding mitochondrial proteins in wild type and ERN1 knockdown U87glioma cells. It was shown that hypoxia significantly increased the level of HIF1A protein in both wild type and ERN1 knockdown glioma cells. Furthermore, exposure of wild type U87 glioma cells under hypoxic condition led to up-regulation only smaller part of studied genes. These changes are correlated with strong up-regulation of HIF1A protein in glioma cells treated by hypoxia. At the same time, the expression of larger part of studied genes was down-regulated in wild type glioma cells. These results clearly demonstrated that there are other mechanisms of hypoxic regulation of gene expressions, in addition to HIF-mediated mechanism. We have also shown that ERN1 inhibition modified the hypoxic regulation of most studied gene expressions in gene-specific manner: removed or decreased the effect of hypoxia on the expression of most studied genes. At the same time, the hypoxic regulation of some gene expressions was similar in both wild type and ERN1 knockdown glioma cells or significantly increased. Moreover, we identified several genes which expression was resistant to hypoxia treatment in wild type glioma cells and shown that ERN1 knockdown introduced sensitivity of these gene expressions to hypoxic regulation. It is possible that endoplasmic reticulum stress reprograms genome function preferentially through ERN1 signaling pathway and modifies hypoxic regulation for elimination the toxic effects of hypoxia. The results of this study clearly demonstrated that hypoxic regulation of gene expressions in glioma cells is possibly realized through different mechanisms and preferentially modified by ERN1 signaling pathways.

P-08.2-55
Preparation of recombinant EpIC-FHL2-β-catenin-Lef1 signaling complex

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Epithelial Cell Adhesion Molecule (EpCAM) is a central molecule in the regulation of epithelial cell-cell adhesion and signaling. Its key signaling pathway is initiated by Regulated Intramembrane Proteolysis (RIP). During the process of RIP, EpCAM's intracellular domain (EpIC) is released to form a signaling complex with key components of Wnt signaling pathway, β-catenin and Lef1. Together with its binding partners FHL2 and β-catenin it is translocated to the nucleus where they interact with Lef1 to form the EpIC-FHL2-β-catenin- Lef1 signaling complex. This in turn leads to transcription of oncogenes, which are responsible for EpCAM-mediated carcinoma cell proliferation. This is also one of the main reasons why EpCAM overexpression is often linked to poor prognosis. EpIC's function in this signaling complex is still unknown. Deciphering its mechanism of action is furthermore hindered by the lack of any structural information on the EpIC-FHL2-β-catenin-Lef1 signaling complex and sparse structural knowledge of β-catenin/Lef1 signaling in general. To get insight into its structure, protocols for the preparation of a stable complex must first be developed. Here we present a comparison of two different approaches for complex formation: overexpression of all interacting partners in a single cell and reconstitution of the complex from individually isolated recombinant proteins. We utilized bacterial, insect, and mammalian expression systems to evaluate the potential influence of post-translational modifications on complex stability. Protocols for protein expression and isolation were also optimized to provide sufficient amounts of proteins for further experiments. Our results provide a solid foundation for structural and functional characterization of the EpIC-FHL2-β-catenin-Lef1 signaling complex, which will enable us to describe the role of EpIC in EpCAM signaling as well as provide novel structural insight into Wnt signaling in general. *The authors marked with an asterisk equally contributed to the work.

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P-08.2-56
New lncRNAs regulating tumor-associated genes mediated by miRNAs in ovarian cancer

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Ovarian cancer (OC) develops asymptomatically and worldwide is accompanied by the highest mortality among oncological diseases of the female reproductive system. The discovery of long noncoding RNAs (lncRNAs) opens up new opportunities in the study of cancer pathogenesis, including the mechanism of gene regulation according to the the ceRNA model. The aim of this work is to identify new lncRNAs potentially involved in the regulation of tumor-associated proteins mediated by miRNAs, namely, 12 miRNAs, which, according to our data, are involved in metastasis of the OC (Loginov et al., 2018). Bioinformatic screening of lncRNAs was performed based on the TCGA transcriptome database (package GENIE3) using modified random forest method. A total of 345 experiments were retrieved containing information on RNAs in OC samples. First, lncRNAs were selected, potentially binding to 10 metastasis-associated miRNAs (miR-124, -125b, -129, -148a, -203, -375 and etc.; rs = 0–1, $P < 0.002$). Second, lncRNA/miRNA/mRNA triplets were selected, including 10 target miRNAs, using all significant pairs "lncRNA-miRNA"and "miRNA-mRNA"(rs = 0–1, $P < 0.002$). Expression level of 10 target miRNAs and selected 15 lncRNAs and 25 protein mRNAs was studied by qRT-PCR using a common set of 40 matched (tumor/normal) samples of OC. Negative correlations were revealed between the levels of RNA of different types (rs = [- 0.33] - [-0.63], $P = 0.00–0.05$), indicating potential interactions within 7 pairs of lncRNA/miRNA, e.g. MALAT1/miR-191, MAFG-DT/miR-148a, OIP-AS1/miR-203, and 11 miRNA/mRNA pairs as miR-148a/BCL2, miR-203/CDK4, miR-375/WNT4. The triple interactions, e.g. MALAT1/miR-203/CDK4, MAFG-DT/miR-148a/BCL2 were also revealed. Of interest, 90 positive correlations between lncRNAs and protein mRNAs were detected. The most significant results would be verified in functional studies using transfection of OC cell lines. This work was supported by the Russian Science Foundation, grant no. 20-15-00368.

P-08.2-57
Under pressure: the role of spliceosomal components in the acquisition of chemoresistance in breast cancer

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The role of tumor intercellular communication in chemoresistance acquisition was underestimated until recently. One model of such communication implies the signal transfer from dying during therapy chemosensitive cells to resistant ones. To investigate this issue, we used two triple-negative breast cancer cell lines with different sensitivity to cisplatin. We analyze differences in proteome profiles of secretomes from sensitive MDA-MB436 cells treated or untreated by cisplatin. LC-MS/MS analysis shows that dying tumor cells secreted more proteins as opposed to control cells. Therapy-induced secretomes were enriched with a cluster of spliceosomal proteins, which is not typical for control secretomes. Next, we assess the influence of secretomes from dying or control sensitive MDA-MB436 cells on resistant MDA-MB231 cells. As expected, secretome from dying sensitive cells led to an acquisition of a more resistant phenotype by recipient MDA-MB231 cells. Interestingly, according to RNAseq data, such changes in sensitivity are accompanied by not only the altered gene expression but also with the massive remodeling of the splicing program in the recipient cell. Further, we generated MDA-MB231-GFP cell line to perform a direct co-cultivation of dying sensitive cells with resistant ones followed by flow cytometry, immunofluorescence microscopy, and cell cycle measurement of recipient cells. As a result, we showed that direct co-cultivation led to a change in proliferation, cell cycle, and expression of proteins responsible for a more aggressive mesenchymal phenotype in recipient cells. Thus, the identification and inhibition of principal players in cancer intercellular communication can be the basis for creating a new strategy for antitumor therapy. The work was supported by the Russian Science Foundation project 19-75-10123 (LC-MS/MS analysis) and grant 075-15-2019-1669 from the Ministry of Science and Higher Education of the Russian Federation (RNA-seq analysis).

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P-08.2-58
SMAD7 and SMAD4 expression and ratio in primary and metastatic colorectal cancer

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SMAD signaling has been identified as an important target for the disruption of the TGF-β pathway in cancers. Since both inhibitory SMAD7 and common-mediator SMAD4 have been shown to play crucial roles in SMAD-dependent TGF-β signaling, this study aimed to profile the expression and ratio of these two genes in colorectal cancer (CRC) cell lines, fresh tumors, and matched non-tumor tissues obtained from primary and metastatic CRC patients. Six CRC cell lines (Caco-2, HT-29, DLD-1, HCT116, SW480, and SW620) and normal human colon epithelial cell line (HCEC-1CT), as well as a total of 66 tissue samples collected from 17 patients with locally advanced rectal cancer (LARC) and 16 patients with colorectal liver metastases (CLM), were used for the expression pattern evaluation. Relative expression levels of SMAD7 and SMAD4 were measured using the quantitative real-time PCR method (qRT-PCR). The mRNA levels of target genes were normalized to housekeeping gene GAPDH and compared between tumor and matched non-tumor tissues and cell lines. SMAD7/SMAD4 ratio was elevated in all CRC cell lines compared to the normal cell line due to drastic upregulation of SMAD7 expression in all CRC cells. In LARC patients, SMAD7 expression was slightly increased, SMAD4 expression was significantly decreased, while SMAD7/SMAD4 ratio was significantly increased in primary tumor tissues compared to matched non-tumor mucosa tissues. In CLM patients, expression of both SMAD7 and SMAD4 was significantly decreased in metastatic tissues compared to matched non-tumor liver tissues, but the SMAD7/SMAD4 ratio did not differ significantly. Observed differences in expression balance of SMAD7 and SMAD4 in various stages of CRC indicate the involvement of these genes in disease progression. Further investigation should validate these results in a larger cohort of patients and propose mechanisms by which balance changes contribute to the disease outcome.

P-08.2-59
Increased abundance of splicing factors in cancer cells contributes to their survival upon DNA damage

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Cancer cells dying during therapy can secrete splicing factors into the extracellular space, and these secretomes make remaining tumor cells more resistant to subsequent therapy [1]. However, the mechanisms underlying this biological effect are not clear. Here we elucidated the role of secreted splicing factors in intercellular communication. First, we investigated how splicing factors could be transferred from dying cells. We noticed that in addition to splicing factors, therapy-induced secretomes were enriched with stress granule proteins. We proposed that splicing factors could be secreted from dying tumor cells as a part of stress granules. To test this, we co-transfected SKOV3 cells with plasmids encoding SR splicing factors and stress granule proteins labeled with RFP and GFP, respectively. After exposure to cisplatin, both groups of proteins were relocalized from the nucleus to the cytoplasm at early time points preceding nuclear fragmentation, and their localization coincides with each other. Next, we showed the presence of SRSF4-RFP and TIA1-GFP secreted by dying cancer cells in recipient cells using fluorescent microscopy. Such recipient cells were more resistant to cisplatin, and the knockout of stress granule proteins in donor cells reverses this effect. Further, we obtained SKOV3 cells with stable overexpression of some splicing proteins and showed that increased amounts of these proteins promote cancer cell resistance to cisplatin. Summing up, splicing factors can be secreted from dying cancer cells as part of stress granules via extracellular vesicles, penetrate recipient tumor cells and contribute to their chemoresistance. The proposed mechanism has potential clinical significance. The work was supported by the Russian Science Foundation project 19-75-10123 (molecular cloning) and grant 075-15-2019-1669 from the Ministry of Science and Higher Education of the Russian Federation (cell tests). [1] Pavlyukov MS et al. (2018) Cancer Cell 34, 119-135.

P-08.2-60
Delaying prostate cancer progression by targeting PP1 complexes using bioportides

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The development of new and effective therapeutic approaches for prostate cancer is currently one of the major challenges of the scientific community in oncology. Phosphatases, and in particular protein phosphatase 1 (PP1) emerged as promising drug targets in this context. Thus, the main goal of this work is to establish

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an efficient strategy to disrupt a key PP1 complex with important roles in prostate cancer progression. To accomplish this goal, a peptide sequence derived from the region that include the PP1-binding motif of caveolin-1 (CAV1) was synthetised using microwave-assisted solid-phase peptide synthesis. This peptide was coupled to the cell penetrating peptide penetratin to enable an efficient cellular delivery and was purified by semi-preparative scale high-performance liquid chromatography. The predicted mass of the peptide was confirmed by mass spectrometry. The ability of this combined peptide (named bioportide) to affect prostate cancer cells progression was evaluated *in vitro*. In this context, prostate cancer cells (PC-3 cell line) were incubated with different concentrations of the bioportide and a mutated homologue (control) and cells viability (PrestoBlue cell viability assay) and the expression of various protein biomarkers (Western blotting) were measured. We found that, despite the incubation with the bioportide for 24h did not significantly affect the prostate cancer cells viability, after 48h incubation, a concentration of 20 μM of the bioportide significantly reduced the prostate cancer cells viability. We will also shed light on the expression of some proteins involved in the signaling pathways modulated by PP1/CAV1 interaction. These results highlight the potential of the synthesized bioportide to negatively impact the prostate cancer cells proliferation and consequently delays cancer progression. Further analyses are now required to confirm the disruption of the target interaction and to better elucidate the mechanisms of cell death.

P-08.2-61
Natural mineral zeolite (clinoptilolite) inhibits αvβ1 integrin-mediated MMP-2 activity in PC3 human prostate cancer cells

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Prostate cancer is the most common type of cancer in men in Western countries. During the development of the disease, the change of tumor from androgen-dependent type to independent type and the development of metastasis limit treatment and survival rates greatly. Therefore, there is an urgent requirement for the new treatment approaches. Matrix metalloproteinases (MMPs), a family of zinc- dependent endopeptidases play an essential role in inflammation, malignant cell proliferation, invasion and metastasis by inducing excessive matrix degradation. The activities of MMPs are controlled by endogenous Tissue Inhibitors of Metalloproteinases (TIMPs). The integrin family of cell adhesion receptors regulates adhesion, proliferation and migration of tumor cells by promoting MMP activation. Thereby, integrins remarkably contribute to the initiation, progression and metastasis of cancer. Clinoptilolite, a natural micronized zeolite is a volcanic mineral. It has cation exchanging and chelating properties. In recent years, it is being used in oral forms especially as a chelation and detoxification agent to remove heavy metals accumulating in the body. Safety of clinoptilolite was approved by the US Food and Drug Administration (FDA). In this study, we aimed to investigate the effects of clinoptilolite on gelatinases (MMP-2/MMP-9) and αvβ1 integrin-mediated gelatinase activity in human PC3 cells. PC3 cells were incubated with or without clinoptilolite (25mg/mL) for 24 hrs in a humidified atmosphere containing 5% CO2 at 37°C. Then the expression/activity of MMP-2/MMP-9 was determined using gelatin zymography. The expression of MMP-14, TIMP-2 and αvβ1 were examined by immunostaining/immunoscoring. Clinoptilolite significantly inhibited MMP-2 activity and the αvβ1 integrin expression. However, it up-regulated expression of TIMP-2 which is a tissue inhibitor of MMP-2. Clinoptilolite could be useful in prostate cancer treatment due to anti-inflammatory and anti-cancer effects. *The authors marked with an asterisk equally contributed to the work.

P-08.2-62
AID expression in several cell lines is independent to pathogen recognizing receptors activation

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Cytidine deaminase AID plays a critical role in the antibodiy class switching and somatic hypermutations in B cells. Initially, AID expression in non-lymphocytes in response to bacterial PAMPs was shown for gastric cancer [1]. Analysis of mutational signature reveals probable AID-dependent transitions in many types of cancers, including ovary, colon, pancreas, kidney, and liver cancers, but breast, cervix, lung, and skin cancers do not demonstrate this signature [2]. We test the hypothesis that activation of pathogen recognizing receptors could increase AID level in B-cell lines or promote AID expression in non-lymphoid cell lines. For the study, we use B-cell lymphomas IM-9, Daudi, RPMI 8226, and Raji, and the cell lines of non-lymphoid origin including A549 (lung), Jurkat (T cell), HeLa (cervix), and MCF10A (breast). For pathogen recognizing receptors activation were used *E. coli* LPS, *E. coli* total lysate, water emulsion of Freund's complete adjuvant, and yeast extract, for positive control was used PMA phorbol ester. Expression levels of AID and other APOBEC family members were measured by RT-PCR with following Western-blot confirmations on the protein level. Basic levels of AID expression vary about 100-fold from highest in IM-9 to lowest in RPMI 8226, for all non-B cell lines it was under the threshold of detection. We found that AID expression level in B-cell lines was not changed in response to used pathogen recognizing receptors activators both on short (10 minutes - 1 hour) and long (1 hour – overnight) exposure times, but it respond on PMA activation in 2–3 fold increasing on short exposures. No one non-B cell line shows reliable AID expression upon treatments. So we prove that several types of cancers have AID-independent mutagenesis even in the case of pathogen recognition induced inflammation. The reported study was funded by RFBR, project number 19-34-51014. References: [1] Matsumoto et al, Nat. Med. 2007 13(4), 470-6 [2] Rogozin et al, Sci. Rep. 2016, 6, 38133

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P-08.2-63
The effect of copper bioavailability on epithelial-to-mesenchymal transition in breast cancer cells

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Copper is an essential transition metal, acting as cofactor of enzymes involved in several biochemical processes, including collagen maturation, free radical detoxification and ATP production in mitochondria. A complex machinery of transporters, chaperones and P-type ATPases concurs to the strict regulation of copper homeostasis. Thus, it is not surprising that genetic disorders (Menkes' and Wilson's diseases), neurodegenerative diseases (Alzheimer's and Parkinson's diseases) and cancer are associated with copper dyshomeostasis. In the context of cancer biology, copper is implicated in tumor growth and metastatization. Of note, several copper-binding proteins are involved in the epithelial-to-mesenchymal transition (EMT), the mechanism underlying metastasis formation. However, no conclusive study on this topic is reported for breast cancer. To fill this gap, we tested the effect of two copper chelators (Tetrathiomolybdate, TTM or Triethylenetetramine, TRIEN) on EMT in breast cancer cell lines, in the presence or absence of TGFβ, a cytokine known to induce EMT. To this aim, we performed morphological analysis of the cells, and immunoblot and immunofluorescence characterization of epithelial (E-cadherin, Occludin) and mesenchymal (Fibronectin, Vimentin) markers. To assess changes in cell migratory behavior, wound healing, transwell cell migration/invasion and gelatin invadopodia assays were also performed. Furthermore, the expression of some copper-binding proteins was analyzed, to verify their modulation during EMT. The data obtained suggest that the treatment of breast cancer cells with copper chelators enhances the acquisition of a mesenchymal phenotype.

P-08.2-64
miR-150 is a fine tuning mediator for the regulation of metastatic potential of MiaPaCa-2 pancreatic cancer cells

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Pancreatic cancer (PC) is a highly aggressive type of cancer that is difficult to diagnose and resistant to chemotherapeutic agents. Dysregulation of mir-150 leads to activation of cancer stem cell (CSC) and EMT mechanism in diverse cancer cells. Palbociclib, a CDK4/6 inhibitor, limits the functions of cyclins that play a critical role in the cell cycle, such as cyclin D and cyclin E, which have high levels of expression in PC. The current study aimed to suppress the metastatic potential of PC cells by increasing the miR-150 levels and treatment of the palbociclib. The EMT mechanism was investigated by immunoblotting and immunofluorescence to analyze key parameters of the EMT process in palbociclib-treated cells. The associations of miR-150 and CDK4/6 inhibition with survival rate and metastasis were determined. Overexpression of miR-150 in Panc-1 and MiaPaCa-2 cells was performed to assess its effect on CSCs potential in leptin-treated cells. The colony and spheroid formation of PC cells was increased by leptin treatment through an increase of Wnt/β-catenin/Notch signaling. Administration of palbociclib reduced the metastatic potential of PC cells, but palbociclib alone was not enough to reduce the leptin-induced CSC phenotype of PC cells. Therefore, an increase of miR-150 inhibited the EMT process and the expression level of CD44 and CD133 in leptin-/leptin+ Panc-1 and MiaPaCa-2 cells. In the light of the obtained results, it is predicted that the therapeutic success of palbociclib will increase when miR-150 upregulated in highly aggressive PC cells. In this study, it was concluded that miR-150 could directly target the CSC by reducing the expression levels of Wnt/β-catenin/Notch signaling as well as CD44 and CD133 levels in the palbociclib-treated PC cells. The combination therapy with miR-150 mimic will increase the efficiency of palbociclib on the elimination of CSC and inhibition of metastasis.

P-08.2-65
Tumor suppressor, RUNX3, induces antioxidant regulation of gene transcription

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Runt-related transcription factor 3 (RUNX3) is feasible tumor suppressor gene since its inactivation was shown to be related to carcinogenesis. Oxidative stress by high levels of reactive oxygen species (ROS) is causally associated with carcinogenesis through DNA damages. Cellular defense against oxidative stress employs nuclear factor E2-related factor 2 (NRF2), a master regulator of a cellular antioxidants. Oncogenic K-RAS-induced ROS is actively suppressed by inducing the transcription of NRF2. However, the molecular mechanisms for the regulation of ROS-induced NRF2 transcription have been poorly studied. In this study, we show that RUNX3, a transcription factor functioning as a tumor suppressor, is required for the NRF2 transcription by oncogenic K-RAS-induced ROS and ectopic oxidative stress. Knockdown of RUNX3 or mutation of RUNX binding sites at the promoter region of NRF2 markedly reduced oxidative stress-induced NRF2 expression. Our study highlights a novel mechanism for NRF2 expression in regulating redox homeostasis, providing insights into new strategies of cancer prevention against the oxidative stress-associated carcinogenesis.

P-08.2-66
Pinus mugo essential oil downregulates constitutive activation of STAT3 and induces apoptosis in DU145 Prostate cancer cell line

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Aberrant activation of the pleiotropic transcription factor STAT3 (Signal Transducer and activator of transcription 3) has been linked to uncontrolled cell proliferation, resistance to apoptosis and induction of chemoresistance in different types of cancers. Prospecting inhibitors of STAT3 activation in phytoproducts is an effective strategy to complement cancer therapeutics and overcome chemoresistance. Essential oils are rich sources of bioactive molecules and have been extensively used in traditional medicine.

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It has been described that some EOs as well as their major constituents can inhibit the proliferation and survival of several types of cancer cells through the downregulation of STAT3 signalling (Previously published in: Hao Chen et al. (2018) Front.Pharmacology 9, article 483). In the present study, we identified the essential oil from *Pinus mugo pumilio* (PMEO), as an inhibitor of STAT3 signalling in human prostate cancer cell line DU145, that expresses constitutively active STAT3. One of the critical steps leading to STAT3 activation is the phosphorylation at Tyr705. Western blot analysis showed that 1 hr PMEO treatment dose-dependently downregulated STAT3 Tyr705 phosphorylation (pSTAT3). The downregulated expression of pSTAT3 sustained up to 4hrs of PMEO treatment, whereas no significant changes were observed in the levels of total STAT3. As evidenced by WST-1 assay, treatment with 60μg/ml (=IC50) PMEO for 24hrs, dose-dependently reduced the viability of DU145 cells. Consistent with this, we observed a significant decrease in the expression of BcL2, an anti-apoptotic protein regulated by STAT3. Results of PI/annexin-V staining and PARP cleavage indicated that PMEO cytotoxicity is mediated through apoptosis. Altogether, our data indicate the potential application of PMEO in cancer therapy. Further analyses are being performed to deepen the molecular mechanism of the anti-STAT3 activity of PMEO treatment.

P-08.2-67
Differential expression of key adhesion markers and apoptotic regulators influences the malignant progression of cutaneous melanoma

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Malignant melanoma (MM) is a highly aggressive type of cancer, whose global incidence substantially increases every year, presenting a tremendous medical challenge. There is an unmet need for reliable molecular prognostic and diagnostic markers that can improve the current clinicopathologic correlates and management of MM. In this context, key biomarkers that mediate the transition from an epithelial to an invasive phenotype, enhancing migration and preventing tumour cell death represent important candidates. The aim of our study was to evaluate the expression of specific adhesion molecules and apoptotic regulators, in order to correlate their level of expression with their potential implication in the malignant progression of cutaneous melanoma. To achieve this, we used primary and metastatic melanoma cell lines. Gene expression analysis was performed using Real-Time PCR and specific primers, while immunostaining coupled with fluorescence microscopy was employed for protein expression evaluation. When analysing the expression of E-cadherin, Fibronectin and MCAM as markers for cellular motility, we found that metastatic melanoma cells possessed higher Fibronectin and MCAM expression, while E-cadherin was markedly decreased, compared to primary cells. Additionally, apoptotic and mitotic regulators Bcl-2 and BIRC5 displayed increased expressions in metastatic samples compared to primary cells, while Bax registered low levels. The gene expression results were confirmed at protein level. Our findings indicate the differential expression of key adhesion markers and apoptotic regulators, which could play a significant role in the progression of cutaneous melanoma from primary to metastatic stages, and might serve as potential prognostic biomarkers. This work was supported by UEFISCDI-PN-III-P1-1.2-PCCDI-2017-0341/PATHDERM.

P-08.2-68
Intracellular mechanism of capsaicin-induced stimulation of the AMPK pathway

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Capsaicin, a secondary metabolite of plants, is the main component responsible for the pungent flavor of chili peppers. In the past few years, antitumor properties have been attributed to this compound, but its underlying mechanisms are yet unknown. Recently, we have shown that capsaicin increases the antiproliferative effects of docetaxel, a chemotherapeutic agent in prostate cancer, and that its antitumor and synergistic properties are associated with the activation of AMP-activated kinase (AMPK). In this study, we aim to explore the mechanism whereby capsaicin activates AMPK in prostate tumor cells. We found that capsaicin dose-dependently inhibited prostate cancer cells growth and increased apoptosis. Both effects were significantly higher in LNCaP and PC3 cells compared to DU-145 cells, in which capsaicin exerted a weaker antiproliferative effect. Accordingly, capsaicin activated AMPK in LNCaP and PC3 cells but not in DU-145 cells, which have a deletion of the liver kinase B1 (LKB1) pointing to an involvement of LKB1 in the capsaicin-induced AMPK activation. To explore this notion, LKB1 was knocked-down with siRNA in LNCaP and PC3 cells. LKB1 knockdown prevented the activation of AMPK by capsaicin. These data suggest that LKB1 could be involved in the mechanism of action of capsaicin in prostate cancer.

P-08.2-69
Melanoma cells differentially induce changes in expression of proteins associated with tumor progression in primary and immortalized keratinocytes in indirect co-culture

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Cutaneous melanoma represents a highly aggressive tumor with an elevated mortality rate among patients with metastatic disease. However, currently used treatment strategies (i.e. chemo-, radio- and immunotherapy) are not able to efficiently eradicate all cancer cells. One of the factors affecting the poor success of anti-melanoma therapies is the protective microenvironment of the tumor. Melanoma cells are surrounded by different types of cells (e.g., fibroblasts, keratinocytes, adipocytes, immune cells) and elements of extracellular matrix (ECM), and can reprogram these stromal cells to promote tumor growth, progression, immune escape, and resistance to therapy. To better understand the crosstalk between melanoma and neighboring cells, we have performed indirect co-cultures of primary and immortalized keratinocytes with four cell lines of melanoma using melanoma-derived conditioned media and cell cultures on inserts. Upon co-culture with melanoma, both variants of keratinocytes exhibited

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a reduction in the level of cytokeratin 10 and a slight increase of cytokeratin 14, a phenotype corresponding with less differentiated and more proliferative cells. Interestingly, melanoma differentially induced changes in levels of receptor tyrosine kinases – EGFR (epidermal growth factor receptor) and MET (hepatocyte growth factor) in stromal cells. The co-culture promoted an increase in receptor levels in immortalized cells, while their expression was reduced in primary keratinocytes. This observation was followed by an evaluation of keratinocytes' sensitivity to EGFR and MET inhibitors in normoxic and hypoxic conditions, also in co-culture with melanoma. Moreover, melanoma cells induced differential expression of ECM digesting enzyme – metalloprotease, and immunosuppressive cytokine – interleukin 1 β in keratinocytes, as well as altered their profile of proteins responsible for adhesion and cell-cell interactions.

P-08.2-70
The role of dual specificity phosphatase 6 in ALK-dependent neuroblastoma cell survival

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Protein Tyrosine Phosphatases (PTPs) function in a coordinated fashion with protein tyrosine kinases to regulate a range of intracellular signalling processes. As these processes are often dysregulated in neuroblastoma (NB), our group has had a long-standing interest in whether PTPs promote the survival of NB tumour-derived cells. In a subset of NB patients, intracellular signalling through the tyrosine kinase receptor ALK drives tumour cell growth. Recent studies have identified an increased frequency of mutations in the ALK signalling pathway in relapsed NB patients, primarily in the RAS-MAPK pathway. Previous studies in our group identified the potential oncogenic role of DUSP6, an ERK-specific PTP, where partial reduction of DUSP6 reduced the growth of NB cell xenografts. Here we demonstrate that DUSP6 could be a regulator of NB cell survival, functioning downstream of ALK. Transient CRISPR/Cas deletion of DUSP6 suppressed cell growth in ALK-driven NB cells. Furthermore, DUSP6 is a transcriptional target of ALK signalling and loss of DUSP6 sensitised ALK-driven NB cells to treatment with Lorlatinib, a 3rd generation ALK inhibitor. Considering that the ERK signalling pathway appears unaffected by DUSP6 depletion in our hands, we are further investigating the mechanism of action of DUSP6 in these cells.

P-08.2-71
Platelets enhance breast cancer cells-induced thrombin generation by providing procoagulant surfaces

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Cancer cells induce platelet aggregation *in vitro* in the presence of plasma. Cancer cells were supposed to trigger thrombin generation by both intrinsic and contact pathways. Role of platelets and contribution of these mechanisms to coagulation and platelet aggregation has not been characterized. To investigate the molecular mechanisms that underlie interactions between breast cancer cells, coagulation system and platelets. Human breast cancer cell line MCF-7 was used. Washed platelets were prepared from human blood, collected in accordance with the local ethic committee. Aggregation was performed by Biola LA-220. Thrombin generation was measured using Thermomax reader. To investigate plasma clot formation induced by cancer cells, we modified the Thrombodynamics method with immobilization of cancer cell on the activator plate. Platelet inhibitors have no effect on tumor-cell induced platelet aggregation in the presence of 1% plasma, while hirudin prevented it completely. We observed thrombin in the systems with platelets, PFA-fixed platelets or inhibited platelets. In the system without platelets, we observed reduced thrombin generation. Replacement of PFP with factor XII or factor VII depleted plasma showed no effect on tumor-cell induced platelet aggregation, while factor X depleted plasma prevented it completely. In addition, there was fibrin clot formation directly from cancer cells in the system with PFP from healthy donors with the same velocity as when TF was used as an activator. Clot growth rate was reduced when blocking factor XIIa. Factor X depleted plasma no detectable clot formation was observed. Tissue factor and coagulation factor XII pathways are the major ways of thrombin generation by cancer cells. There is no thrombin-like activity in MCF-7 cells. Platelets are likely to provide anionic phospholipids for assembling complexes of coagulation factors. The study was supported by Russian Science Foundation (grant 20-45-01014). *The authors marked with an asterisk equally contributed to the work.

P-08.2-72
The influence of ZEB1 transcription factor on the resistance to genotoxic drugs during the epithelial-mesenchymal transition

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Epithelial-mesenchymal transition (EMT) is the major mechanism for metastasis formation which is the main cause of death in cancer. Upon EMT, the loss of intercellular contacts occurs and cells acquire the ability to migrate and invade. ZEB1 transcription factor is one of the key factors which are responsible for EMT initiation. As was shown, ZEB1 is capable of affecting a number of target genes responsible for cell cycle regulation and apoptosis. We have studied the effect of ZEB1 on the cell cycle of MCF7 breast cancer cells exposed to genotoxic drugs used in clinic - doxorubicin and etoposide. We have used MCF7 cells with inducible expression of the ZEB1 gene, which is activated using doxycycline. According to the results of the MTT test, at various concentrations of doxorubicin (0.5–4 μM), cell survival increased two-fold upon activation of ZEB1. One of the reasons for this increase in resistance may be the cell cycle arrest in the G1 phase. Using flow cytometry, it was shown that the activation of ZEB1 led to the increase of the cell's number in the G1 phase and to decrease of cells in S and G2/M phases. Moreover, the most effect was observed at 72 hours after ZEB1 activation. Upon doxorubicin and etoposide treatment at concentrations of 0.5–1 μM and 50–100 μM, respectively, it was observed that the number of cells in the S-phase increased relatively to control.

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However, upon simultaneous treatment with one of the genotoxic drugs and ZEB1 activation, there was a significant reduction of the cells in S- and G2/M phases. Using Annexin V and 7AAD we have shown that the activation of ZEB1 expression decreased the number of apoptotic cells upon doxorubicin treatment. We conclude that ZEB1-induced resistance to genotoxic drugs may occur because cells stop dividing, whereas the effect of these drugs is directed ultimately towards proliferating cells. This work was supported by grant RSF № 19-45-02011. Key words: ZEB1, EMT, cancer, doxorubicin, etoposide, metastasis.

P-08.2-73
Regulation of BCL-2 proteins by oncogenic BRAF signaling in colorectal cancer cells

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Oncogenic BRAF mutations ($BRAF^{V600E}$) are found in approximately 10% of colorectal cancers (CRCs) patients and are associated with therapy resistance, high aggressiveness and poor prognosis, highlighting the need for novel therapeutic strategies. The BRAF-regulated RAS/MEK/ERK signaling pathway plays a key role in controlling several cellular processes including cell survival or cell death decisions, however, its role in the regulation of BCL-2 family proteins is not well understood. Here, we report that although BRAF inhibition by vemurafenib strongly upregulates the expression of pro-apoptotic proteins including BIM, BMF, and in some cases PUMA, CRC cells undergo minimal apoptosis. Surprisingly, we also found a time-dependent accumulation of anti-apoptotic BCL-XL protein upon BRAF inhibition, which might contribute to treatment resistance of BRAF mutated CRCs. Therefore, we hypothesized that BCL-XL inhibition may synergize with vemurafenib to trigger apoptotic cell death in CRC cells. Indeed, combined pharmacological or genetic BRAF inhibition and subtoxic concentration of the specific BCL-XL inhibitor A-1331852 significantly induced apoptosis. We also demonstrated that pre-treatment with vemurafenib before A-1331852 addition resulted in a significant increase in apoptosis as compared to simultaneous treatment with this combination. These results are correlated with the observed delayed accumulation of BCL-XL expression upon vemurafenib. Collectively, we demonstrate that mutant BRAF downregulate the expression of the BCL-2 family proteins BIM and BMF, serving to protect the colorectal cancer cells from apoptosis. While BRAF suppression results in upregulation of BCL-XL, promoting resistance to vemurafenib, co-inhibition of BCL-XL and BRAF effectively triggers apoptotic cell death. Thus, these results may open new perspectives for the treatment of $BRAF^{V600E}$-positive CRC patients.

P-08.2-74
Prognostic significance of SERPINB1 expression in gliomas

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Glioblastoma (GB) is the most prevalent and aggressive brain tumor with high morbidity. Determination of tumor initiative and prognostic factors are ultimately needed for therapy options. The effects of Serine Protease Inhibitor (SERPIN) B1 on tumor progression have been reprted in several cancer types including breast and lung cancers. However, the relation between SERPINB1 expression levels and glioma progression is still to be elucidated. In this study, we aimed to determine the SERPINB1 expression levels in glioma patients and investigate its prognostic effect on patient survival. We first examined the expression of SERPINB1 in glioma and glioblastoma tissue samples (low grade (I,II); n = 6, high grade (III,IV) n = 4 and glioblastoma (grade IV) n = 4) by western blotting and immunohistochemistry. Expression of SERPINB1 in tissue lysates was significantly higher in glioblastoma samples than in low grade glioma ($P = 0.0056$). Additionally, SERPINB1 overexpression was associated with high glioma grades in the overall pattern. Survival analysis by using TCGA (French) Glioma cohort data (303 patients) showed that the mRNA expression of SERPINB1 correlates with decreased survivals in both GBM and non-GBM patient groups ($P < 0.0001$). Our results showed that the SERPINB1 expression is significantly high in glioblastoma patients correlated with poor prognosis. Here, we suggest that SERPINB1 may be a prognostic biomarker for glioma and may offer application in clinics. Further investigations will provide more insights about its function and mechanism of action. Keywords Glioma, glioblastoma, SERPINB1, tumor progression, biomarker

P-08.2-75
Functional effects of a rare NBN variant rs61754966 in hepatocellular carcinoma

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rs61754966 (p.I171V) variant in NBN, which encodes a subunit of MRN complex involved in double-strand break (DSB) repair, has been previously associated with the risk of developing several types of cancer (Gao, 2013). However, the data on its functional impact in different model systems are conflicting. In order to determine rs61754966 allele frequency (AF) in patients with hepatocellular carcinoma (HCC) we genotyped 87 paired tumor and liver samples using ddPCR with TaqMan probes. As a result, AF was 1.72% in HCC patients as compared to 0.14% in healthy controls according to ExAC global data and 0.60% in the Russian population (Zhernakova, 2020). Using CRISPR-Cas9 editing we generated NBN knockout (KO) in HepG2, a human hepatoma

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cell line. KO cells were transduced with lentiviral vectors to produce cell lines which stably expressed wild-type (WT-NBN) and mutant (NBN-I171V) NBN, and an empty control vector (CV). DSB were induced with 25 μg/ml bleomycin (bleo), the activity of MRN complex was inhibited with 100 μM mirin. Neutral comet assay demonstrated that DNA repair in HepG2, HepG2-WT-NBN and HepG2-NBN-I171V treated with bleomycin was more efficient than in KO and CV cells ($P < 0.01$, mean tail moment, Mann-Whitney). Cell viability and caspase 3/7 activity were similar for all cell cultures within treatment groups at different time points. The percentage of BrDU-positive S-phase cells in the studied cell lines was similar under normal conditions. However, KO, CV and HepG2-NBN-I171V cell lines demonstrated a two-fold larger decrease in S-phase cells than HepG2 or HepG2-WT-NBN after 24 hours of bleo, mirin and bleo-mirin treatment. Thus, HepG2-NBN-I171V demonstrates features of KO and WT-NBN cell cultures. Further research including assaying NHEJ/HR ratio is needed to elucidate its impact on chromosomal instability. The reported study was partially funded by RFBR (projects # 18-34-00816 & 18-29-09164) and President's Scholarship for PhD students and young scientists.

P-08.2-76
Fine metabolism reprogramming of citrate in cancer cells

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Citrate is the first intermediate of the tricarboxylic cycle in the mitochondria and is also a key metabolic regulator for glycolysis, gluconeogenesis and fatty acid synthesis. Within the cytosol, citrate is cleaved by ATP citrate lyase (ACLY) into oxaloacetate (OAA) and acetyl-CoA, which is the precursor of some processes, including the synthesis of fatty acids and histones acetylation, required to sustain the rapid division and the epigenetic metabolic reprogramming of cancer cells. We recently evaluated the effect of different concentrations of citrate in hepatoma cells (HepG2) and in non-tumor immortalized human hepatocyte (IHH). Our results suggested a complex effect of exogenous citrate: at low concentration both lipid deposition and Histone H4 acetylation increased, while they decreased at high concentration. In the same condition, ACLY expression decreased in HepG2 cells, while it remained unchanged in IHH cells, thus suggesting that cancer cells, through the epigenetic regulation of ACLY, are able to adapt their metabolism for specific cellular requirements. We proposed a different fate for exogenous citrate, compared to that deriving from the TCA: considering the strong demand for acetyl-CoA but not for OAA, citrate acts as a Trojan horse for cancer cells releasing OAA in the cytoplasm, which can be only removed after reduction to malate by NADH produced during the glycolisys1. We performed further experiment to prove that hypothesis. What would it happen if NADH was not the limiting factor for the removal of oxaloacetate from the cytoplasm? To answer this question we repeated experiment in presence of inhibitors of lactate dehydrogenase, which consumes NADH to reduce pyruvate deriving from glycolysis. Results partially confirm what stated, and suggest a fine biochemical regulation of metabolism in cancer cells. Previously published in: 1. Petillo A et al (2020) Front Mol Biosci7:593866

Immune and inflammatory disorders

P-08.3-01
Functionalization of reconstituted HDL for therapy in cardiovascular disease

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Aim: ApoA-I containing HDL lipoproteins are the major cholesterol acceptors from extra-hepatic tissues. In normal condition, their role in reverse cholesterol transport (RCT) favours lipid homeostasis. Furthermore, they are one of the key effectors in atheroprotection when dyslipidemia occurs and accordingly they have been widely studied [1]. Nowadays, *in vitro* reconstituted-HDL (rHDL) can be adequately produced and applied in biomedicine research. Referred to the cardiovascular field, it has been demonstrated that treatments based on rHDL are beneficial both in vitro and in animal studies. However, despite promising, rHDL infusions have failed to reach the prespecified efficacy endpoints when mortality benefit has been tested in clinical trials [2]. Therefore, with the aim to overcome this handicap, we have set up a methodology to functionalize rHDL with a microRNA in order to improve their therapeutic outcomes. Methods: *In vitro* production of rHDL was studied by mixing apoA-I with different lipids and microRNA. Biophysical characterization was performed by circular dichroism, dynamic light scattering and electron microscopy; and cholesterol efflux assays on macrophages were carried out to analyse their potential on RCT. Results: We have obtained stable rHDL that have been functionalized with microRNA to favour RCT from foam cells. Conclusions: Functionalized rHDL are suitable molecule-nanocarriers that efficiently enhance cholesterol efflux from foam cells. Meanwhile, delivery efficiency of the encapsulated molecules can be tracked and quantified thus highlighting their potential use in theranostics. In addition, labelling different molecules within the particle will offer the advantage of traceability by imaging if infused. [1] Rosenson RS et al. (2012) Circulation 125(15), 1905-1919. [2] Karalis I and Jukema J (2018) Curr Cardiol Rep 20(8), 66.

P-08.3-02
Intense physical exercise induces an anti-inflammatory change in IgG N-glycosylation profile

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Exercise is known to improve many aspects of human health, including modulation of the immune system and inflammatory status. Despite the general understanding that exercise reduces inflammation, the relation of the two is not yet fully understood.

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N-glycosylation of immunoglobulin G (IgG) and total plasma proteins was previously shown to reflect changes in inflammatory pathways, which could provide valuable information to further clarify effects of exercise. In order to better understand the relationship between physical activity and inflammation, we examined the effect of intense exercise, in the form of repeated sprint training (RST), on IgG and total plasma proteins N-glycosylation. Twenty-nine male physical education students were separated into treatment (RST, N = 15) and control (N = 14) groups. The RST group completed a 6-week exercise protocol while the control group was instructed to refrain from organized physical activity for the duration of the study. Three blood samples were taken at different time points: prior to start of the training program, the final week of the exercise intervention, and at the end of the four-week recovery period. Following the recovery period IgG N-glycosylation profiles showed anti-inflammatory changes in RST group compared to the control group, which manifested as a decrease in agalactosylated and an increase in digalactosylated and monosialylated N-glycans. Observed changes show the potential of intense physical exercise to reduce levels of systemic basal inflammation, as well as the potential for IgG N-glycosylation to serve as a sensitive longitudinal systemic inflammation marker.

P-08.3-03
N-glycosylation of plasma proteins and immunoglobulin G in multiple sclerosis

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Multiple sclerosis (MS), a disease affecting the central nervous system, is characterized by demyelination and inflammation. The mechanism underlying the development of MS is not yet fully understood, but it is considered to be influenced by both genetic and environmental factors. N-glycosylation, a highly regulated process, is one of the most complex co- and post-translational modifications. N-glycans do not only increase protein structural diversity but can also modify their function. Multiple studies have shown the importance of N-glycan changes in different autoimmune and inflammatory diseases, which highlights N-glycans as potential diagnostic and/or prognostic biomarkers. Even though plasma protein and immunoglobulin G (IgG) N-glycosylation changes are well investigated in many diseases, the studies on N-glycan changes in MS are scarce. Hence, we aimed to investigate N-glycosylation patterns of plasma proteins and IgG in MS. We have analyzed plasma and IgG N-glycosylation profiles in 83 multiple sclerosis cases, 88 age- and sex-matched controls and 85 sex-matched controls, but who passed the majority of lifetime risk for MS development (>70 years of age). Core fucosylation showed to be the most prominently altered IgG glycosylation trait, as it was significantly decreased in MS subjects compared to the healthy controls (adjusted $P = 0.0069$). Among all generated plasma glycosylation traits, the most significant changes were observed in antennary fucosylated and high branched N-glycans, which were both increased in the MS group (adjusted $P = 0.0067$ and 0.0219, respectively). On the other hand, low branched N-glycans were significantly decreased in MS subjects (adjusted $P = 0.0167$). Our results demonstrated that plasma protein and IgG N-glycosylation markedly changes in multiple sclerosis. However, additional studies are needed to determine the background of these changes, their role in multiple sclerosis development and their potential use as diagnostic biomarkers.

P-08.3-04
Low-dose model of allergy in mice

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The development of type I allergy, mediated by the formation of IgE to safe respiratory and food allergens, is directly related to the use of an adequate animal model. We previously showed that patients with allergy had significantly increased titers of specific IgE, but not IgG or IgA, compared to healthy controls. Current mouse models of allergy use high-dose adjuvant priming, which leads to the formation of both IgE and IgG to the target allergen. Since natural sensitization is induced by very low concentrations of allergens, it is necessary to develop a model of mouse sensitization without high-dose priming. To develop such a model, we used BALB/c mice, which were immunized with allergens in a phosphate buffer in the withers, intraperitoneally (ip) or foot pad (fp) in doses of 0, 100, and 10000 ng/injection two to three times a week for a long time. Ovalbumin (OVA) was used as the model allergens. No IgE antibodies were formed during fp immunization of BALB/c mice. The formation of IgE in BALB/c mice was registered during ip immunization or after immunization into the withers, starting after 17–20 injections. IgE titers increased upon more prolonged immunization. Maximal IgE titers were observed after immunization into the withers and in the dose of 100 ng/injection for all model allergens, which decreased by an order of magnitude at immunization dose of 10000 ng/injection. Formation of IgG was recorded in both BALB/c and C57BL/6; it directly depended on the immunizing dose and appeared starting from 100 ng/injection . The dominant class was IgG1. Accordingly, to form a humoral response, as close as possible to the one observed in patients with allergies, it is recommended to inject an allergen without adjuvant into the withers of BALB/c mice at a dose of 50–200 ng/injection two to three times a week during 1–1,5 months. Foundings: "The reported study was funded by RFBR according to the research projects № 19-015-00099 and 19-05-50064».

P-08.3-05
Effects of high-fat diet on functional and molecular metabolic parameters in rats with constitutionally altered serotonin homeostasis

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Serotonin (5HT) is bioamine implicated in many fundamental biological functions including maintaining of energy homeostasis. Recent studies in mice suggest that pharmachological inhibition of peripheral 5HT synthesis protectes against obesity and type 2 diabetes, however little is known about role of endogenous 5HT tone in development of obesity and obesity-related health conditions. Here we investigated the relationship between endogenous 5HT activity and body weight regulation using sublines of rats with constitutionally up-regulated (high-5HT) or down-regulated

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(low-5HT) blood 5HT leves. Male rats from both sublines were fed a high fat diet (HFD, 45% kcal from fat) for 11 weeks. Control diet (CD)-fed high 5HT rats had elevated body weight as compared to CD-fed low 5HT rats, however only low-5HT rats showed significantly increased body weight in response to HFD-feeding. In line, impaired glucose and insulin tolerance in response to HFD-feeding was observed only in low-5HT animals. Blood cholesterol, trygliceride and leptin levels were in both sublines increased by HFD-feeding while blood levels of insulin and glucagon were affected by HFD-feeding only in low-5HT rats. HFD had no effect on platelet 5HT levels, but it significantly increased 5Ht uptake rates in platelets of low-5HT rats. Brown adipose tissue thermogenesis, as measured by infrared thermography, was in both sublines similarly elevated by HFD-feeding while expression levels of multiple energy homeostasis-related genes in hypothalamus and adipose tissue were affected by HFD-feeding only in low-5HT rats. Our results suggest that low-5HT rats are more susceptible to deletorious effects of HFD than high-5HT animals. Research was funded by Croatian Science Foundation, grant no IP-2014-09-7827

P-08.3-06
Catalytic immunoglobulins from the blood of schizophrenia patients

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Damage-associated molecular patterns (DAMPs) such as DNA, RNA, histones are known to induce immune responses. DAMPs release from the cells during apoptosis, NETosis, pyroptosis, necrosis and other process of cell death. Various evidence of an enhanced inflammatory response has been found in schizophrenia (SCZ). Here, we present the evidence that polyclonal IgG isolated from serum of SCZ patients not only bind nucleic acids (DNA, RNA and microRNA), but also hydrolyze these antigens. Using different criteria we confirmed experimentally that this nuclease activity belongs to antibodies itself. IgG preparations were obtained by affinity chromatography and FPLC and analyzed by MALDI MS. Nuclease activity of IgG were revealed by the degree of hydrolysis of DNA, RNA and fluorescent labeled microRNAs as a substrate. The products of the hydrolysis were analyzed by electrophoresis. DNase activity was observed in 80% of IgGs in schizophrenia. RNase activity was detected in 100% of IgGs of patients. It has been shown that IgGs efficiently hydrolyze total RNA from neuronal T98G cells. There was revealed site-specific hydrolysis of microRNAs associated with SCZ (miR-137, miR-9, miR-219). The kinetic parameters of nuclease reactions catalyzed by IgG were significantly lower than that for native nucleases. Interestingly, the level of nuclease activity correlated with the clinical parameters of SCZ. The generation of catalytic IgG with nuclease activities is new evidence of immunological derangements in SCZ. This can be used as a diagnostic marker for stratification of patients and administration of anti-inflammatory therapy. Besides, we assume that IgG with nuclease activity are involved in the removal DAMPs (DNA and RNA), from the circulation, thereby reducing activation of pattern recognition receptors and reducing the inflammation. This work was supported by RFBR grant (20-015-00156) and scholarship of the President of the Russian Federation (SP-2258.2019.4).

P-08.3-07
Ubiquitin signalling in inflammation

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It is commonly accepted that tight regulation of proteostasis is key to ensure intact cellular function. Posttranslational modifications (PTMs) such as ubiquitination give rise to a complex layer of regulation. PTMs regulate protein function by orchestrating maturation, stability, localization and (in)activation. Ubiquitin regulatory versatility arises through the formation of ubiquitin chains upon ubiquitination of one of the 7 internal lysin residues or the N-terminal methionine. Specificity of chain types allows for a specific ubiquitin code comprised of at least eight different types of ubiquitin chains: Lys6, Lys11, Lys27, Lys29, Lys33, Lys48, Lys63 and Met-1. In addition to its well-studied function as a marker for proteasomal degradation, linear ubiquitin-chains, arising from Met-1 coordinated linkage, are required for activation of NFkB signalling during innate immune response. Dedicated writers, readers and erasers (dis)assemble the code targeting various substrates. Since the discovery of Met-1 chain dependence of innate immune signalling, the mode of action of the respective ubiquitin modifying enzymes has been readily studied, leading to the discovery of several important regulators of inflammatory response whose absence is connected to chronic inflammatory disease. Using an integrated structural, biochemical and biophysical approach, we aim to understand, at the mechanistic level, how the ubiquitin code is regulated and how the ubiquitin signal controls inflammatory signalling pathways. Structure guided point mutations can be generated to dissect complicated biological pathways without abrogating the general function in the overall physiological context. The overall aim is to unravel regulation of protein function within the cell. By adding to the understanding of ubiquitin signalling in inflammation we aim to contribute towards deciphering how dysregulation leads to disease.

P-08.3-08
Combination of xanthohumol and phenethyl isothiocyanate inhibits NF-κB more effectively than single compounds in pancreatic cancer cells

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Chronic inflammation is an important risk factor for pancreatic cancer (PC). Survival rates in PC patients remain poor, thus searching new strategies of prevention and therapy is still needed. Phytochemicals, such as phenethyl isothiocyanate (PEITC) and xanthohumol (XAN), were shown to have anti-inflammatory and anti-tumorigenic activities in different PC cell lines. Recent studies indicate that using combinations of phytochemicals is a more efficient strategy of cancer chemoprevention. In this study we evaluated the effects of XAN and PEITC, alone and in combination, on the activation and expression of NF-κB, cell cycle distribution and apoptosis in human pancreatic cancer cell line PSN-1. The cells were treated with XAN and PEITC, alone or in combination, at the concentration selected based on MTT assay. The

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activation of NF-κB was assessed by measuring the level of binding of its active subunits p65 and p50 to specific DNA sequence using the ELISA assay, and their translocation from cytosol to nucleus by Western blot. The Muse Cell Analyzer was used for flow cytometric analysis of apoptosis and cell cycle progression. Gene expression was measured by RT-PCR, protein level by Western blot and Luminex MAGPIX® System. Both tested compounds, alone and in combination, reduced the activation and expression of NF-κB resulting in diminished expression of COX-2. Moreover, lower level of IKK was observed after treatment with combination of compounds. Cell cycle analysis showed the induction of cell accumulation in S/G2 phase and the increased level of late apoptosis. These results indicate that combination of XAN and PEITC more effectively inhibit NF-κB than single compounds and may induce cell cycle arrest and apoptosis in pancreatic cancer cells. Thus, the mixture of these phytochemicals can be considered as a potential chemopreventive and/or supporting conventional therapy strategy in the prophylaxis of PC. Funding: Polish NCN 2016/21/B/NZ5/01390.

P-08.3-09
Obesity is associated with male secondary hypogonadism, altered fertility and lipid homeostasis

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Introduction: Obesity is an inflammatory disease associated with male secondary hypogonadism, decrease sperm concentration, motility, altered sperm morphology and hormonal profile. There is a close relationship between lipid metabolism, development of gametes and fertility. Objectives: To study the sperm characteristics and changes in reproductive hormones in obese men after bariatric surgery and obese mice after low fat diet. Material and Methods: Twenty severely obese men (body mass index (BMI) $\geq$35 kg/m^2) were evaluated before and 2 years after bariatric surgery. The obese mice model was: 1) LFD 10 weeks; 2) HFD 10 weeks; 3) LFD 18 weeks; 4) HFD 18 weeks; 5) HFD 10 weeks and LFD 8 weeks. Results: Obese patients showed decreased serum total testosterone, calculated free testosterone and leptin, whereas glucose and fasting insulin, HOMA-IR, were increased. After surgery, serum total testosterone, calculated free testosterone, inhibin B, and kisspeptin increased, whereas fasting insulin, HOMA-IR, and leptin concentrations decreased. Despite these improvements, the low sperm concentration and altered sperm characteristics remained unchanged after surgery in 60% of the obese patients. HFD mice showed insulin resistance, altered testis morphology, increased plasma cholesterol, HDL and LDL which recovered when the HDF replaced by LFD. Plasma testosterone and spermatozoa concentration decreased in HFD at 10 and 18 weeks compared to LFD, and remained low in HFD+LFD. There was not reversion of plasma testosterone levels and spermatozoa concentration with the change of the diet. Conclusions: Obese patients and mice do not improve the low sperm concentration after bariatric surgery or diet, despite the beneficial changes of reproductive hormones.These alterations may underlie the infertility associated to obesity. Acknowledgement: CIBEROBN. Financed by PI16/00154. Instituto de Salud Carlos III. Ministerio de Economía y Competitividad, Spain. E-mail: m.e.casado4@gmail.com; antonia.martin@hrc.es

P-08.3-10
New biochemical markers of HIV infection – bispecific antibodies and its catalytic activity

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The search for new markers of viral and autoimmune diseases is an important problem of modern medicine. Autoimmune processes may cause severe damage to the nervous system, particularly it is relevant for the natural catalytic antibodies hydrolyzing neuro specific substrates. We have isolated antibodies hydrolyzing myelin basic protein, histones, and myelin oligodendrocyte glycoprotein from the blood of the patients with systemic lupus erythematosus, multiple sclerosis, and HIV/AIDS. Also we have shown that the blood, milk, and placenta of healthy donors, as well as the blood of the patients with systemic lupus erythematosus and multiple sclerosis, contain natural kappa-lambda bispecific antibodies. Here we compare the catalytic activities of natural bispecific antibodies of the blood of patients with autoimmune disorders. According to our recent results, the content of bispecific kappa-lambda IgG in HIV/AIDS is significantly higher than in the blood of healthy donors. Since that we cannot exclude that the similarity of pathological processes in HIV/AIDS and in autoimmune diseases like multiple sclerosis and systemic lupus erythematosus might be due to content and activity of bispecific antibodies. Comparison of bispecific antibodies content and its catalytic activity in HIV/AIDS and other autoimmune diseases may play a role in the determination of the HAART effectiveness and of particular interest for personalized medicine. The research was supported by Grant of RFBR № 20-34-70115.

P-08.3-11
Targeting extracellular vesicles for protein delivery into macrophages and dendritic cells

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Proteins and peptides are promising therapeutics for a wide range of diseases, including autoimmune disorders. Multiple sclerosis (MS) is one of the most prevalent autoimmune neurodegenerative diseases, affecting young adults. Previously we described

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prospective therapeutic, which successfully passed the second phase of clinical trials – Xemys. The drug medicine consists of the myelin basic protein (MBP) fragments encapsulated in mannosylated liposomes to increase the efficiency of delivery to dendritic cells and the subsequent induction of autotolerance. One of the main disadvantages of this potential medicine is the need for lifelong multiple subcutaneous administrations of the drug. The usage of genetically-encoded extracellular vesicles (GEEV) can solve this problem. GEEV represents highly ordered protein nanocages enveloped with a cell's membrane. Described vesicles are able to release from eukaryotic cells and deliver the desired protein. When using immunodominant MBP peptides as a therapeutic agent of GEEV, it is necessary to deliver drug to the antigen-presenting cells – dendritic cells and macrophages. These cells are characterized by high level of CD206 expression. Thereby we developed genetic constructs for the production of extracellular vesicles with anti-CD206 antibodies anchored on the membrane surface and tested the ability of the obtained GEEV to deliver proteins into target cells. Preliminary experiments demonstrated that almost all targeted proteins were delivered in $CD206^+$ cells from human peripheral blood. Besides delivery to $CD206^+$ cells by targeted GEEV was more effective in comparison with non-targeted GEEV. We developed targeted GEEV loaded with therapeutic MBP peptides that allow to examine this drug on the animal model of MS. The reported study was funded by RFBR, project number 20-315-90115.

P-08.3-12
Structural insight in the complex between major histocompatibility complex class II molecules and invariant chain

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The adaptive immune response relies on recognizing invading pathogens by their unique protein signature generated by professional antigen presenting cells. In these cells, the peptides generated by endosomal processing enzymes are loaded onto major histocompatibility complex class II molecules (MHCII) and presented at the cell surface for recognition by T-cell receptors. MHCII molecules are membrane anchored heterodimers consisting of α and β chains. They assemble in the endoplasmic reticulum where they associate with a chaperone like protein, the invariant chain (Ii), thus forming presumably a nine-subunit complex consisting of three αβ dimers and Ii trimer. Roche PA et al. (1991) Nature 354, 392-394 primarily reported the stoichiometry of nonameric MHCII-Ii complex, nonetheless, pentameric and heptameric assembly of MHCII-Ii was also documented by Cloutier M et al. (2014) Immunol Cell Biol 92(6), 553–556. The MHCII-Ii complex is then transported into the late endosomal compartment, where it is further processed by the lysosomal cysteine proteases (Hsing LC and Rudensky AY (2005) Immunol Rev 207, 229–241). The processing scheme of MHCII-Ii was established quite well by cell biology studies, but at the molecular level the detailed mechanism of its processing and its synchronization with antigen processing is not yet understood. Structural insight of the MHCII-Ii complex will help us explain the steps of MHCII maturation and loading, whereas biochemical insight in Ii processing may reveal importance of cysteine proteases. We have established a system for soluble production of the MHCII-Ii complex in insect cells. Using cryo-electron microscopy we have obtained preliminary 3D structure of the MHCII-Ii complex, but the optimization of sample and data collection are still ongoing. *The authors marked with an asterisk equally contributed to the work.

P-08.3-13
microRNA-19b-3p as a biomarker of chronic obstructive pulmonary diseases

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Chronic obstructive pulmonary disease (COPD) is a respiratory condition characterized by the persistent inflammation and destructive processes in the airways and pulmonary parenchyma. Despite the fact that the main mechanisms of COPD are already known, many molecular aspects remain unclear, including the participation of miRNAs in the development of the disease. MicroRNAs are a large class of molecules involved in post-transcriptional repression of genes and regulated many biological processes. The aim of this study was to evaluate the expression of cell-free circulating miR-19b-3p in the blood plasma of patients with COPD. The study included 50 COPD patients aged 59 to 82 years. The material was collected in the Pulmonology Department of the City Hospital № 2 (Nur-Sultan). A diagnosis of COPD has been made in accordance with the recommendations of the Global Initiative for COPD. The control group consisted of 37 healthy individuals. Plasma miR-19-3p expression was determined by qRT-PCR with using the comparative threshold cycle (Ct) method. U6 small nuclear RNA was taken as endogenous control. We found that a level of cell-free circulating miRNA-19b-3p in the blood plasma of COPD patients was significantly 2.3 times higher compared with the control group ($P < 0.0001$). Thus, miR-19b-3p is differentially expressed in patients and healthy individuals, and can be considered as a biomarker of COPD.

P-08.3-14
Effects of inflammation in neuronal tissue on the level of molecular markers in drug-resistant epilepsy

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Temporal lobe epilepsy is considered to be the heaviest form of epilepsy, which cannot be treated by anticonvulsant drugs. The one of theories of drug-resistant epilepsy's genesis is neuroinflammation. Resistance to anticonvulsant drugs may be caused by neuroinflammation, that affected neuronal tissue. Due to neuroinflammation, AMPA and NMDA receptors are damaged by cytokines, produced by immunocompetent neuronal cells. Our aim was to identify neuroinflammation markers in brain temporal cortex in patients with drug-resistant epilepsy. We studied

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temporal cortex biopsies obtained from patients with drug-resistant epilepsy (experimental group), and temporal cortex biopsies from patients with traumatic brain injury but without autoimmune diseases (observational group). We investigated expression of proinflammatory cytokine TNF-α, TNF receptor CD95, transcription factor STAT1, and antiapoptopic factor p53 in cortex epileptic focus and perifocal zone. Data was analyzed via R jamovi (Version 1.2) software. Datasets were assessed using Shapiro-Wilk normality test to determine significant deviations from a normal distribution. We obtained increase of expression of TNF-α and its receptor CD95, which leads to increase of transcription factor STAT1 level in the epileptic focus. These changes can be evidence of neuroinflammation process in cortex epileptic focus. We may suggest that STAT1 dimerizes and up-regulates transcription of p53 locus, because we have shown high level of p53. So, this increase of proinflammatory proteins level in epileptic focus demonstrates activation of apoptosis by extrinsic and p53-mediated pathways. This pathogenesis may be the cause of neurodegeneration. We obtain similar data in perifocal zone of cortex, however, CD95 level is lower. Probably, TNF-α bonds its receptor, and it may explain this low level of CD95 and can be evidence of development and progression of adjacent tissue inflammatory damage. This work was supported by RFBR № 20-015-00127. *The authors marked with an asterisk equally contributed to the work.

P-08.3-15
Cystatins in inflammasome activation and sepsis

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Progressive myoclonus epilepsy of Unverricht–Lundborg type (EPM1) is an autosomal recessively inherited neurodegenerative disease, characterized by the cerebellar granule neurons apoptosis, progressive ataxia and myoclonic epilepsy. Mutations in the cysteine proteinase inhibitor stefin B/cystatin B (CTSB) are found in patients with EPM1[1]. CSTB gene is located on human chromosome 21 and it is overexpressed in the brain of Down Syndrome individuals. Stefin B was shown to interact with cathepsin L and histones H3, H2B, and H2A.Z in the cell nucleus. A mouse model of the EPM1 disease, stefin B deficient mice, recapitulates the principal symptoms of EPM1, myoclonic seizures and progressive ataxia. Stefin B-deficient mice were found more sensitive to lipopolysaccharide (LPS)-induced sepsis as a consequence of increased expression of caspase-11 and nucleotide-binding oligomerization domain-like receptor 3 (NLRP3) inflammasome activation and higher levels of mitochondrial reactive oxygen species (ROS) [2]. In addition we determined that the the lack of stefin B leads to a significant increase in the expression of the mitochondrial antioxidant proteins to LPS challenge [3]. In our study we used stefin B deficient mice (StB KO), as well as mice with an additional copy of stefin B gene, stefin B- trisomic mice (StB 3n). In macrophages from stefin B trisomic mice we determined lower caspase 11 expression and non canonical inflammasome activation. Stefin B suppressed mammalian target of rapamycin (mTOR) activity and induced autophagic activity for mitophagy by increase of unc-51 like autophagy activating kinase 1 (Ulk1) phosphorylation. As a conclusion, we propose that stefin B plays an important role in regulation of autophagy and non canonical inflammasome activation. [1] Pennacchio LA, et al. (1997) Science 271, 1731–1734. [2] Maher K, et al. (2014) J Biol Chem 289, 31736–31750. [3] Trstenjak Prebanda et al (2019) Cells, E1476.

P-08.3-16
Study of the immune system under emotional stress

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The purpose of this study was to study the state of the immunological reactivity of the organism in the early period after emotional stress.Two series of experiments on white rats, weighing 200–250 gr, were performed. I gr.-intact, II gr.- those who suffered emotional stress. It was found that the number of leukocytes in animals of gr. II was increased by 41.49% ($P < 0.05$) in comparison with gr. I and the number of lymphocytes increased by 60.07% ($P < 0.01$). The number of CD3+ lymphocytes in experimental animals increased by 1.18 times ($P < 0.05$), on the part of CD4+ lymphocytes the total number of lymphocytes increased by 31.57% ($P < 0.05$). On the CD8+ side of lymphocytes, the same picture was revealed: in gr. II was an increase of 24.07% ($P < 0.05$). The analysis of research results showed that at the early stages of stress in animals, the number of CD19+ lymphocyte in the blood increased by 1.65 times correspondingly in comparison with control ($P < 0.001$). Changes were observed in the nonspecific phagocytic immunity link in the body. Thus, an hour after stress, an increase in nonspecific phagocytic immunity link was observed in animals. Phagocytosis (59.32 ± 4.36) and phagocytic number (2.53 ± 0.20) were correspondingly reliably above the control parameters (36.10 ± 2.46) and (1.39 ± 0.11), the NBT-test parameter increased from 4.80 ± 0.37 to 8.48 ± 0.64 ($P < 0.01$), that testifies to activation of functional activity of blood neutrophils in stressed animals. The functional activity of immunity T-system was studied using LMIR reaction to PHA, where the leukocyte migration index was determined in response to PHA exposure. The decrease of this index from 0.86 ± 0.05 to 0.65 ± 0.05 testified to the increase of MIF producing capacity of leukocytes, which determined the functional activity of the T-system of immunity. The conducted researches have shown that activation in all indexes, both in cellular and nonspecific phagocytic links of immunity, is observed at the early period of emotional stress. *The authors marked with an asterisk equally contributed to the work.

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P-08.3-17
Safety assessment of novel food: the use of multiplex analysis in immunological researc

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The model of systemic anaphylaxis caused by intraperitoneal administration of ovalbumin (OVA) to Wistar rats has been traditionally used for the research of genetically modified food allergenic potential in the Russian Federation. For the expanding of anaphylaxis indicators list the model studies to determine rats cytokine profile were conducted. In the 31-day experiment were used 35 males, at the beginning of the study they were 6-week old. The animals were divided into 6 groups-control (10 rats) and 5 test groups (5 rats each). The test groups were sensitized by intraperitoneal administration of OVA on 1,3,5 and 21st days of the experiment. The rats of the control group were injected with a physiologic saline instead of OVA. On the 29th day the 1–5 test groups animals got intraperitoneal administration of OVA increasing dose-12, 24,36,48,60 mg/kg, respectively. The Bio-Plex Pro Rat Cytokine 23-Plex (Bio-Rad) panel and Luminex 200 multiplex analyzer were used to determine cytokines in serum samples. In this publication is presented analysis of the Wistar rat cytokines profile after intraperitoneal OVA sensitization. All statistical analyses were conducted with SPSS Statistics. Statistical significance assessed with a Mann-Whitney and Kruskal-Wallis. Statistical significance was defined as $P < 0.05$. In all test groups was observed the increase of the indicators, typical for inflammation pathogenesis: in the 1st group-IL-6, TNF-α, MIP1α, MIP3α, RANTES; in the 2nd group-IL-5, IL-6, IL-12, Inf-γ, MIP1α, TNF-α, GM-CSF; in the 3rd group-MIP1α, TNF-α, GM-CSF, Inf-γ; in the 4th group-G-CSF, GM-CSF, IL-1α, MIP1α, MCP1; in the 5th group-G-CSF, GM-CSF, M-CSF, MCP1, MIP1α, TNF-α. Therefore, in the research of systemic anaphylaxis simulation was obtained a new information on rats' cytokine profile changes and immune status disorders. This work was carried out within the state assignment of Ministry of Science and Higher Education of the Russian Federation (theme No. 0529-2019-0056).

P-08.3-18
Expression of the Th17/Treg-related transcriptional factors in the whole blood in patients with rheumatoid arthritis

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The induction and progression of rheumatoid arthritis (RA) are characterized by an abnormal Treg response with a shift towards a Th17 response. Our aim was to investigate Th17/Treg-related transcriptional factors expression in the whole blood and characterize their clinical significance. 45 RA patients, 27 osteoarthritis (OA) patients and 46 healthy subjects were recruited into the study. Flow cytometry was used to analyze the frequencies of Th17 and Treg cells. The expression of FOXP3, RORc2, SMAD2/3/4, SOCS1, HIF-1A, STAT3 and STAT5 was analyzed by real-time PCR. Concentrations of IL-17A/F, IL-21, IL-22, IL-6, IL-10, IL-35, IL-2, IL-23, TGF-β, and IFN-γ were measured by specific ELISAs. A higher ratio of Th17/Treg was present in the peripheral blood of RA patients compared to OA and healthy subjects. Expression of the HIF-1A, SOCS1, SMAD3, STAT3, and STAT5a was higher RA, while expression of the FOXP3 and RORc2 was lower in RA than in OA. A high positive correlation between HIF1 and SMAD3, HIF-1 and STAT3, SMAD3 and STAT3 was observed in RA and OA patients. In OA patients, FOXP3 and RORC correlated strongly. A positive correlation was observed between the Th17/Treg ratio and STAT5a expression. We observed increased STAT5a expression in rheumatoid factor (RF) positive RA. A significant correlation between FOXP3 and RORc was detected in RA patients without RF, while in RA patients with RF a high correlation between STAT5a and SMAD2 and SMAD4 was detected. The serum level of IL-2, IFN-γ, IL-17, and IL-21 was higher in RA patients than in OA patients. A significant positive correlation was detected between STAT3 expression and serum IL-6 levels in RA patients, and between SMAD2 expression and serum IL-35 levels in OA patients. Moreover, our data revealed that SMAD3 and STAT3 may be possible diagnostic biomarkers for RA. Supported by grant 2015/19/B/NZ5/00247 from National Science Centre, Poland.

P-08.3-19
Intracellular and cell free circulating microRNA involved in Treg/Th17 imbalance in patients with rheumatoid arthritis

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Rheumatoid arthritis (RA) is a disease characterised by progressive inflammation of the joints which leads to irreversible destruction of bone and cartilage. Epigenetic factor has an important impact on the autoimmune disease pathology. miRNAs are one of the components in the gene expression networks that govern lymphocytes differentiation and establish central tolerance. On the other hand, the origin and function of circulating miRNAs in various body fluids are still not well understand. The aim of this study was to determine the profile expression of selected microRNA in serum and in Treg and Th17 lymphocytes in rheumatoid arthritis (RA), osteoarthritis (OA)patients and healthy control (HC). The expression level was analyzed based on miRCURY SYBR Green probes and ΔCt method. miR-100 and miR-326 were at a very low level both in Treg and Th17 in RA, OA and HC. In HC miR-24 and miR-31 levels were significantly higher in Th17 cells in comparison with Treg. In Treg cells, miR-26 level was significantly upregulated in HC when compared to this observed in RA. In the case of Th17 cells, HC was characterized by a significantly higher level of miR-155 than RA patients. In RA and OA patients miR-146a was significantly higher in Treg than in Th17 cells. Interestingly, miR-146 was undetermined in serum in most RA patients. miR-10 was detected only in RA serum but not in OA and HC. Interestingly, high negative correlation between the expression of miR-10 and miR-155 in RA serum has been noted. Higher level of miR-10 has been observed in RA patients with high disease activity, but

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the difference was not statistically significant. RA patients with present rheumatoid factor (RF) were characterized by a significantly higher level of miR-326 in serum than patients with negative RF. Therefore high miR-146a expression leads to increase Treg suppressor function we suggest that miR-146a may be a therapeutic target for RA. Funded: Polish National Science Centre 2015/B/NZ5/00247.

P-08.3-20
The effectivity of triggering of anti-viral response in cells by nonstructural protein 1 of tick-borne encephalitis virus of different subtypes

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Tick-borne encephalitis virus (TBEV) infection treatment remains a problem of healthcare. For prophylaxis of TBEV the vaccines based on killed viruses of European or Far Eastern subtypes are used, meanwhile therapy includes only symptomatic treatment. For effective specific therapy it is important to understand the molecular aspects of the pathogenesis of the virus at the early stage of infection. Among the primary markers of infection, the detection of nonstructural protein 1 (NS1) in the blood can be highlighted. It also should be pointed that severity and the case fatality rate of disease are correlated with the virus subtype. In a current study the ability of NS1 of European, Siberian and Far Eastern subtypes to trigger cellular response to viral infections was compared. Activation of immunoproteasomes, oxidative stress, cytokines and related factors were evaluated in NS1 expressing cells. Differences in NS1 protein accumulation levels, ROS production, expression of oxidative stress response genes and some transcription factor genes were observed. NS1 of Far Eastern subtype was accumulated in cells at least at a fold lower level. Using proteasome inhibitors its higher proteasome degradation rate was shown. Interestingly the lowest expressed NS1 of Far Eastern subtype induced the highest levels ROS production and Nqo1 gene expression. The differences in expression of GATA3 (Th2 cells -immune response transcription factor) for Far Eastern NS1 were also detected. Among TBEV subtypes the Far Eastern subtype tends to cause most severe disease with a highest fatality rate. Obtained results pointed on NS1 importance in disease progression that raises the significance of development of NS1specific therapeutics for TBEV infection. The reported study was funded by RFBR, project number 20-04-00766.

P-08.3-21
The Sirtuin 2 inhibitor suppresses allergic airway inflammation in mast cell-mediated asthma

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Asthma is characterized by airway hyperresponsiveness and allergic inflammation, which aggravate the patient's life quality. Therefore, drug development for the treatment of asthma is needed for a healthy life. Recent studies have demonstrated that sirtuin2 (SIRT2) aggravates asthmatic inflammation by up-regulation of T-helper type 2 responses and macrophage polarization, but not demonstrated the mast cell activation. In this study, we investigated that AGK2 as an inhibitor of SIRT2 regulates mast cell-mediated allergic airway inflammation *in vitro* and *in vivo*. In mast cells, pre-treatment of AGK2 inhibited degranulation of mast cell, and the expression of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-1β, IL-4, IL-5, IL-6 and IL-8 through inhibiting FcεRI signaling pathway via suppressing Lyn, Syk, PI3K, PLCγ, Akt, IκBα, and NF-κB. To verify the effect of AGK2 on mast cell activation *in vivo*, we used the anaphylaxis and allergic asthma model. In passive cutaneous anaphylaxis and acute lung injury models, AGK2 attenuated Evans blue pigmentation by mast cell activation and the lung barrier dysfunction by inflammatory responses in respective animal models. In ovalbumin (OVA)-induced allergic airway inflammation murine model, AGK2 alleviated airway constriction, immune cell infiltration and mast cell activation in the lung. AGK2 reduced the level of pro-inflammatory mediators such as b-hexosaminidase, myeloperoxidase and eosinophil peroxidase, and pro-inflammatory cytokines including TNF-α, IL-1β, IL-4, IL-5 and IL-6 in bronchoalveolar lavage fluid and lung tissues. AGK2 also decreased the level of immunoglobulin (Ig) such as IgE, OVA-specific IgE, IgG1 and IgG2a in serum. In conclusion, our findings suggest that SIRT2 could facilitate the target of mast cell-mediated airway inflammatory diseases. Therefore, AGK2 could be candidates for treating allergic asthma and lung inflammation.

P-08.3-22
Legumain as a modulator of immune response

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Legumain or asparaginyl endopeptidase (AEP) is a member of the CD clan of cysteine proteases and cleaves protein substrates exclusively after asparagine or (to a minor extent) after aspartic acid residues. Mammalian legumain is abundantly expressed in immune cells, however its exact role in molecular mechanisms of immune response remains largely unknown, since only a few of its physiological substrates have been identified to date. We performed a comprehensive proteomic analysis of tissue samples from legumain null mice and detected significant upregulation of antimicrobial peroxidases. Peroxidases are commonly expressed in various types of immune cells where they catalyse the formation of hypohalous acids which are detrimental for invading pathogens. Our further degradomic analysis also revealed that legumain regulates degradation of chitinase-like proteins (CLPs), which are potent effectors of immune cell function and are known to regulate inflammation as well as immune response against infection. Our experiments have shown that legumain deficiency causes accumulation of CLPs, which than induce the expression of peroxidases in immune cells. This mechanism could be the cause of increased inflammation observed in legumain

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deficient mice and might also influence the ability of the immune system to clear pathogen infection.

P-08.3-23
Comparison of saliva and plasma antioxidant markers in patients with periodontal diseases

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Chronic periodontal diseases are very common worldwide, leading to the loss of supprotive tissues and teeth, which in turn lead to the restriction of food intake following by nutritional deficiency and associated health complications. The main cause of this disease is the presence of microbial plaque containing bacteria causing inflammatory changes in the gums. Up to date a sample of more than 100 patients with diagnosed gingivitis, chronic and aggressive form of periodontitis has been examined for changes in selected antioxidant markers (superoxide dismutase, glutathione peroxidase, glutathione reductase, reduced glutathione) in saliva and plasma in comparison to healthy individuals. Although the number of patients investigated in each group is low, the nature of the enzyme activity suggests inhibition of glutathione peroxidase due to excess substrate (peroxides) and consequently increased glutathione reductase activity as a compensatory mechanism for using reduced glutathione as a direct reducing agent. However, a possible explanation may also be the assay methodology used, according to which we have determined only the activity of the selenium glutathione peroxidase isoforms, and not the total. The overall activity of glutathione peroxidases can also be increased. It is apparent that changes in the activities of antioxidant enzymes relative to reduced glutathione concentrations compared to healthy individuals indicate oxidative stress conditions. There are also positive correlations between saliva and plasma superoxide dismutase, glutathione peroxidase and glutathione reductase activities. If this would be confirmed in a larger number of patients examined, it would create the possibility of using the results within groups in favor of analyzing more readily available biological material. The study was supported by VEGA 1/0559/18. *The authors marked with an asterisk equally contributed to the work.

P-08.3-24
The relationship between serum concentration of tumor necrosis factor-α and oxidative damage in the renal cortex of rats with type 2 diabetes mellitus

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Tumor necrosis factor-α (TNF-α) is one of the most important mediators involved in the pathogenesis of type 2 diabetes complications. TNF-α is mainly produced in adipocytes and peripheral tissues including renal cells and induces tissue inflammation through the involvement of reactive oxygen spaces. The present study aimed to examine the relationship between serum concentration of TNF-α and oxidative stress biomarkers in the renal cortex of rats with streptozotocin/nicotinamide induced type 2 diabetes mellitus. The animals were divided into two experimental groups (10 animals/group) namely control (C) and diabetes mellitus (DM) and were killed 6 weeks after the induction of diabetes. The raised level of TNF-α was found in the DM group ($P < 0.001$) when compared to control. The serum concentration of TNF-α was in correlation with the specific rate of body mass gain ($r = 0.579$, $P < 0.001$), fasting blood glucose level ($r = 0.549$; $P < 0.001$) and serum fructosamine ($r = 0.388$; $P = 0.019$). Furthermore, the serum concentration of TNF-α was positively correlated to increased concentrations of oxidative stress biomarkers, thiobarbituric acid reactive substances ($r = 0.400$; $P = 0.017$), and advanced oxidation protein products ($r = 0.381$; $P = 0.024$) in the renal cortex of diabetic rats. In conclusion, increased concentration of TNF-α may be related to the increase in oxidative damage of the renal cortex in type 2 diabetes mellitus which might be of importance in developing new therapeutic approaches for treating diabetic nephropathy.

P-08.3-25
A new role of the succinate-SUCNR1 axis in human pregnancy: assessing the effects of gestational diabetes

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Background: Succinate has recently emerged as an extracellular signaling metabolite governing local stress, tissue damage and immunologic danger via its cognate receptor SUCNR1. Increased levels of circulating succinate and disturbed SUCNR1 signaling have been described in obesity and type 2 diabetes, which are directly related to the chronic inflammatory processes that accompany them. The aim of this work is to explore the potential implication of succinate in normal and gestational diabetes mellitus (GDM)-affected pregnancies, and to investigate the possible alterations in the succinate-SUCNR1 axis in umbilical cord and their consequences for fetal health. Methods: 54 pregnant with GDM and 54 with normal glucose tolerance (controls) and their offspring were included. Maternal blood was collected at different points of pregnancy. Cord blood and tissue were collected immediately after delivery. Data related to pregnancy evolution was recorded. Circulating succinate concentration and levels of succinate transporters, SUCNR1 and downstream targets were determined. Results: The highest succinate concentration in maternal blood was observed in the peripartum period, with no differences between GDM and controls. Similar levels were found in umbilical cord blood. Vaginal delivery was associated with higher circulating succinate levels compared with elective caesarean. Maternal and cord blood succinate levels were associated with birth weight and subscapular fold, respectively. Higher succinate release by umbilical cord was also found in GDM but, however, SUCNR1 and its downstream targets PTGS2 and VEGF were downregulated, along with the extracellular succinate transporters SLC13A3 and SLC13A5. Conclusion: Our results suggest a potential physiological role of succinate-SUCNR1 axis at the end of pregnancy. GDM could impair its

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signaling pathway in umbilical cord which might affect the physiological mechanisms of childbirth and have a direct impact on fetal health.

P-08.3-26
Effects of human albumin modified with methyl glyoxal on the functional activity of normal neutrophils *in vitro*

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Neutrophils disfunction in diabetes mellitus (impairment of their chemotactic and bactericidal activity, decreased degranulation and reduced production of reactive oxygen species) could result from stimulation of leukocytes with advanced glycation products (AGEs). Receptor for AGEs (RAGE) is expressed on neutrophil membrane and AGEs/RAGE interaction alters neutrophil responses in calcium-dependent manner. Human serum albumin (HSA), the most abundant protein in blood plasma, can be modified with methylglyoxal (MG), a crucial intermediate of AGEs generation but little is known about direct effects of HSA-MG on human neutrophils. In the current study formation of HSA-MG was registered by fluorescence of Schiff bases and its influence on normal human neutrophils (NP) in vitro was analyzed by luminol chemiluminescence (LCL), scopoletin fluorescence (ScF), IFA assay of exocytosis/degranulation of lactoferrin (LF) and myeloperoxidase (MPO). HSA-MG did not induce neutrophil extracellular traps formation which was demonstrated by microscopy of Romanowsky-stained smears. HSA modified with MG for 3–24 hours (100 ug/ml) stimulated H2O2 production which was recorded by a decrease in SF. EDTA (Ca^{2+} chelator), diphenyleneiodonium chloride (NADPH-oxidase inhibitor), genistein (tyrosine kinase inhibitor) wortmannin (inhibitor of phosphatidylinositol 3 kinase) reduced HSA-MG-stimulated H_2O_2 production by ~85%, ~95, ~80% and ~50% correspondingly indicating possible role of AGEs/RAGE signaling pathway in NP stimulation with HSA-MG. At the same time, phorbol myristate acetate -activated production of HOCl and of H2O2 measured as LCL and SF as well as LF and MPO degranulation were significantly impaired in the presence of HSA-MG, in dose-dependent mode. Thus, impairment in neutrophil functions in diabetes could be associated to HSA-MG circulating in bloodstream. The study was supported by Russian Science Foundation grant № 20-15-00390. *The authors marked with an asterisk equally contributed to the work.

P-08.3-27
Prognostic factors for amputation on lower extremity in patients with type 2 diabetes mellitus

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Among potential predictors for amputation on lower extremity in patients with diabetes foot ulcers, plasma interleukin-6 (IL-6) and white blood cell count (WBCC) have given reliable results (published in: Karakas A. et al. (2014) Pak J Med Sci 30(3), 578–582). Our study aimed to evaluate the predictive value of these parameters in combination with that of other biomarkers (myeloperoxidase (MPO), interleukin-1 beta (IL-1b), thiols in plasma and reduced glutathione (GSH) in erythrocytes) in patients with type 2 diabetes mellitus. Approval was obtained from the local research ethics committee. Patients with foot ulcers were divided retrospectively into two groups: without amputation (group 1, n = 10) and with amputation on the lower extremity (group 2, n = 5). Healthy subjects matched with patients in gender and age were included into the control group (group 3, n = 16). Blood was drawn at admission. Plasma and erythrocytes were collected and frozen at -80°C until further analysis. IL-1b, IL-6 and MPO were measured by ELISA, total thiols and GSH by Ellman's test. Both WBCC and IL-6 (unlike IL-1b and GSH) showed significant difference between groups 1 and 2 ($P < 0.05$) being higher than in control ($P < 0.05$). The median for IL-6 in groups 1, 2 and 3 was 12.1, 48.2 and 1.0 pg/ml, respectively. A noticeable increase ($P < 0.05$) in MPO (medians of 31.5 and 50.6 ng/ml in groups 1 and 2 vs 22.3 ng/ml in control) as well as a decrease in plasma thiols (medians of 185 and 142 micromol/L in groups 1 and 2 vs 237 micromol/L in control) was found. Il-6 and MPO as well as plasma thiols could be useful in the development of molecular panel for prognosis for diabetic patients with foot ulcers. The study was supported by Russian Science Foundation grant № 20-15-00390.

P-08.3-28
Assessment of MM9 and SIRT-1 contributing to the tissue homeostasis in saliva of patents with periodontal diseases

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Periodontitis is a group of commonly occurred severe inflammatory gum diseases due to present infection. It is characterized by the soft tissue damage, and, if untreated, the teeth loss and bone destruction. The traditional diagnostic tools are non-invasive and economic, but they enable to assess only current disease status. Therefore, the biomarkers for the classification of the disease, the assessment of the disease risk, or monitoring of a response to the treatment are searched. In our study, we investigated the level of

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matrix metalloproteinase 9 (MMP-9) which participates on the extracellular matrix remodeling and degradation and its indirect negative regulator sirtuin 1 (SIRT-1) in saliva of patients with gingivitis, agressive form of paradontitis, chronic paradontitis in comparison to healthy controls. Apparently, the highest extracellular matrix degradation is present in the patients with agressive form of paradontitis which is approximately 2-fold higher than in the patients with chronic form and approximately 7-fold higher than in healthy controls. SIRT-1 saliva levels were not significantly different between the groups of patients but they differed among the individuals suggesting personalized basis of the observed event. Taken together, in this pilot study, we showed the correlation of periodontitis severity with MMP-9 level which might be used as the biomarker in the periodontic patients state in the future. The study was supported by grant VEGA 1/0559/18. *The authors marked with an asterisk equally contributed to the work.

P-08.3-29
Parathyroid hormone prevents the effect of bacterial lipopolysaccharide on the functional activity of the heart

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Pro-inflammatory factors of endogenous and exogenous origin have a key role in development of heart pathologies including heart failure. Considering the cardio-protective effect of parathyroid hormone (PTH) in chronic heart failure shown in our earlier research, the current study aim to investigate the impact of PTH on the action of a lipopolysaccharide, an exogenous factor of bacterial origin on functional activity of isolated heart. The dynamic changes in rhythm and amplitude of heart contractions were recorded by deviation in rate and degree of the laser ray in accordance with the heart contractions by photoelectrical device. The action of LPS was studied both *in vivo* and *in vitro*. The administration of LPS to a frog intraperitoneally a day before the experiment at a dose of 10 μg per 100 g, as well as the application of 10 μg per ml of the incubation medium *in vitro*, decreased the amplitude and frequency of an isolated heart contractions. PTH administered intraperitoneally at a dose of 10 ng per 100 g not only prevented the impact of LPS, but also increased the strength of cardiac contractions compared with the control. The combined action of LPS and PTH (10 ng per ml of the incubation medium) in vitro blocked the manifestation of the LPS effect. Likely, the inhibiting effect of LPS on the functional activity of heart might be caused by an impairment of membrane calcium mechanisms, which might be corrected by the calcium-regulating parathyroid hormone.

P-08.3-30
Production of extracellular domains of human desmoglein type 3 in CHO cells and the study of their specificity with serum from pemphigus vulgaris patients

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Pemphigus vulgaris (PV) is a severe blistering autoimmune disease of the skin and mucosa. The key role in the pathogenesis of pemphigus is played by IgG class autoantibodies directed against the main structural protein of desmosomes, desmoglein 3 (Dsg3). An attractive perspective of PV treatment is extracorporeal immunoadsorption based on specific antigen-antibody interaction between circulating autoantibodies and artificial Dsg3 target. For this purpose, the recombinant immunodominant extracellular domains EC1 - EC4 of human Dsg3 were obtained. The coding sequences (P32926 from the UniProt database) of the extracellular domains of human Dsg3 were synthesized from a set of overlapping primers using a PCR method. Recombinant Dsg 3 domains EC1-EC4 were transiently expressed in CHO cells by using an expression system based on pcDNA3.4 vector (Thermo Scientific, USA). Recombinant domains were produced in the form of N- or C-His tagged proteins, as well as in the form of fuses with the Fc domain of human IgG1. Recombinant proteins were purified from cell culture supernatant using metal affinity chromatography or on MabSelect SuRe columns (GE Healthcare, USA). Protein purity was studied by using of size exclusion chromatography and electrophoretic method. The recombinant full-length extracellular Dsg3 fragment (EC1-EC5) and single EC1, EC2, EC3, EC4 domains were tested for immunoreactivity with sera of PV patients. The domain specificity of PV autoantibodies was shown and individual immunereactivity profiles in PV patients were characterized. This work was supported by a subsidy of the Ministry of Education and Science of the Russian Federation (No. 075-15-2019-1942, Project ID No. RFMEFI60719X0325).

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P-08.3-31
Positive feedback between acidosis and hypoxia during the transition of COVID-19 to a severe form of the disease

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SARS-CoV-2 virus causes disease that has several distinctive characteristics compared to diseases caused by other viruses. We have put forward a hypothesis that relates COVID-19 pathogenesis with acidosis, which frequently characterizes severe cases of this disease. It has been shown that hypoxia and acidosis affect the progression of severe COVID-19 at various physiological levels such as organs, tissues, and cells. The physiological effects of acidosis and hypoxia range from the level of compensatory capabilities of the whole organism to the functioning of a single hemoglobin molecule. In our work, we consider several mechanisms that link the damaging factors of COVID-19 with acidosis. These mechanisms reveal step-by-step processes with a pronounced positive feedback. In accordance with the well-known Bohr effect, a decrease in blood pH leads to a drop in blood oxygen saturation. At the same time, this drop in saturation contributes to the further development of acidosis. This indicates a depletion of the body's compensatory capabilities to regulate acidosis and leads to deterioration of the patient's condition. In addition, a decrease in pH can cause conformational changes in the viral S-protein, followed by changes in ability of some antibodies to recognize the virus. This might lead to the decrease in antibodies affinity and avidity, negatively affecting virus clearance. Low levels of pH and hypoxia in blood and tissues can induce a pro-inflammatory innate response even in the absence of antigen stimulation. Therefore, hypoxia and acidosis can lead to a restructuring of the immune system and multidirectional pro- and anti-inflammatory responses, which often, instead of recovery, lead to the disease aggravation.

P-08.3-32
Inflammatory response in the urinary bladder of young and old mice after chitosan treatment

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Chitosan is natural non-toxic polymer and suitable inducer of urothelial desquamation, which in combination with antibiotics highly enhances the treatment of experimentally induced bacterial cystitis of young mice. The effects of chitosan have already been described, but not in old mice. Therefore, the aim of our study was to check if chitosan is really non-toxic as mentioned in the literature and also to evaluate potential inflammatory response to chitosan treatment in young and old mice. We analysed classic hallmarks of acute inflammation in the urinary bladder wall, characterized histologically by edema of the lamina propria and extravascular migration of neutrophil granulocytes. Moreover, we analysed the expression of serum amyloid A3 (SAA3), the main protein of acute inflammation in humans and most mammals including mice, and cyclooxygenase 2 (COX-2), the key enzyme for the formation of prostaglandins during inflammation. We proved the inflammation in the urinary bladder wall in both age groups. By measuring the thickness of lamina propria, the evaluation of extravascular migration of neutrophil granulocytes and increased expression of COX-2 and SAA3, we have confirmed inflammatory response already 2 hours after chitosan treatment that passes within 10 days. In conclusion, chitosan was proven as low-toxic agent since it caused short-term acute inflammation in urinary bladder of young and old mice allowing the use of chitosan also in old mice. This could be the added value of potential treatment with chitosan in translational medicine of bacterial cystitis in the future. *The authors marked with an asterisk equally contributed to the work.

P-08.3-33
Role of inflammatory biomarkers in nephropathy and atherosclerosis in type 2 diabetes mellitus patients with microalbuminuria

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Type 2 Diabetes Mellitus (Type 2DM) is a common chronic disease in the world. Microalbuminuria (MAU) which is due to endothelial damage in Type 2DM leads to progressive diabetic nephropathy (DN). These two are the most important microvascular complications of the disease. The aim of this study was to investigate the relationship between serum inflammation biomarkers (Monocyte Chemoattractant Protein-MCP-1), Interleukin-6 (Interleukin-6-IL-6), Interleukin-10 (Interleukin-10-IL-10), Tumor Necrosis Factor (TNF-α), High Sensitive C-Reactive Protein (Hs-CRP) and DN and atherosclerosis. The levels of inflammatory biomarkers were detected by Multipex Assay method in patients with Type2DM (MAU (+), n = 40; MAU (-) n = 40). Ankle Brachial Index (ABI) which is an indicator of atherosclerosis was also determined along with routine cardiometabolic markers. Student t Test and Pearson test was used for statistics. No significant difference was found in the levels of inflammatory biomarkers between the 2 groups (MAU (+), MAU (-)). Similarly, in both groups, ABI values were within the normal range of 0.9–1.3 and thus no significant difference between the groups. A moderate or strong correlation was detected between inflammatory biomarkers IL-6, IL-10, MCP-1 and TNF-α and cardiometabolic markers Tkol, TG and LDL in patients with MAU (+). Cardiometabolic markers increased significantly parallel to the inflammatory biomarkers' increase. However, no significant relationship was found between ABI, the

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determining factor in atherosclerosis, in patients with MAU (+), and inflammatory biomarkers and cardiometabolic markers. As a result, MAU, ABI and inflammatory markers in patients with Type 2DM have been investigated together for the first time. New studies with large cohorts, MAU subgroups, or prospective designs may lead to a better understanding of the relationship between DN and atherosclerosis.

P-08.3-34
Jumping over protein production bottleneck: the development of competitive inhibitor of IL-20R2 binding cytokines

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Developments of competitive inhibitors for cytokines usually require enhancing its affinity towards one receptor and destroying the affinity to the second receptor. Only then the inhibitor can displace original cytokine from its signaling complex without initialization of signalization event. This requires purified bait and prey proteins for display techniques, which can be a bottleneck for difficult target proteins, because high protein quality and its defined state are crucial for successful in vitro evolution process. Expression and purification of most cytokines and their receptors is not a simple task and do require multiple expression attempps, time, costs and sometimes co-expression with their interacting partners. All above mentioned difficulties are truth for IL-24 signaling complex which is because of that very difficult target for in vitro protein evolution. We have adopted an alternative strategy to address this challenging task. Engineered protein variants, more amenable to downstream manipulation was used instead of wild type counterparts and only the small-scale expression of wild-type proteins in human HeLa cells were used for validation and performance comparison. In our previous work we designed an interleukin 24 which can be expressed in large quantities in *E. coli*. Similar strategy was applied on IL-20R2. We designed and tested a new IL-20R2D variant with 19 mutations which showed considerable stability and binding to IL-24B4 (FEBS paper). Both engineered proteins were used in several rounds of affinity maturation by yeast-display *in vitro* evolution method. This approach led to development of competitive inhibitor with sub-nanomolar affinities which is capable to inhibit IL-20 subfamily cytokine signalization in tissue culture based experiments. Our inhibitor outperforms antibody based inhibitors be its costs, stability and also affinity. Protein engineering applied before *in vitro* evolution seems to be viable strategy for difficult targets.

P-08.3-35
MHCII allele DRB1_01:01, known as a protective allele in multiple sclerosis, kinetically discriminate the autoantigen myelin peptides and exogenous antigenic peptides

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Risk of multiple sclerosis (MS) development is known to be increased in individuals carrying distinct MHC class II human leukocyte antigen variants, whereas some of them may have a protective effect. Here we analyzed distribution of a highly polymorphous HLA-DRB1 locus in more than one thousand relapsing-remitting MS patients and healthy individuals of Russian ethnicity. Carriage of HLA-DRB1*15 and HLA-DRB1*03 alleles was associated with MS risk, whereas carriage of HLA-DRB1*01 and HLA-DRB1*11 was found to be protective. Analysis of genotypes revealed the compensatory effect of risk and resistance alleles in trans. We have identified previously unknown MBP153−161 peptide located at the C-terminus of MBP protein and MBP90−98 peptide that bound to recombinant HLA-DRB1*01:01 protein with affinity comparable to that of classical antigenic peptide 306–318 from the hemagglutinin (HA) of the influenza virus demonstrating the ability of HLA-DRB1*01:01 to present newly identified MBP153−161 and MBP90−98 peptides. Measurements of kinetic parameters of MBP and HA peptides binding to HLA-DRB1*01:01 catalyzed by HLA-DM revealed a significantly lower rate of CLIP exchange for MBP153−161 and MBP90−98 peptides as opposed to HA peptide. Analysis of the binding of chimeric MBP-HA peptides demonstrated that the observed difference between MBP153−161, MBP90−98, and HA peptide epitopes is caused by the lack of anchor residues in the C-terminal part of the MBP peptides resulting in a moderate occupation of P6/7 and P9 pockets of HLA-DRB1*01:01 by MBP153−161 and MBP90−98 peptides in contrast to HA308−316 peptide. This leads to the P1 and P4 docking failure and rapid peptide dissociation and release of empty HLA-DM–HLA-DR complex. We propose that protective properties of the HLA-DRB1*01 allele could be linked to the ability of HLA-DRB1*01:01 to kinetically discriminate between antigenic exogenous peptides and endogenous MBP derived peptides. Supported by the RSF project 17-74-30019; *The authors marked with an asterisk equally contributed to the work.

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P-08.3-36
Plasma IL-10, IL-17, IL-35, IL-36 levels and their relationship with disease severity in psoriasis

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Psoriasis is a common, immune-mediated inflammatory disease which is characterized by red coloured plaques with well-defined borders and silvery-white dry scales on the skin. Even though pro-inflammatory cytokines and anti-inflammatory cytokines are thought to be related to the pathogenesis of psoriasis, there are contradictory results about circulating levels of IL-10 and IL-17. Only few studies have investigated circulating levels of IL-35 and IL-36, which are newly discovered interleukins, in psoriasis. Our aim was to determine the plasma levels of IL-10, IL-17, IL-35 and IL-36 in psoriasis patients and their relationship with the disease severity. Peripheral blood samples were collected from 38 patients with psoriasis and 20 control subjects. Plasma levels of IL-10, IL-17A, IL-35 and IL-36γ were determined by ELISA. The severity of chronic plaque psoriasis was graded according to psoriasis area and severity index (PASI). On the basis of the PASI score, patients have been stratified into two groups: mild disease (PASI $\leq$ 10) and moderate-to-severe disease (PASI>10). Only IL-10 was found to be significantly lower in patients ($P < 0.01$). No significant differences were detected in plasma IL-17A, IL-35, IL-36γ levels between the patient group and control group or between the mild disease group and the moderate-to-severe disease group (p>0.05). There was positive correlation between IL-35 levels and PASI scores in all patients (r = 0.648, $P < 0.05$); IL-17A levels and PASI scores in patients with mild psoriasis (r = 0.499, $P < 0.05$); IL-36γ levels and PASI scores in patients with moderate-to-severe psoriasis (r = 0.532, $P < 0.05$). In conclusion, the production of IL-10 seems to be impaired in patients with psoriasis. The correlations of PASI scores with IL-17A in the mild psoriasis group and IL-36γ in the moderate-to-severe psoriasis group may indicate increased circulating levels of different pro-inflammatory cytokines in association with disease severity.

P-08.3-37
Comparison of TCR-pMHC interactions associated with HIV control or progression

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Specific recognition of peptide-MHC class I (pMHC-I) complex by T cell receptor (TCR) is a crucial step of Cytotoxic T lymphocyte (CTL) response. An optimal pattern of TCR-pMHC interaction is therefore necessary for efficient control of viral infection and cancer. However, individuals suffering from viral infections or cancer might not possess in their repertoire TRCs capable of such "favorable" interaction. Defining parameters of optimal TCR-pMHC interactions can, therefore, prove useful for the development of T cell-based therapeutics. Specifically, obtaining solved crystal structures of both TCR-pMHC complexes associated with control and progression of infection can allow us to utilize the Quantum mechanics/Molecular mechanics approach to calculate the optimal pattern of receptor-ligand interaction. This data can later be used for virtual maturation of TCR CDRs, producing receptors capable of facilitating efficient CTL response to "unfavorable" pMHC-I. In our work, we focus on TCR-pMHC systems linked to "elite control" of HIV infection. Namely, CTL clones D3 and AAA83 recognizing HIV Gag-derived peptides SLYTNVATL (SL9) and KAFSPEVIPMF (KF11) presented by HLA-A*0201 and HLA-B*5703 molecules, respectively, and MHC mutants that are associated with the differential ability to control HIV infection. In this work, soluble TCR and MHC molecules were expressed in the Drosophila S2 line and purified from concentrated cell media. For evaluating how different MHC variants affect TCR signaling kinetics we assembled nanolipoprotein particles (NLPs) containing functional nickel chelating lipids, bearing His-tagged pMHC-I variants. This system allowed us to measure Ca2+ signaling kinetics while mimicking pMHC-I clusters on the surface of antigen presenting cells, with varying density and ratios of stimulatory and nonstimulatory pMHC-I complexes. This work was supported by CA217714 NIH and Ministry of Science and Higher Education Russian Federation No. 075-15-2020-773. *The authors marked with an asterisk equally contributed to the work

P-08.3-38
The paradoxical effect of increasing of the final yield of reaction product during inhibition of self-inactivating bifunctional enzyme prostaglandin H synthase by nonsteroidal anti-inflammatory drugs

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Dimeric enzyme prostaglandin H synthase (PGHS) catalyzes two sequential reactions – cyclooxygenase (COX) reaction and peroxidase (POX) reaction – in prostaglandin biosynthesis. PGHS undergo irreversibly inactivation during both catalyzed reactions. COX reaction is inhibited by nonsteroidal anti-inflammatory drugs (NSAIDs). There is some evidence of interactions between active sites of PGHS and subunits in PGHS dimer. In this study, we used the PGHS-1 isoform isolated from sheep vesicular glands. The cyclooxygenase reaction was detected amperometrically by consumption of dissolved molecular oxygen. The peroxidase reaction was detected spectrophotometrically by accumulation of oxidized electron donor. We observed a paradoxical effect of increasing of final yield of COX reaction in presence of some COX inhibitors (naproxen, tolmetin, ibuprofen, and fenoprofen but not for indomethacin, diclofenac) while the initial rate predictably decreased. It is consistent with the discovery of the negative cooperativity for naproxen (previously published in: Filimonov IS et al. (2018) Biochemistry (Mosc) 83,

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119–128) and tolmetin. This paradoxical effect can be explained by slower inactivation of the second enzyme subunit during the reaction in case of association of an inhibitor molecule with the first subunit. It was also shown that addition of COX inhibitor naproxen increases final yield of POX reaction product while initial rate does not change. It can be explained by allosteric interactions between the active sites of COX and POX reactions within the same subunit. The obtained results should be taken into account when investigating the pharmacological action of NSAIDs in vivo. The reported study was funded by RFBR according to the research project № 19-04-01150a. It was carried out using the equipment purchased via the Moscow State University Development Program and the equipment of the shared research facilities of HPC computing resources at Lomonosov Moscow State University.

P-08.3-39
Chemical elements in exhaled breath condensate of calves with infectious bronchopneumonia

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The study was aimed to evaluate the elemental composition in the exhaled breath condensate (EBC) in calves with naturally occurring infectious bronchopneumonia (NOIB) and healthy subjects to expand the knowledge on the pathogenesis of NOIB. The authors examined 18 Holstein calves with NOIB, caused by M. bovis and bovine adenovirus 3, and 12 healthy calves aged 14–42 days. NOIB was diagnosed in calves based on clinical findings, results of thoracic auscultation, and X-ray imaging. EBC samples were obtained from fasting calves using a special probing tool (patent RU 134772 U1). The quantitative content of chemical elements (Al, As, B, Ca, Cd, Co, Cr, Cu, Fe, Hg, I, K, Li, Mg, Mn, Mo, Na, Ni, P, Pb, Se, Si, Sn, Sr, V, Zn) in EBC samples was estimated by ICP-MS (Nexion 300D, Perkin Elmer, USA). All the values were expressed in μg/L as the mean ± standard error of the mean. The significance of differences between the groups was identified using independent-samples Mann-Whitney U-test in IBM SPSS Statistics 20.0. It was established that the studied elements, except for Cd (< 0.016) and Hg (< 0.12), were contained in EBC samples in the concentrations available for the analysis. In comparison with healthy animals, the calves with NOIB were characterized by a decrease in Se (0.62 ± 0.09 vs 1.40 ± 0.31, $P = 0.013$) and Zn (41.3 ± 2.4 vs 55.0 ± 5.4, $P = 0.044$) and an increase in Al (50.0 ± 4.9 vs 32.5 ± 5.4, $P = 0.015$), Co (0.145 ± 0.024 vs 0.033 ± 0.005, $P = 0.0001$), Mn (9.50 ± 0.67 vs 6.25 ± 0.85, $P = 0.004$), Mo (0.60 ± 0.08 vs 0.23 ± 0.06, $P = 0.002$), Na (2622 ± 529 vs 1155 ± 169, $P = 0.031$), P (685.0 ± 89.2 vs 387.5 ± 32.3, $P = 0.013$), Pb (2.13 ± 0.39 vs 0.58 ± 0.07, $P = 0.0001$), and Sn (0.143 ± 0.018 vs 0.055 ± 0.012, $P = 0.0001$) in EBC. The content of other elements in EBC did not change significantly. It was established that the elemental content in EBC samples in calves with NOIB had a specific profile that differed from the profile of healthy calves. This work was supported by Russian Science Foundation, grant 18-76-10015.

P-08.3-40
Correlation between the intestinal region-specific thickening of ganglionic basement membrane and regionally decreased matrix metalloproteinase 9 expression in myenteric ganglia in type 1 diabetes

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The importance of the neuronal microenvironment has been recently highlighted in gut region-specific diabetic enteric neuropathy. Regionally distinct thickening of endothelial basement membrane (BM) of intestinal capillaries supplying the myenteric ganglia coincide with neuronal damage in different intestinal segments. Reduced degradation of matrix components may contribute to the imbalance of extracellular matrix dynamics resulting in BM thickening. Therefore, we aimed to evaluate the effects of type 1 diabetes and immediate insulin replacement on BM thickness surrounding myenteric ganglia as well as the expression of matrix metalloproteinase 9 (MMP9) and its tissue inhibitor (TIMP1) in myenteric ganglia and their environment along the small intestine using fluorescent immunohistochemistry, post-embedding immunogold electron microscopy and qPCR. Ten weeks after the onset of hyperglycaemia, the ganglionic BM was significantly thickened in the diabetic ileum, while it remained intact in the duodenum. The immediate insulin treatment prevented the diabetes-related thickening of the BM surrounding the ileal myenteric ganglia. Quantification of particle density showed an increasing tendency for MMP9 and a decreasing tendency for TIMP1 from the proximal to the distal small intestine under control conditions. In the diabetic ileum, the number of MMP9-indicating gold particles decreased in all cell types, however, it remained unchanged in all duodenal compartments. The MMP9/TIMP1 ratio was also decreased in ileal ganglia only. However, a marked segment-specific induction was revealed in MMP9 and TIMP1 at the mRNA levels. These findings support that the regional decrease in MMP9 expression in myenteric ganglia and their microenvironment may contribute to extracellular matrix accumulation, resulting in a region-specific thickening of ganglionic BM and diabetic damage of enteric neurons.

P-08.3-41
Effects of Harpagophytum procumbens extract on inflammation and nociceptive system in human primary synoviocytes

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Osteoarthritis (OA) is a chronical-degenerative and inflammatory disease affecting joints, where cartilage is degraded and bones friction each other, causing pain. At present date, there is no

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specific anti-OA therapy, so painkilling and anti-inflammatory drugs are dispensed. The aim of this research is to study the possible analgesic and anti-inflammatory properties of nutraceutical substances, in *in vitro* cell models. We studied the therapeutic properties of the Harpagophytum procumbens extract (HPE), commonly known as devil's claw, a plant worldwide used as a traditional remedy for joint pain, on primary human synoviocytes (FLSs) from osteoarthritis patients. HPE powder has been dissolved in deionized water (HPE_{H2O}), DMSO (HPE_{DMSO}), 100% v/v EtOH ($HPE_{EtOH100}$), and 50% v/v EtOH (HPE_{EtOH50}). HPE_{H2O} and HPE_{DMSO} were able to increase gene and protein expression of CB2, a cannabinoid receptor involved in the modulation of the pain and inflammation in FLSs, and to inhibit the expression of phospholipases C β2 (PI-PLC β2), where the other two extracts did not produce the same effects. Moreover, we observed that PI-PLC β2 has low concentrations in healthy synovial membrane and high concentrations in osteoarthritic tissues, suggesting its direct involvement in the development of the inflammatory process in OA. On the other hand, only HPE_{H2O} was able to decrease the gene expression of FAAH enzyme, involved in endocannabinoids degradation, while all the other extracts were ineffective. Nonetheless, all extracts used in these experiments were able to interfere with FAAH activity in a fluorescence assay. Since these preliminary results are very promising, a future perspective is to analyze some intracellular pathways, modulated by HPE, in order to better explain the anti-inflammatory activity in addition to the anti-nociceptive one as described in this study. Mariano A. et al., Nutrients, 2020, doi: 10.3390/nu12092545.

P-08.3-42
The role of annexins during calcification of human coronary artery smooth muscle cells

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Vascular calcification (VC) is accompanied by the expression of tissue nonspecific alkaline phosphatase (TNAP), a crucial enzyme for atherosclerotic plaque formation by vascular smooth muscle cells (VSMCs). Furthermore, it has been shown that membrane-binding annexin A6 (AnxA6) is abundant at sites of VC and that its depletion reduces VSMC mineralization. In chondrocytes, where the physiological process of mineralization takes place, the non-specific calcium channel blocker K201 inhibits up-regulation of terminal differentiation marker genes (i.e. TNAP, AnxA2, AnxA4 and AnxA6) expression. Extracellular vesicles (EVs) released from K201-treated chondrocytes are characterized by lower TNAP activity and lower amounts of AnxA2, AnxA4 and AnxA6 proteins [Previously published in: Wang et al. (2003) J Biol Chem 278, 3762–3769]. The aim of this study was to identify the functions of annexins in VC. We used human coronary artery smooth muscle cells (HCASMCs) cultured in an osteogenic medium for 7–21 days to promote their transition to osteochondrocyte-like cells. In order to inhibit the activity of annexins, K201 was added at various concentrations. The level of VC in the cultures was examined via Alizarin Red-S staining. Subsequently, the minerals were extracted to analyze their quantity. Transmission electron microscopy (TEM) was used to identify EVs and minerals in samples of HCASMCs. The TNAP activity was examined in HCASMC lysates by ELISA assay. The obtained results show that K201 markedly reduces VC, as well as TNAP activity in HCASMC cultures. Calcium minerals could be observed under TEM only in HCASMCs lysates not treated with K201. Addition of proteoliposomes containing AnxA6-FITC to atherosclerotic cells stimulated their mineralization which was prevented by K201. In conclusion, annexins seem to play a significant role in VC evoked by TNAP but the molecular mechanisms of their activity should be further investigated.

P-08.3-43
How effects of BCG vaccination depend upon host genetics: performance of MHC haplotypes in congenic mouse strains B10.M (H2f) and B10 (H2b)

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The most widely applied vaccine in the world is the *Mycobacterium bovis*-derived baccillus Calmette-Guerin (BCG), which is used for exactly one hundred years to defend humans against tuberculosis (TB). Important issues regarding its highly variable performance in different populations are possible influence of host genetics and remarkable anrigenic differences between attenuated *M. bovis* BCG and virulent *M. tuberculosis* strains circulating over the globe. Previously, our laboratory described an unusual phenotype of the mouse strain B10.M, carrying the $H2^f$ allelic variant of the H2 complex: the lack of BCG protective effect against subsequent challenge with virulent *M. tuberculosis* H37Rv. Congenic mice of the B10 strain, carrying the $H2^b$ allelic variant of the H2 complex, demonstrated a normal level of BCG protective effect. Thus, we compared vaccinated and non-vaccinated congenic mice of two strains regarding post-challenge survival time, CFU counts in the lungs, T-lymphocyte recognition of mycobacterial antigens and capacity to produce INF-γ in response to specific and non-specific stimulation via T-cell receptor. Our results show that T-cells of B10.M mice are specifically defective for INF-γ production in spleens and lungs in responce to prolonged stimulation with mycobacterial antigens during chronic infection. However, immunologic recognition of these antigens was unaltered, as well as the capacity to secrete INF-γ after non-specific signalilng via T-cell receptor following anti-CD3 antibodies stimulation. A defect in INF-γ production might be due to the events that occur at the late phases of chronic infection and rather depend not upon prior BCG vaccination, but specific T-cell immune exhaustion during a long-lasting course of the disease. (B10.M x B10) F_1 hybrids displayed the phenotypes close to those displayed by B10 mice, demonstrating the dominant inheritance of the $H2^b$ haplotype.

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P-08.3-44
Allergenicity assessment of mealworm proteins used as a novel dietary protein source for dogs

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Edible insects, such as the yellow mealworm (*Tenebrio molitor*), have been proposed as a high-quality and sustainable alternative protein source for human and animal consumption. Unfortunately, cross-reactivity and/or co-sensitization of some insect proteins has been demonstrated in house dust mite and seafood allergic people. Since there are insect proteins commercially available in pet feed formulations, allergenic risk assessment is needed to prevent new food allergies. Therefore, the aim of this study was to evaluate the potential cross-reactivity to mealworm proteins in dogs sensitized to storage mites. Raw and frozen *Tenebrio molitor* larvae were ground and defatted. Proteins were extracted and digested in vitro with pepsin, trypsin and α-chymotrypsin. The protein extracts and digests were analysed by SDS–PAGE and immunoblots were performed with canine sera. Two groups of dogs were included in the study: clinically healthy dogs and dogs with clinical signs of allergy. The mealworm proteins were identified by liquid chromatography coupled with tandem mass spectrometry analysis (LC-MS/MS). IgEs from all tested dog sera strongly cross-reacted with several proteins of 20–30 kDa in the non-digested mealworm acidic extract. IgE binding to proteins between 34 and 55 kDa and to a 14 kDa protein obtained after digestion with enzymes differed between sera from clinically allergic and clinically healthy dogs, but not significantly. The proteomic approach resulted in identification of several mealworm proteins, including some known invertebrate pan-allergens. Among them proteins such as tropomyosin and α-amylase, were previously recognised as IgE-binding cross-reacting allergens in humans. In conclusion, our results suggest that mealworm proteins used as ingredients in dog feed pose a risk to the existing allergic population.

P-08.3-45
Cardiac myofibroblasts enhance hypertrophy and systolic dysfunction, but not fibrosis in experimental autoimmune myocarditis

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Myocarditis is a common cause of dilated cardiomyopathy which is characterized by ventricular stiffening, cardiac fibrosis and heart failure. In experimental autoimmune myocarditis (EAM) susceptible mice are immunized with alpha myosin heavy chain (αMyHC) and complete Freund's adjuvant (CFA). CD4+ T cell-mediated acute cardiac inflammation is followed by fibrosis and systolic dysfunction. The aim was to investigate the role of fibroblasts and myofibroblasts in myocarditis and postinflammatory cardiomyopathy in EAM model. EAM was induced in BALB/c mice by immunization with αMyHC/CFA. We used reporter strains expressing EGFP under the type I collagen promoter and RFP under α-smooth muscle actin (αSMA) promoter and transgenic αSMA-TK mice with ganciclovir-inducible myofibroblasts ablation. Comparing unaffected heart, the number of cardiac fibroblasts (EGFP+) and the subset of myofibroblasts (EGFP+αSMA+) was unchanged at inflammatory (day 21) and fibrotic stages (day 40). EGFP+ fibroblasts were sorted from control and myocarditis-positive hearts (d21) and analyzed for the whole genome transcriptomics by RNA sequencing. Analysis of differentially expressed genes (min. 2x fold change, p value < 0.05) suggested activation of immune processes (mainly chemokine production), response to stress, cytoskeletal and extracellular matrix re-organization in cardiac fibroblasts in response to myocarditis. Ablation of myofibroblasts in αSMA-TK mice with ganciclovir at the acute myocarditis (day 21 of EAM) showed no effect on cardiac fibrosis (Trichrome Masson's staining, hydroxyproline assay), but markedly reduced heart weight, decreased cardiomyocyte hypertrophy and improved ejection fraction and cardiac output at day 40. In EAM model cardiac fibroblasts participate in proinflammatory and profibrotic responses, while activated myofibroblasts drive progression of myocarditis to dilated cardiomyopathy phenotype independently of cardiac fibrosis.

P-08.3-46
Relative hypoglycemia due to viral infection promotes the innate anti-viral immune response

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Viral infection has a major impact on systemic metabolism. In humans, severe infection may lead to both hypoglycemia and hyperglycemia, but how this is regulated on a molecular level and what possible benefits to the host might be is mostly unknown. We have recently shown that mild viral infection alters endocrine regulation of systemic blood glucose levels, even though this does not lead to dysglycemia. In this work, we investigated how severe infection impacts regulation of blood glucose levels. We showed that infection of mice with high, but non-lethal titres of mCMV or LCMV causes transient, relative hypoglycemia. Low blood glucose levels were beneficial to the host as enforced hyperglycemia during infection resulted in a significant increase of viral titres in peripheral organs. This effect was dependent on IFNg secreted by gdT cells, as TCRd-/- mice and animals treated with neutralizing antibodies against IFNg failed to develop hypoglycemia. Infection-induced IFNg causes specific insulin resistance in muscle, but not in liver which leads to increased insulin secretion by the pancreas. As a result of infection-induced hyperinsulinemia, glycogenolysis and thus liver glucose output is reduced, leading to a reduction in systemic glucose levels. Limited glucose availability amplifies the cellular stress response of infected cells, leading to higher production of type I interferons which promotes the antiviral response. When glucose levels were experimentally increased, viral loads were strongly increased, both in vitro and in vivo because of an impaired Type I interferon response. Our findings indicate that the reduction of

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blood sugar levels during infection is a well-regulated process that is a part of the body's natural anti-viral response. *The authors marked with an asterisk equally contributed to the work.

P-08.3-47
Obesity-induced memory CD8 T cell dysfunction in mice is independent of direct insulin signaling

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Diabetes mellitus type 2 (T2D) causes an increased risk of morbidity and mortality in response to viral infection, in part because of impaired T cell functionality, but much of the underlying mechanism is unknown. T2D is associated with insulin resistance and compensatory hyperinsulinemia and previously we have shown that insulin is able to directly modulate effector CD8 T cell function. We therefore hypothesized that chronic, obesity-associated hyperinsulinemia impairs the anti-viral memory CD8 T cell response. In a mouse model for diet-induced obesity and hyperinsulinemia we could show that memory CD8 T cell function was significantly reduced in response to re-challenge by viral infection or with B16 melanoma cells. Deficiency of the insulin receptor of CD8 T cells resulted in a reduced ability to upregulate GLUT-1 and to take up glucose in memory precursors early after activation. However, this did not result in a difference in metabolism, memory formation, cytokine production or recall responses. Inversely, insulin injection increased GLUT-1 expression and glucose uptake in memory precursors, but memory formation and functionality was not affected. Importantly, in context of obesity, insulin receptor deficiency on CD8 T cells did not affect the functional capacity of memory CD8 T cells. Our findings indicate that obesity impairs the memory CD8 T cell response against viral infection and cancer, but this is not the result of direct insulin stimulation.

P-08.3-48
Role of Mcpip1 in obesity-induced hepatic steatosis as determined by myeloid and liver-specific conditional knockouts

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Monocyte chemoattractant protein-induced protein 1 (MCPIP1) is a negative regulator of inflammation, acting through cleavage of transcripts coding for proinflammatory cytokines and by inhibition of NFκB activity. Moreover, it was demonstrated, that MCPIP1 regulates lipid metabolism both in adipose tissue and hepatocytes. In this study, we investigated the effects of tissue-specific Mcpip1 deletion on the regulation of hepatic metabolism and development of non-alcoholic fatty liver disease (NAFLD). We also analyzed MCPIP1 level in liver biopsies of NAFLD patients. We used control Mcpip1fl/fl mice and animals with deletion of Mcpip1 in myeloid leukocytes (Mcpip1fl/flLysMCre) and in liver cells (Mcpip1fl/flAlbCre), which were fed chow or a high-fat diet (HFD) for 12 weeks. For analyses of MCPIP1 level in human liver we enrolled 37 patients. We demonstrated that MCPIP1 level is lower in livers of NAFLD patients compared to control subjects. Then, we showed that Mcpip1fl/flLysMCre mice fed a chow diet were characterized by reduced hepatic expression of genes regulating lipid and glucose metabolism. These animals displayed also systemic inflammation manifested by increased serum levels of e.g. IL-6, IL-12, IL-16, TNF-alpha. Although we detected a reduced hepatic expression of genes regulating glucose metabolism and β-oxidation in the Mcpip1fl/flAlbCre mice, they did not develop any abnormalities related to lipid metabolism. Despite feeding with HFD for 12 weeks, Mcpip1fl/flLysMCre mice did not develop obesity nor hepatic steatosis. Mcpip1fl/flAlbCre animals, following a HFD accumulated lipids in the liver at the same level as Mcpip1fl/fl mice. In conclusion, we demonstrated that MCPIP1 protein can be considered as a new player involved in fatty liver development. Depletion of Mcpip1 in myeloid leukocytes, followed by systemic inflammation, has a more pronounced effect on controlling liver metabolism and homeostasis than its deletion in liver cells.

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P-08.3-49
The proportions of MR1-restricted TCRVα7.2+ and VγVδ1-δ2-TCR subtypes are altered in human psoriatic skin and vary with age

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Innate-like T cells, such as γδ and MR1-restricted TCRVα7.2 subsets, perform vital homeostatis functions at epithelial sites, but their pathogenic relevance in vulgar psoriasis (PV), a common autoinflammatory skin disorder, remains unclear. Here, we used flow cytometry (FACS Canto II, FlowLogic) to investigate the phenotype and numbers of MR1-restricted TCRVα7.2+ and γδ T cells by examining skin samples from 26 adult PV patients and 16 healthy, age and sex-matched controls. Cell suspensions were obtained from lesional and normal skin (Whole Skin Dissociation Kit, gentleMACS Dissociator), and stored at -80°C before thawing and cell counting. For staining, fixable viability dye, FcR blocking reagent, MR1-5-OP-RU tetramers, CD3ε, CD4, CD8a, TCRVα7.2, TCRγδ, TCRVδ1, and TCRVδ2 monoclonal antibodies were used. As expected, CD3+T cells were more frequent in lesional skin compared to healthy-looking skin [80.6 (68.3–89.1) vs. 26.5 (16.8–58) %, median (interquartile range), PV vs controls, $P = 0.00008$, Mann-Whitney test]. There was a marked change in composition of the γδ TCR subtypes in psoriatic skin, reflecting a strong enrichment of Vδ1-Vδ2- cells within the γδ T compartment [1.6 (1.2–3.2) vs. 1 (0.7–1.4) %, $P = 0.001$], followed by a decrease in Vδ2+T cell abundance [0.065 (0.03–0.12) vs. 0.22 (0.05–0.5) %, $P = 0.0091$]. Aged lesional skin contained fewer Vδ1+T cells (Spearman's $\rho = -0.72$, $P = 0.00004$, $n = 26$), a phenomenon not observed in healthy surgical specimens. PV skin samples were also enriched in CD8+MR1-tet+TCRVα7.2+ cell numbers compared to controls [3.4 (2.2–4.7) vs 2.2 (1.4–3.2) % of T cells, $P = 0.039$], more so in younger patients ($\rho = -0.45$, $P = 0.021$, $n = 26$). CD4+MR1-tet+TCRVα7.2+ T cells were rare in psoriatic plaques and appeared more readily in severely affected patients ($\rho = 0.5$, $P = 0.011$, $n = 26$), as judged by PASI. Altogether, our preliminary results suggest a potential role for MAIT and γδ T cell subsets in PV and warrant further analysis of their clonotypes and function. *The authors marked with an asterisk equally contributed to the work.

P-08.3-50
Comparative analysis of B cell receptor repertoires revealed delay in transitional regulatory B lymphocyte maturation during multiple sclerosis development.

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Regulatory functions of B lymphocytes play an important role in the development and suppression of the immune response. Previously it has been demonstrated that B regulatory cells' (Bregs) lesion or decrease of its anti-inflammatory activity can lead to a number of immunological pathologies, in particular autoimmune diseases. Unfortunately to the date the exact mechanism of Breg functioning and development is unknown. Almost nothing is known about Breg specificity and its BCR structure. In this study using high-throughput sequencing we analyzed B cell repertoire at the Ig gene level of regulatory transitional CD19(+)CD24(high)CD38(high) B cells of patients with multiple sclerosis (MS). For the first time, the study reveals that MS patients get less maturated variable IgG fragments in analyzed transitional Bregs in comparison with healthy donors. Additional analyses reveal that the percentage of CD19(+)CD24(high)CD38(high) cells is increased and the frequency of CD27+ transitional B cells is decreased in MS patients compared with those in matched healthy subjects. Thereby, the study indicates that impaired maturation of regulatory B lymphocytes could be associated with MS progression. Current study was supported by Russian Science Foundation grant #17-74-30019.

P-08.3-51
E2F2 protects activated T-lymphocytes from apoptosis through repression of Fas/FasL-dependent mechanisms

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Lymphocyte homeostasis requires synchronized regulation of cellular proliferation and apoptosis to prevent immune abnormalities such as lymphoma or autoimmunity. We have previously shown that targeted disruption of E2f2 in mice causes resting T cells to overexpress E2F target genes and to enter S phase inappropriately. Nevertheless, E2f2-/- mice do not develop a lymphoproliferative condition, suggesting that an apoptotic mechanism could be activated in these mice. The goal of our study was to elucidate the mechanism by which E2F2 regulates T cell homeostasis and, in particular, to evaluate the role of p53 and Fas-FasL in this process. Apoptosis and gene expression were analyzed in E2f2-knockout and E2f2/p53-double knockout T cells activated through the T-cell receptor (TCR). Antagonistic antibody to FasL and agonistic antibody to Fas were used to address the implication of Fas-FasL. Here we show that E2F2 plays an antiapoptotic function *in vivo* and *in vitro*. E2f2-/- T cells undergo apoptosis upon activation of the TCR and

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concomitantly with p53 accumulation and increased expression of Fas and FasL. Disruption of p53 in E2f2-/- mice prevents only partially the induction of apoptosis. By contrast, treatment with anti-FasL antagonistic antibody completely prevents apoptosis of E2f2-/- T cells, and agonistic activation of Fas markedly increases cell apoptosis and accelerates death of E2f2-/- mice. Importantly, E2F2 directly binds to the Fas promoter. These results point to a novel role for E2F2 in the negative regulation of apoptosis through the Fas-FasL pathway by directly repressing Fas expression, and thereby promoting proliferation of activated T-cells. In conclusion, E2F2 transcription factor contributes to immune cell homeostasis via regulation of p53- and Fas/FasL-dependent pathways. These results may provide insight into our understanding of the apoptotic mechanisms regulating immune cell homeostasis

P-08.3-52
Deletion of Mcpip1 in mice induces primary biliary cholangitis

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Background: Primary biliary cholangitis (PBC) is an autoimmune disease characterized by progressive destruction of the intrahepatic bile ducts. The pathology of PBC involves excessive inflammation; thus, negative regulators of inflammatory response, such as Monocyte Chemoattractant Protein-1-Induced Protein-1 (MCPIP1) may play important roles in the development of PBC. The aim of this work was to verify whether Mcpip1 expression protects against development of PBC. Method: Genetic deletion of Zc3h12a was used to characterize the role of Mcpip1 in the pathogenesis of PBC in 6–52-week-old mice. Results: We found that Mcpip1 deficiency in the liver (Mcpip1fl/flAlbCre) recapitulates most of the features of human PBC, in contrast to mice with Mcpip1 deficiency in myeloid cells (Mcpip1fl/flLysMCre mice), which present with robust myeloid cell-driven systemic inflammation. In Mcpip1fl/flAlbCre livers, intrahepatic bile ducts displayed proliferative changes with inflammatory infiltration, bile duct destruction, and fibrosis leading to cholestasis. In plasma, increased concentrations of IgG, IgM, and AMA autoantibodies (anti-PDC-E2) were detected. Interestingly, the phenotype of Mcpip1fl/flAlbCre mice was robust in 6-week-old, but milder in 12–24-week-old mice, suggesting early postnatal origin of the phenotype. Hepatic transcriptome analysis of 6-week-old and 24-week-old Mcpip1fl/flAlbCre mice showed 812 and 8 differentially expressed genes, respectively, compared with age-matched control mice, and revealed a distinct set of genes compared to those previously associated with development of PBC. Conclusion: Mcpip1fl/flAlbCre mice display early postnatal phenotype that recapitulates most of the features of human PBC. Acknowledgment: This work was supported by research grants from National Science Centre, Poland no. 2015/19/D/NZ5/00254 and 2017/27/B/NZ5/01440 to JeKo, and 2018/29/B/NZ6/01622 to JoKo.

P-08.3-53
IgG modulation of reproductive disorders in male mice induced by proinflammatory cytokines in prenatal period

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Systemic inflammation induced by bacterial lipopolysaccharide (LPS) leads to increased synthesis of proinflammatory cytokines, including interleukin (IL) 6, IL-1β, tumor necrosis factor alpha, monocyte chemotaxis protein 1 and leukemia inhibitory factor. The elevated cytokine levels in the early, critical periods of ontogenesis, can cause developmental disorders of reproductive system in rodents, with persist in adulthood (Izvolskaia et al., 2019, Ignatiuk et al., 2019). Immunoglobulin G (IgG) is known to have protective and anti-inflammatory effects during bacterial infections. This study aim was to define prenatal modulating effect of mouse IgG on reproductive disorders in male offspring after prenatal LPS-treated. Intravenous administration of IgG (20 μg/mouse) after LPS (100 μg/kg) exposure to pregnant mice on day 11.5 had a positive impact on male reproductive maturity: the decreased Sertoli cell number, testosterone level and sexual attempt number were increased, while the increased symplastic spermatid number and estradiol level were decreased. In an in vitro model, IgG (100 μg/ml) reduced elevated cyclic adenosine monophosphate (cAMP) level in LPS-activated (0.5 μg/ml) murine peritoneal macrophages. The IgG effect was most prominent in 40 minutes after LPS exposure, when the synthesis of proinflammatory cytokines has not yet reached its peak. Thus, IgG modulates reproductive disorders caused by inflammation in early development. The cAMP pathway may be involved in IgG modulation. The reported study was funded by RFBR, project number 19-34-90006. Previously published in: Izvolskaia MS et al. (2019) Andrologia 51(3), e:13204. Ignatiuk VM et al. (2019) Stress 22(1), 133–141.

P-08.3-54
The extracellular protease EpiP from *S. aureus* triggers blood coagulation by proteolytically activating prothrombin (ProT) and platelet protease-activated receptor 1

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BACKGROUND *Staphylococcus aureus* is a Gram+ bacterium known for being responsible for both mild and systemic bacteremia and sepsis. During the last decades, clinical evidence

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correlates *S. aureus* infections to thrombotic complications, such as DIC. Among several virulence factors, extracellular proteases might play a role in triggering thrombotic events in infectious diseases, whereby bacterial proteases could activate the coagulation cascade by proteolytically converting ProT zymogen into thrombin species and stimulating platelets aggregation. AIMS In this work, we investigated if the serine protease EpiP from *S. aureus* is involved in thrombus formation during staphylococcal infections, directly activating ProT and platelets. METHODS Biochemical techniques: limited proteolysis, enzymatic chromogenic assay, mass spectrometry; coagulation assays: fibrin generation and platelet aggregation; fluorescence microscopy (FM). RESULTS Staphylococcal EpiP converts ProT into an active species that can hydrolyze the thrombin-specific substrate S2238. The time-course analysis of ProT activation allowed to identify the EpiP cleavage sites, identical to those hydrolyzed by factor Xa under physiological conditions. The activation products of ProT by EpiP can induce fibrin clot formation and platelet aggregation. Surprisingly, EpiP can proteolyze PAR1(38–60) peptide at the same site of thrombin cleavage and electrostatically interact with GpIbα(268–282) peptide as demonstrated by SPR. Ultimately, we directly observed EpiP-mediated platelets agglutination by FM. CONCLUSIONS EpiP can proteolyze the inactive ProT into an active thrombin species which is able to trigger blood clotting, and directly induces platelet aggregation activating PAR1 receptor after binding to GpIbα on platelets. These results widen our understanding of the biochemical mechanisms whereby *S. aureus* proteases can initiate coagulation, establishing a direct link between infections and higher thrombotic risk.

P-08.3-55
β2-Glicoprotein I (β2GpI) is a fibrinogen binding protein

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Background β2GpI is an abundant plasma protein and the main autoantigen in the antiphospholipid syndrome (APS), a severe thrombotic autoimmune disease. We have recently discovered that β2GpI tightly binds to αThrombin (αT) at exosite-2. This result suggests that β2GpI may function as a scavenger of αT for binding to the GpIbα platelet receptor. Aims The major aims of this study are: i) check whether β2GpI interacts with fibrinogen (Fb); ii) identify the binding regions on β2GpI and Fb involved in β2GpI-Fb complex formation; iii) study the effect of β2GpI binding to Fb on fibrin structure. Methods Binding measurement were performed by fluorescence and Surface Plasmon Resonance (SPR). Fibrin structure was studied by turbidimetry, Dynamic Light Scattering (DLS) and Scanning Electron Microscopy (SEM). Fb fragment-X and β2GpI nicked at Domain V were both prepared by limited proteolysis with plasmin, while the β2GpI Domain I was produced by solid-phase synthesis. Results Fluorescence and SPR analyses indicate that β2GpI interacts with Fb according to a two-sites non-equivalent binding model, with $Kd^1 = 12 \pm 4$ nM and $Kd^2 > 1\mu M$. Identical results were obtained with the synthetic Domain I and β2GpI clipped at Domain V. Conversely, binding of intact β2GpI to Fb fragment-X, lacking the Ca-domains, resulted in a drop of affinity. Turbidimetric, DLS, and SEM measurements indicate that physiological concentrations of β2GpI induce the formation of thinner and shorter fibrin fibers. Conclusions Given the physiological plasma concentrations of β2GpI (4 μM) and Fb (7 μM), and the affinity of β2GpI for Fb (Kd = 12 nM), it can be concluded that β2GpI circulates in the bloodstream bound to fibrinogen. Domain I on β2GpI and the Ca-domains on Fb drive β2GpI-Fb complex formation. Our results challenge current view of the physiological function of β2GpI and its role in the pathogenesis of APS. *The authors marked with an asterisk equally contributed to the work.

P-08.3-56
Systemic effect of Tff3 deficiency in high-fat diet mouse model

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Trefoil factor family protein 3 is a small peptide from a family of proteins characterized by a trefoil domain. It plays a role in various physiological processes, including apoptosis, cytoprotection, cell survival, migration and immune response. Tff3 is expressed in multiple tissues and involved in various pathologies. Complete attenuation of liver Tff3 expression was noticed as one of the first events in the early phase of diabetes development in a multigenic mouse model of diabesity (Tally Ho strain). Tff3 expression was changed in liver steatosis, and its low levels were associated with neurodegeneration in Alzheimer's disease patients. To shed light on the systemic effect of Tff3 deficiency in different pathological pathways we have simulated type 2 diabetes conditions in congenic mouse model Tff3-/-/C57Bl6/N, developed from mixed background strain (C57Bl6/J /SV129), and its wild type counterpart. Male and female 11-week-old mice were fed high fat diet for 9 weeks. They were subjected to glucose and insulin tolerance tests, sacrificed, and relevant tissues were collected and analysed. Initial glucose tolerance differed significantly between the wild type and Tff3-/- mice, but the high-fat diet treatment decreased the differences. The groups have also shown differences in weight gain during the experiment. We have monitored the expression of different oxidative and endoplasmic reticulum (ER) stress genes, relevant inflammation, and apoptosis markers in liver tissue of wild-type and Tff3 deficient mice. The most dramatic changes were noticed at the level of apoptosis relevant genes. Additionally, proteomic analysis, performed on the liver, hippocampus and colon samples revealed differences in the cytoskeleton and mitochondrial proteins, which are, directly or indirectly, associated with apoptosis. Our data reveal, for the first time, the impact of Tff3 deficiency on different organs and disease relevant pathways in the context of metabolism and neurodegeneration. *The authors marked with an asterisk equally contributed to the work.

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P-08.3-57
Effect of Trefoil factor 3 deficiency in liver of streptozotocin induced Type I diabetes mice model

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Trefoil Factor 3 (Tff3) is a small protein predominantly expressed in gastrointestinal epithelial cells with a multifunctional role in the protection and repair of the mucosa through participation in an immune response. However, Tff3 is expressed in other various tissue where it exhibits different biological effects. The complete diminishment of liver Tff3 was observed in the early phase of the diabesity mice model and since then its role in metabolic processes has emerged. Downregulated expression of TFF3 is reported in the serum of Type I diabetes (T1D) patients. T1D is a complex disease characterized by absolute insulin deficiency and altered immune response. The liver, as a main metabolic organ, is one of the key factors in the pathophysiology of T1D. Hence, our goal was to analyze the impact of Tff3 deficiency on different relevant endoplasmic reticulum (ER) stress, apoptosis and inflammation markers in the liver of the T1D model. To address this issue, firstly, we generated new congenic mouse model Tff3-/-/C57Bl6/N from existing mixed background strain by speed congenics. The new model is more representative for metabolic studies due to less altered metabolic phenotype. Multiple intraperitoneal injections of streptozotocin (STZ) were administered to Tff3-/- mice and wild type control. STZ is selectively toxic to the insulin-producing β cells and it is widely utilized for inducing T1D in animal models. We have monitored different metabolic parameters and after 6 weeks of STZ-induced hyperglycemia, mice were sacrificed and relevant tissues were analyzed. DNA damage-inducible transcript 3 (CHOP), a key ER stress transcription factor involved in apoptosis was downregulated in Tff3 deficient mice. Altered ER stress, presence in different tissues and involvement in the immune response are triggering the question of Tff3 systemic action in the organism and new congenic Tff3 deficient mice represent a valuable tool in that scientific pursuit. *The authors marked with an asterisk equally contributed to the work.

P-08.3-58
Binding studies of *Hirudo medicinalis* related cationic antimicrobial peptides and human plasma proteins

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Cationic antimicrobial peptides (cAMPs) are a promising alternative to conventional antibiotics since they cause little or no microbial resistance. The interaction of cAMPs with plasma proteins is of interest because such interaction plays an important role in the pharmacokinetics of therapeutic agents. The aim of this study was to determine blood plasma proteins that bind cAMPs developed by us based on bioinformatics analysis of the genome of the leech *Hirudo medicinalis*. The cAMPs used in this work were as follows: KKGKSFKQLHIIVHLVKSWLRTILTHI and FLIGKAIKRKFCLRSVWNA. The peptides were immobilized on agarose gels via an aminocaproic acid spacer. Affinity chromatography of human blood plasma on the resins obtained revealed a 67-kDa protein to be a single protein that binds with cAMPs. The protein was identified as human serum albumin (HSA) by its molecular mass, by staining with bromophenol blue (BPB) and by reaction with monoclonal antibodies against HSA. Chromatography of blood plasma on a control sorbent (without immobilized cAMP) showed no adsorption of HSA. The complex HSA-cAMP dissociated with increasing NaCl concentration, thereby indicating the electrostatic nature of the interaction. When blood plasma was titrated with increasing amounts of cAMP, disc electrophoresis in detergent-free gels displayed a decrease in the intensity of the BPB-stained HSA band, and, conversely, a dose-dependent increase in the BPB-stained precipitate that did not migrate into the gel was observed. To avoid a possible influence of the loading dye on complexing, Cy5-labeled cAMPs were used, which also formed complexes with HSA under electrophoretic conditions. Thus, the results show that among blood plasma proteins, only HSA binds cAMPs. It is possible to assume that HSA can be a transport molecule for cAMPs in blood plasma. The study was supported by the Russian Science Foundation grant No. 20-15-00270.

P-08.3-59
Protective effects of olive-derived compounds in molecular mechanisms underlying osteoarthritis.

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Osteoarthritis (OA) is the most common disease of the joints and first cause of motor disability in the elderly. Chondrocytes, the only cell type present in cartilage tissue, are primarily affected by the disease and responsible for local inflammation and ECM remodeling, thus leading to degeneration of articular cartilage. Up to date, available treatments for OA mainly focus on symptom management. Recently, a valid alternative to restore joint homeostasis has emerged from dietary nutraceuticals with known anti-inflammatory properties. Our study aims to understand the potential protective role of two olive-derived (poly)phenols, hydroxytyrosol (HT), oleuropein (OLEU), in the pathogenesis and progression of OA. Primary chondrocytes were obtained from cartilage samples of OA patients. High density cell cultures were set up, pre-treated with HT or OLEU and then, stimulated with lipopolysaccharide (LPS). Our findings indicate that HT and OLEU decrease the gene expression of LPS-induced inflammatory markers, such as iNOS and COX-2 and significantly reduce oxidative stress after 48 h. Furthermore our data suggest the involvement of the NOTCH signaling pathway in the etiopathogenesis of OA. Of the four genes in the NOTCH family, the over-expression of NOTCH-1 in OA cartilage suggests a possible role in the onset and progression of the disease. Indeed, in

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our experimental model, the silencing of NOTCH-1 resulted in the attenuation of its target HES-1 as well as of several OA markers, such as MMP13, VEGF, ADAMTS-5 and RUNX-2, and inflammation-related genes, like NFKB1, IKK-α, IL6 and IL8. HT and OLEU were able to decrease NOTCH-1 expression in LPS-stimulated-chondrocytes. Thus we can suppose an involvement of NOTCH-1 in biochemical modifications exerted by these treatments. In conclusion, these results open an interesting outlook for further investigations on the molecular mechanisms involved in OA and for future development of novel therapeutic strategies.

P-08.3-60
The gene expression of inflammatory cytokines and tight junctions proteins as a markers of impaired fish immunity

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The bacterial diseases are known to be associated with an increase in the permeability of the fish intestinal barrier, often due to decrease of the tight junctions proteins (TJ) expression. TJ such as transmembrane occludin (ocln) and claudin (cldn1) and cytosolic zonula occludens-1 (zo-1) provide intercellular contacts and thus regulate epithelial barriers, including blood-tissue barrier. Therefore, bacterial infection can cause systemic effect on structural integrity of all tissues in fish organism. In this study we compare expression of pro-inflammatory interleukin 1b and interleukin 8 (IL1b and IL8) and anti-inflammatory cytokin transforming growth factor (TGFb) with expression of TJ proteins in liver and spleen of farmed rainbow trout with vibriosis. Among fish collected from the same cage there were individuals with different extent of development of visual signs of infection progression (skin and organ color, ulcers, hemorrhages), indicating two group of fishes: sick and resistant to bacterial infection. The same, 60–100 fold difference of mRNA content of IL1b and IL8 (enchanced in sick fish) was found in the liver and spleen of fishes from these two groups by analysis of gene expression. The 4 fold increase in anti-inflammatory TGFb also was detected in diseased individuals in comparison with more healthy. Except of weak positive corrlation zo-1 with IL8 in fish spleen ($r = 0.44$), no correlation was detected between expression of TJ proteins and inflammatory cytokines in fish liver and spleen, indicating that the effect of decreased TJ expression in the intestine of infected fish, reported previously, was local. This suggests that disturbance of intestinal epithelium integrity during infection could be provoked by altered intestinal microflora, rather than cytokines, systemically circulating in the blood. The interdisciplinary research was supported by a grant from the Russian Science Foundation (No. 20-66-47012) in partnership with Irkutsk State University. *The authors marked with an asterisk equally contributed to the work.

P-08.3-61
Development of new experimental approaches to study Ca^{2+} regulation and B-cell fate *ex vivo*

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B cells play a crucial role in a humoral immune response against pathogens. Antigen (Ag)-specific B cells need to recognize Ag via B cell receptor (BCR) and then get T cell help to be recruited into long-term humoral response. The fate of Ag-exposed B cells (activation, anergy or death) is determined by the type, size and valency of Ag, duration of Ag interaction with BCR and timing of T cell help, as well as their extracellular environment. Combination of these signals stimulates intracellular calcium signaling in B cells. In the simplest case, activated by foreign Ag BCRs modulate PLCγ, IP_3 production and subsequent calcium mobilization from intracellular store. A significant increase in cytosolic Ca^{2+} can lead to mitochondrial energization and collapse. How do B cells integrate all incoming signals to decide on their activation or tolerance is insufficiently understood. Analysis of the Ca^{2+} and other signaling events in B cells *ex vivo* is complicated by relatively rapid death of B-cells. Here we aimed to develop a better experimental approach to study Ca^{2+} signaling in B-cells during activation *ex vivo*. Previous studies indicated that coculturing murine B cells with fibroblast reticular cells (FRCs) can better mimic physiological environment and support their survival *ex vivo* [1]. Therefore, we have undertaken to utilize FRC-B cells co-culture approach to study intracellular signaling and cell fate decisions in human B cells. Preliminary analysis confirmed better survival of human B cells co-cultured with FRCs from human lymph nodes *ex vivo*. By utilizing co-culture approach, flow cytometry and confocal microscopy we are planning to study various scenarios of Ag and T cell help acquisition by human B cells and dissect regulation of cytosolic and mitochondrial calcium in determining B cell fate. The study was funded by RFBR and the Royal Society of London (RS), project number 21-51-10005. [1] V. Cremasco et al., Nat.imm. (2014), vol.15, pp. 973–983

Aging stress and neurodegeneration

P-08.4-01
A new genetic drug for Hutchinson-Gilford progeria syndrome

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Hutchinson–Gilford progeria syndrome (HGPS) is a rare genetic disorder caused by a point mutation in the LMNA gene. A point

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mutation (C1824T) in exon 11 leads to cryptic splice site appearance and as a result, part of exon 11 is deleted in progerin (mutated lamin A). The missing sequence plays an important role in post-translational modification. After C-terminus farnesylation and transport into the nucleus, lamin A is released by the proteolytic cleavage and can form the nuclear lamina. Progerin remains attached to the nuclear membrane because it lacks the sequence recognized by protease. The accumulation of progerin leads to nuclear envelope disorganization, abnormal chromatin organization and changes in gene expression. Nowadays HGPS therapy is based mostly on the prevention of cardiovascular diseases because they are the main cause of patient's deaths. Reported preclinical therapies were based on the reducing progerin protein level or farnesylation inhibition. Our point is to develop the genetic drug to suppress the progerin expression by RNA interference. siRNAs were designed to recognize the joint of exon 11 and 12 of progerin mRNA. Their specificity and efficiency were assayed with flow cytometry by measuring the fluorescence intensity changes after transfection. Obtained results were confirmed with western blotting. We also examined the effect of the combination of siRNA and lonafarnib, the clinical drug. We aimed to identify if two drugs act as antagonists, is they action additive, or do they display a synergistic activity. We demonstrated that progerin level could be sufficiently decreased by siRNA without changes in lamin A level. Besides, our results showed an additive effect of genetic drug combination with the clinically approved lonafarnib treatment. This suggests that genetic drug treatment presumably could be performed with approved HGPS treatment. The research is supported by grant ERA-NET-E-RARE-3/III/TREATHGPS/10/2018 from the Polish Agency NCBR.

P-08.4-02
Novel multifunctional compounds with potential therapeutic activity for targeted delivery to dopaminergic neurons

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Although the etiology and pathogenesis of Parkinson's disease is not completely known, it has been established that oxidative stress caused by negative environmental effects or age-related factor leads to the progressive loss of dopaminergic neurons. It should be noted that the clue role in the antioxidant cell protection plays the Nrf2/Keap1 antioxidant system. Obviously, therapy for Parkinson's disease should include not only drugs that increase dopamine levels, but also neuroprotective drugs that activate the cell's defense system against oxidative stress. Here we develop the concept of "multifunctional drugs" in contrast to the idea of a "golden bullet". The goal of this work is to develop targeted multifunctional compounds with potential drug activity for the treatment of Parkinson's disease. The role of the address fragment and, at the same time, pharmacophore, whose action leads to an increase in the level of dopamine, is performed by the modified structure of the dopamine reuptake inhibitor GBR12909. First of all, we synthesized a novel fluorescent derivative of the dopamine reuptake inhibitor GBR12909 and completed the proof of our concept in DAT-expressing pheochromocytoma cells PC12 and dopaminergic neurons. Then we developed a set of GBR12909 derivatives with various linkers between the address and pharmacophore parts carrying amino, thio and maleimide functional groups. These multifunctional linkers were introduced to attach low molecular weight pharmacophores or polypeptides that activate the Nrf2 / Keap1 antioxidant system, as well as fluorophores to image the transport of the aim molecule. This work was partially supported by the Research Program of the Presidium of the RAS #18 "Biomedicine Technologies: innovative developments". *The authors marked with an asterisk equally contributed to the work.

P-08.4-03
Tauopathy in Niemann-Pick type C disease carriers: analysis in a mouse model

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Niemann-Pick type C disease (NPC) is a rare, fatal lipid storage disorder characterized primarily by progressive neruodegeneration of Purkinje neurons in the cerebellum and neuroinflammation (activation of microglia and astrocytes). NPC is caused by dysfunction of cholesterol transport proteins NPC1 and/or NPC2, which leads to accumulation of free cholesterol and other lipids within late endosomes and lysosomes. Assuming that heterozygous mutations in NPC1 and/or NPC2 genes do not cause any symptoms, the carriers of NPC disease are considered healthy. Our goal is to characterize the brains of NPC1 heterozygous mice for pathological features of NPC (including neurodegeneration, neuroinflammation, endolysosomal disfunction and tau hyperphosphorylation) and to identify the earliest changes upon loss of single NPC1 allele. We used the BALB/cNctr-Npc1$^{N/+}$ mouse model (NPC1$^{+/-}$, Jackson Laboratory, Bar Harbor, Maine, USA) and analyzed the brains of 60- and 100-weeks old NPC1$^{+/-}$ and NPC1$^{+/+}$ (wt, control) mice by western blotting and immunohistochemistry. Here, we demonstrate that 60-weeks old NPC1$^{+/-}$ mice show increased tau hyperphosphorylation in contrast to age-matched control mice. The differences in neuroinflammation level, as well as endolysosomal dysfunction between the two groups of mice were not observed. These preliminary findings suggest that hyperphosphorylation of tau may be the earliest pathological feature that occurs in NPC1 heterozygous mice. We, thus, conclude that the aged murine carriers of NPC disease may not be considered healthy and that human NPC1 heterozygous mutation may be a risk factor for neurodegenerative disorders (such as tauopathy) in the aged population.

P-08.4-04
The pre-retirement stress, immune-senescence, inflammation and biological age

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The rise of life expectancy in many countries increases the retirement age. However, the number of healthy working people does not always grow accordingly. To continue an active lifestyle and

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prevent early disability, it is necessary to control the development of senile diseases (cardiovascular neurodegenerative, oncological, acute, and chronic infections). Moreover, we found that an increase of retirement age is accompanied by pre-retirement stress (PRS). Stress triggers the release of cortisol and suppresses the sensitivity of lymphocytes and granulocytes to activation signals. According to our data, PRS correlates with an increase of biological age and an elevation of the number of senescent effector NK and T cells. Chronic stress can also induce an inflammatory response and the secretion of pro-inflammatory factors. The combination of chronic inflammation, accumulation of senescent cells, and exhaustion of immune cells is an unfavorable background for the development of senile diseases. Testing of inflammatory factors and cortisol levels with the analysis of effector blood cells is necessary to create a multifactor model for the analysis of predisposition to age-related diseases. *Ex vivo* screening of natural, synthetic compounds and FDA drugs using blood monocytes has proven to be an effective test system. The study of the level of phagocytosis, secretion of cytokines, the ability to proliferate will allow to propose candidate drugs for early prevention of immune aging, age-related, and stress-induced diseases. We hope that the combination of anti-inflammatory treatment, psychological assistance, cortisol control, and immune stimulation will help reduce nonspecific inflammation and enhance antiviral control, which will be beneficial for extending active life expectancy in the Russian Federation in the context of the recent increase of retirement age. Supported by Russian Science Foundation № 19-18-00058.

P-08.4-05
Neurotoxic effect of extracellular alpha-synuclein can be alleviated by AMPK and autophagy

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Alpha-synuclein (ASYN) is regarded as one of the key culprits in pathogenesis of synucleinopathies, including Parkinson's disease, and impaired regulation of autophagy is associated with the ASYN aggregation. Autophagy is regulated by complex mechanisms, including AMP activated protein kinase (AMPK), a key energy sensor regulating cellular metabolism to maintain energy homeostasis. The aim of our study was to investigate the role of AMPK and autophagy in neurotoxic effect of secreted ASYN, as well as dopamine-modified and nitrated recombinant wild-type ASYN oligomers, on retinoic acid (RA)-differentiated SH-SY5Y cells. The culture supernatant from neuroblastoma cells stably expressing wt ASYN was collected and used as conditioned medium (CM). The presence of wt ASYN in CM was confirmed by immunoblot, following lyophilisation. The CM, as well as recombinant dopamine-modified or nitrated ASYN, all reduced viability in differentiated SH-SY5Y cells. This decrease in viability was accompanied by reduced AMPK activation, increased conversion of LC3-I to LC3-II and increase in Beclin-1 level, as demonstrated by immunoblot. Pharmacological activators of AMPK and autophagy (metformin and AICAR) significantly increased the cells' viability in the presence of CM and modified ASYN forms. Level of AMPK-activated pULK was reduced in presence of CM, but pharmacological activators of AMPK reversed that effect. Pharmacological inhibitors of autophagy, further reduced cell viability in the presence of extracellular ASYN. The shRNA-mediated LC3 downregulation, as well as the RNA interference-mediated knockdown of ATG7 gene, both important for autophagosome biogenesis/maturation, increased sensitivity of SH-SY5Y cells to the extracellular ASYN-induced toxicity. These data demonstrate the protective role of AMPK and autophagy against the toxicity of extracellular ASYN, suggesting that their modulation may be a promising neuroprotective strategy in Parkinson's disease.

P-08.4-06
Phenotypic changes in peripheral blood T-lymphocytes in patients with Parkinson's disease.

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The neurotoxic effect of regional inflammation in the brain is considered as one of the central links in the pathogenesis of Parkinson's disease. It has been established that in addition to neuroinflammation, PD is characterized by chronic systemic inflammation. Currently, an active search is being made for associations of PD development with peripheral markers of the physiological state of the patient's body, which could be used to diagnose and control the course of the disease. It is known that $CD57^+CD28^-$ T-lymphocytes, so-called senescence T-lymphocytes, were reported to be associated with persistent viral infection and with chronic immune activation, especially in aged patients. Therefore, we conducted a comparative analysis of the subpopulation composition of peripheral blood T-lymphocytes, in patients with PD and healthy donors who also are elderly people. We examined 31 PD and 33 healthy donors. The average age in the group of PD was 57 (+/-2.4) years, in the healthy group average age - 54 (+/- 1.9) years. Flow cytometry analysis demonstrated that the percentage of CD57 positive cells from total T-lymphocytes ($CD3^+CD56^-$) number was lower in the group of PD patients compared with that in the group of healthy donors (8.8 and 13.1, $P = 0.03$). Analysis of the data measured by ELISA in serum, that 76% (25/33) of the healthy donors were seropositive for CMV IgG. The percentage of CMV positive samples in the group of patients with PD that were characterized by decreased CD57 was 100% (31/31). In our study we used samples of blood of PD patients who are elderly people, we found that the percentage of CD57 positive cells from T-lymphocytes was reduced. Thus, opposite to healthy people, in the course of PD, we revealed the absence of replicative senescent T-lymphocytes. Such a phenomenon which is atypical for the elderly population of healthy donors and independent of CMV

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infection. The reported study was funded by RFBR, project number 20-315-90072

P-08.4-07
Central and peripheral effects of oral galactose in cognitively unaltered rats

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Brain insulin resistance is a core of sporadic Alzheimer's disease (sAD). Our previous research showed a therapeutic potential of oral galactose in an intracerebroventricular-streptozotocin (STZ-icv) rat model of early sAD, which affected brain glucose hypometabolism and induced metabolic changes. We explored a time-course of peripheral and central effects following a single oral galactose dose in healthy rats used as controls to the STZ-icv model. Three-month old male Wistar rats received 0.05M citric buffer icv. A month later, plasma, cerebrospinal fluid (CSF) and hippocampal samples were taken before, 15, 30, 60 or 120 minutes after 200 mg/kg galactose by oral gavage. We measured glucose, galactose and total glucagon-like peptide-1 (GLP-1) levels (spectrophotometry, ELISA) in plasma/CSF and hippocampal GLP-1 receptor levels (Western blot). Galactose levels showed a significant increase both in plasma and CSF after 15 min (+257%; +137%, $P < 0.05$) but were normalized later (decline below control found only in CSF at 120min/-58%, $P < 0.05$/). Increase in glucose levels was significant only in CSF at 15 and 30min (+40%; +31%, $P < 0.05$), while similar but lower tendency in plasma remained insignificant. Total GLP-1 levels showed similar but insignificant acute tendencies of increase in plasma and CSF (+63%), followed by significant increase in hippocampal GLP-1R expression at 15 and 30min (+72%; +15%, $P < 0.05$) with subsequent normalization. A single dose of oral galactose does not disrupt glucose and GLP-1 homeostasis but acutely activates GLP-1R signalization in healthy rats. This work has been supported in part by Croatian Science Foundation under the project IP-2018-01-8938. Research was co-financed by the Scientific Centre of Excellence for Basic, Clinical and Translational Neuroscience (project "Experimental and clinical research of hypoxic-ischemic damage in perinatal and adult brain"; GA KK01.1.1.01.0007). Presented at: 9th Croatian Congress of Pharmacology.

P-08.4-08
3-Bromo-4,5-dihydroxybenzaldehyde inhibits particulate matter-mediated cellular againg

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A natural bromophenol found in seaweeds, 3-bromo-4,5-dihydroxybenzaldehyde (3-BDB), has been shown to possess various biological effects. Exposure to particulate matter (PM) is associated with increased senescence in human skin cells. The aim of this study was to elucidate the mechanism by which particulate matter 2.5 ($PM_{2.5}$) induces cellular aging in human HaCaT keratinocytes and mice skin tissues, and to evaluate the preventive capacity of the 3-BDB. 3-BDB inhibited cellular aging induced by $PM_{2.5}$ treatment. Also, $PM_{2.5}$ induced a decrease in DNA methyltransferase (DNMT) expression and an increase in DNA demethylase (ten-eleven translocation; TET) expression, leading to hypomethylation of the p16INK4A promoter region. However, 3-BDB decreased hypomethylation of the p16INK4A promoter region and TET expression. In addition, 3-BDB decreased epigenetic transcriptional activator MLL1 expression increased by $PM_{2.5}$. Furthermore, 3-BDB increased binding of DNMT1, DNMT3B, and histone H3 lysine 27 trimethylation (H3K27Me3) to the promoter region of p16INK4A decreased in $PM_{2.5}$-treated keratinocytes, whereas TET1 and histone H3 lysine 4 trimethylation (H3K4Me3) binding decreased in the promoter of p16INK4A. Altogether, our work shows that a natural products 3-BDB inhibited skin aging induced by environmental $PM_{2.5}$ through the epigenetic modification of senescence-associated gene expression.

P-08.4-09
Alpha-synuclein level in plasma extracellular vesicles in Parkinson's disease and other synucleinopathies

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Objectives. Misfolding and aggregation of alpha-synuclein resulting in cytotoxicity is a hallmark of Parkinson's disease (PD) and related synucleinopathies. The recent body of evidence indicates that alpha-synuclein can be released from neuronal cells by non-conventional exocytosis involving extracellular vesicles, such as exosomes. CNS exosomes derived from PD patients were shown to transfer increased amount of alpha-synuclein protein that may be relevant to disease pathogenesis. Mutations in the LRRK2 and GBA genes are linked to inherited forms of PD and may influence autophagy and exosomal protein content. Methods. Total alpha-synuclein levels in plasma exosomes obtained from 5 patients with LRRK2-associated PD, 8 patients GBA-associated PD, 14 patients with dementia with Lewy bodies (DLB), 11 patients with multiple system atrophy (MSA), 15 PD-patients with dementia (PDD), 18 patients sporadic PD and 16 healthy individuals (controls) were measured by enzyme-linked immunosorbent assay with SensoLyte Anti-alpha-Synuclein Quantitative ELISA Kit (Human) (AnaSpec, USA) and normalized to total protein. To isolate the exosomes from human plasma we used the Exo-Prep (HansaBioMed, Estonia). Lysis was carried out by Total Protein Extraction Kit (Chemicon (Millipore), USA). Results. No significant difference was found for alpha-synuclein/total protein ratio in plasma exosomes of patients with LRRK2- and GBA- associated PD, MSA, DLB, PDD, sporadic PD, compared to controls (28.5 ± 7.6 pg/mkg, 27.3 ± 14.1 pg/mkg, 29.8 ± 3.4 pg/mkg, 35.8 ± 6.9 pg/mkg, 28.6 ± 4.0 pg/mkg, 26.5 ± 3.6 pg/mkg and 27.5 ± 4.1 pg/mkg ($P > 0.05$), respectively). Conclusions. Our result indicate that exosomal alpha-synuclein from blood plasma was not associated with PD and related synucleinopathies. Acknowledgments. The work was supported by RFBR № 19-315-90059.

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P-08.4-10
Chronic immobilization stress causes aging acceleration and induces depressive-like behaviors and mild cognitive impairments in mouse

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Chronic immobilization stress (CIS), repeated-2 h restraint for consecutive 15 days, decreased body weight and increased blood corticosterone level compared with non-stressed group (CTL). Telomere length and mitochondrial DNA copy number were changed with normal aging, but CIS accelerated the changes in the leukocyte, liver, kidney, skeletal muscle, prefrontal cortex (PFC), aorta, and spleen, implying aging acceleration in each tissue. CIS-treated mice showed depressive-like behaviors including helplessness and anhedonia compared with CTL. Lower levels of glutamate and glutamine, GFAP expression, glutamine synthetase activity, and glutamatergic signaling were also found in the PFC after CIS. Moreover, CIS induced higher levels of ROS/RNS in the blood, PFC and hippocampus. Especially, neuronal damages were found in the CA1 pyramidal neurons in the hippocampus of CIS-treated mice. The synaptic puncta were also decreased in the PFC and hippocampus by CIS. Consequently, learning and memory function was impaired in CIS-treated mice. These results suggested that CIS model used in these studies would be a very useful model to develop a treatment strategy for chronic stress to prevent such pathological conditions.

P-08.4-11
Recombinant human Hsp70 as a promising neuroprotective agent in neurodegenerative diseases and brain ischemia

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Pathogenesis of brain ischemia and injury has a lot of features in common with Alzheimer's Disease (AD) and several other degenerative diseases. These pathologies are characterized by high levels of inflammation in brain tissues and neurodegeneration observed in the effected brain areas. Heat shock protein 70 (HSP70) provids cell survival under a variety of different stress conditions. Besides, HSP70 may be secreted out from cells and exhibit cytoprotective activity, probably by decreasing the levels of some proinflammatory cytokines through the interaction with several receptors specific for innate immune system (Previously published in: Rozhkova et al. (2010) Ann N Y Acad Sci 1197, 94–107). In the mouse models of Alzheimer disease the recombinant HSP70 was demonstrated to penetrate into the brain after intranasal administration and cause neuroprotective effect, decrease of neuroinflammation and restoration of cognitive parameters of the model animals (Previously published in: Evgen'ev et al. (2017) J Alzheimers Dis 59, 1415–1426). Herein, we demonstrated that intranasal administration of human HSP70 significantly (two folds) decreased the volume of the local ischemia induced by photothrombosis in the mouse prefrontal brain cortex. The obtained results revealed that intranasal injections of HSP70 decreased the apoptosis level in the ischemic penumbra. Furthermore, recombinant HSP70 administration also stimulated axonogenesis and increased the number of neurons producing synaptophysin. In the isolated crayfish stretch receptor consisting of a single sensory neuron surrounded by the glial envelop, exogenous HSP70 significantly decreased photoinduced apoptosis and necrosis of glial cells. The obtained data enable one to consider human recombinant HSP70 as a promising compound which could be translated into clinical therapies. The work was supported by the grant of Russian Science Foundation #19-14-00167.

P-08.4-12
Structural studies of ALS-associated annexin A11

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Amyotrophic lateral sclerosis (ALS) is a fatal neurodegenerative disease of motor neurons. Mutations in annexin A11 gene (ANXA11) have only recently been associated with ALS. Annexin A11 (ANXA11) is a member of annexin family of calcium dependant phospholipid-binding proteins. It has a conserved C-terminal alpha-helical core and a structurally disordered N-terminal domain, which is the longest in the annexin family. We wanted to determine the crystal structure of ANXA11, explain the functional changes caused by known ALS-associated mutations and evaluate their effect on aggregation of ANXA11. We expressed wild type and N-terminally truncated forms of ANXA11 in *E. coli* to obtain soluble forms for crystallization experiments. Only N-terminally truncated ANXA11 successfully crystallised. Crystals diffracted to 2,2 Å and the structure was solved using molecular replacement. Structure of ΔN-ANXA11 has a conserved core that constitutes of four homologous alpha-helical annexin repeats. Predicted N-terminal structurally disordered part explains the unsuccessful crystallisation of the wild type ANXA11. ALS-associated mutation R235Q results in disrupted structure as arginine 235 is crucial for the position of the first annexin repeat. ANXA11 is a predominantly nuclear protein that forms insoluble cytoplasmic aggregates in patients with ALS. Therefore, we have also focused our studies on the aggregation of ANXA11 and its mutated forms in *in vitro* conditions.

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P-08.4-13
Regulated cell death in the naked mole rat

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The naked mole rat (NMR), *Heterocephalus glaber*, is the longest-living rodent species, and is extraordinarily resistant to cancer and aging-related diseases. The molecular basis for these unique phenotypic traits of the NMR is under extensive research. However, the role of regulated cell death (RCD) in the longevity and the protection from cancer in the NMR is still largely unknown. RCD is a mechanism restricting the proliferation of damaged or premalignant cells, which counteracts aging and oncotransformation. In this study, DNA damage-induced cell death in NMR fibroblasts was investigated in comparison to RCD in fibroblasts from Mus musculus. The effects of methyl methanesulfonate, 5-fluorouracil, and etoposide in both cell types were examined using contemporary cell death analyses. Skin fibroblasts from *Heterocephalus glaber* were found to be more resistant to the action of DNA damaging agents compared to fibroblasts from *Mus musculus*. Strikingly, our results revealed that NMR cells also exhibit a limited apoptotic response and seem to undergo regulated necrosis. Taken together, this study provides new insights into the mechanisms of cell death in NMR expanding our understanding of longevity, and it paves the way towards the development of innovative therapeutic approaches. This study was supported by Russian Science Foundation project №19-74-10056

P-08.4-14
Interactomes of wt and C-terminally truncated FUS

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Neuronal degeneration has been recognized as a predominant driver of disability and disease progression in central nervous system diseases such as amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD). Successful treatments for these disorders have yet to be developed. The aggregation of RNA binding proteins (RBPs) has been recognized as a hallmark pathological feature in these disorders, defining them as proteinopathies. Fused in sarcoma (FUS), normally a nucleus residing RBP, is known to aggregate into physiological granules and pathological inclusions, which can impair cell homeostasis leading to neuronal cell death. Mutations in FUS that alter its C-terminal nuclear localization signal (NLS) are autosomal dominant in ALS and disrupt its nucleo-cytoplasmic shuttling leading to its cytoplasmic mislocalization. Since protein interactors of FUS and the exact signaling pathways involved in cytoplasmic toxicity of FUS remain unknown, using BioID2 proximity labeling, we aimed to identify the interactomes of FUS and FUSdNLS (lacking NLS) proteins overexpressed in a model cell line. This technique harnesses the ability of the enzyme biotin ligase (birA) to biotinylate proximal endogenous proteins. Bioinformatic analyses of proteomic data identified interaction candidates involved in RNA processing and degradation, protein translation and various signal transduction pathways. Selected interactions were validated by pull-down assay and cell co-localization analyses in vitro. The interactome differences between FUS and FUSdNLS, provide detailed insight into FUS function most likely relevant to disease, that could be targeted in therapeutic interventions.

P-08.4-15
Dimebon derivative (DF402) tunes up mitochondrial function and increases the lifespan of FUS-transgenic mice

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Amyotrophic lateral sclerosis (ALS) is a severe neurodegenerative disease leading to the eventual death of motor neurons and skeletal muscle paralysis. ALS is mostly incurable and the patients receive only supporting therapy. Described cases of familial ALS have emphasized the significance of protein misfolding and aggregation of two functionally related proteins, FUS and TDP-43, implicated in RNA metabolism. Herein, using the *in vivo* mouse model of FUS-mediated proteinopathy we performed the comprehensive analysis encompassing the onset of the first clinical symptoms as well as changes in gene expression profile in motor neurons and surrounding microglia on the background of the treatment by DF402, a derivative of antihistamine drug Dimebon (Latrepirdine). We demonstrated that chronic administration of DF402 results in delaying the onset of the clinical symptoms of motor dysfunction up to 30 days and significantly increases the overall lifespan of transgenic animals. Comparative analysis of gene expression profiles demonstrates that DF402 administration affects gene expression in neural cells even stronger than the expression of FUS-transgene. Genes affected by DF402 are involved in protein folding and oxidative phosphorylation suggesting that fine-tuning the protein homeostasis and mitochondria function underlie a positive effect on neuronal function and delays the onset of the clinical symptoms of ALS in the transgenic mice. Overall, our data provide insights into the putative mechanisms of the adaptation of motor neurons to the accumulation of misfolded proteins and enable one to conclude that DF402 treatment may be a safe and effective therapeutic against ALS neuropathology. This work was supported by Grant of Russian Foundation for Basic Research №20-34-90028

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P-08.4-16
ALS-linked TDP-43 inclusions dysregulate astroglial noradrenergic signalling and metabolism

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In most cases of amyotrophic lateral sclerosis (ALS) and frontotemporal dementia (FTD) cytoplasmic TDP-43 (TAR DNA-binding protein 43) inclusions are present not only in neurons, but also in non-neuronal glial cells, including astrocytes, which metabolically support neurons with nutrients. Neuronal metabolism largely depends on the activation of locus coeruleus noradrenergic system and astroglial adrenergic receptors (ARs), the primary target of noradrenaline (NA) released from the noradrenergic neurons. Activation of astroglial ARs with NA triggers cAMP and Ca^{2+} signaling and augments aerobic glycolysis with production of lactate, an important neuronal energy fuel. Cytoplasmic TDP-43 inclusions in astrocytes alone can cause motor neuron death, however, whether astroglial metabolism and metabolic support of neurons is altered in astrocytes with TDP-43 inclusions, is unclear. We measured lipid droplet and glucose metabolisms in astrocytes expressing the inclusion-forming C-terminal fragment of TDP-43 or the WT TDP-43 using fluorescent dyes or genetically encoded nanosensors. Astrocytes with TDP-43 inclusions exhibited a 3-fold increase in the accumulation of lipid droplets vs. astrocytes expressing WT TDP-43, indicating altered lipid droplet metabolism. In these cells the NA-mediated increases in intracellular cAMP and Ca^{2+} levels were reduced by 35% and 31%, respectively, likely due to the downregulation of β_2-ARs. Although NA triggered a similar increase in intracellular lactate levels in astrocytes with and without TDP-43 inclusions, the probability of activating aerobic glycolysis was facilitated by 1.6-fold in astrocytes with TDP-43 inclusions and lactate MCT1 transporters were downregulated. Thus, while in astrocytes with TDP-43 inclusions noradrenergic signaling is reduced, aerobic glycolysis and lipid droplet accumulation are facilitated, suggesting dysregulated astroglial metabolism that likely impairs astroglial metabolic support of neurons in ALS and FTD. *The authors marked with an asterisk equally contributed to the work.

P-08.4-17
Interactome screening of C9orf72 dipeptide repeats reveals VCP sequestration and functional impairment by polyGA

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Amyotrophic lateral sclerosis (ALS) and frontotemporal lobar degeneration (FTLD) are neurodegenerative disorders, related by signs of deteriorating motor and cognitive functions, and short survival. Mutations of more than 30 different genes were linked to familial ALS/FTLD, including a hexanucleotide GGGGCC repeat expansion in the C9orf72 gene. One of the proposed mechanisms of GGGGCC repeats is their translation to produce unnatural dipeptide repeat (DPR) proteins that accumulate in aggregates and contribute to the pathology. There are 5 different DPRs polyGA, polyGR, polyPR, polyPA, polyGP, which are variably toxic. They all have been previously shown to interact with ribosomes, stress granules and low-complexity proteins. In our study we are identified the *in vivo* interactome of all five DPRs, consisting of 125 repeats, overexpressed in HEK293T cells using BioID2 proximity labeling. We identified 140 interacting partners for polyGR, 130 for polyGA, 138 for polyPR, 62 for polyPA, and 38 for polyGP. Gene ontology enrichment analysis of proteomic data identified interaction candidates involved in protein translation, signal transduction pathways, protein catabolic processes, amide metabolic processes and RNA-binding. Selected protein candidates, some of which have already been linked to ALS, were validated by immunoblotting and immunocolocalization. Finally, we proceeded with determination of mechanism and pathological significance of our most potent interacting partners. Using autopsy brain tissue from patients with C9orf72 expansion complemented with cell culture analysis, we evaluated the interactions between polyGA and valosin-containing protein (VCP). Functional analysis of this interaction showed sequestration of VCP with polyGA aggregates, thereby altering the levels of the soluble VCP protein. VCP function is decisive in autophagy processes, and in line with this, we observed altered autophagy in cells expressing polyGA. *The authors marked with an asterisk equally contributed to the work.

P-08.4-18
Stress-related lipid droplet accumulation in rat brain astrocytes

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Astrocytes are an abundant subtype of neuroglial cells in the central nervous system (CNS) involved in the regulation of CNS

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energy metabolism. Compared to glucose metabolism, mechanisms underlying the regulation of lipid metabolism in astrocytes are largely unexplored. During CNS pathologies lipid droplets (LDs), free fatty acids (FFAs) and cholesterol storing organelles, tend to accumulate in glial cells (not neurons). However, the mechanisms leading to LD accumulation in glial cells during CNS pathologies are unknown. The aim of the study was to investigate whether different stressors linked to CNS pathologies promote LD accumulation and affect LD mobility in astrocytes. Astrocytes in culture and organotypic brain tissue slices were exposed for 24 h to nutrient deprivation (replacement of growth medium with 10 or 0 mM glucose in extracellular solution), excess of FFAs (300 μM oleic acid) or L-lactate (20 mM), hypoxia (1% O_2) and adrenergic agonists/antagonists to determine whether noradrenaline, the major stress-related CNS neuromodulator, affects LD content in astrocytes. LDs in astrocytes were labeled with fluorescent markers (Nile Red, BODIPY493/503), and the mobility and the content of LDs evaluated by confocal microscopy. Nutrient stress (nutrient deprivation, a surplus of FFAs or L-lactate), hypoxia, and adrenergic cAMP signaling increased the formation of LDs in isolated and brain tissue astrocytes over 2-fold. The mobility of LDs in astrocytes was reduced under nutrient deprivation. Our results show that the mobility of LDs is decreased, while LD content is increased in isolated and brain tissue astrocytes by environmental stressors, indicating LD accumulation in astrocytes, similar as observed in CNS pathologies. During stress, FFAs from LDs can be utilized in mitochondrial β-oxidation as an alternative energy source. Furthermore, LDs may protect cells against FFA lipotoxicity, which may increase the viability of astrocytes.

P-08.4-19
CHRFAM7A modifies the α7 nAChR to a hypomorphic receptor for Aβ1-42 uptake

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The α7 nicotinic acetylcholine receptor (α7nAChR) is implicated in cognition and neuropsychiatric disorders. It has been an active drug target for decades, however the effect observed in animal models failed to translate into human clinical trials. CHRFAM7A, a human specific fusion gene between CHRNA7 and FAM7A, incorporates in different copy number variations and orientation into α7nAChR and modulates its activity. Understanding CHRFAM7A functional impact is imperative for interpreting α7nAChR targeting clinical trials. To study the effect of CHRFAM7A on drug response in the human context, two iPSC lines (UB068, 0 copy, and UB052, 1 copy direct) were developed. UB068 was further genome edited to express CHRFAM7A by using TALENS. iPSCs were differentiated into MGE progenitors, the precursors of BFCNs and GABA interneurons. As readouts for genotype-phenotype correlation, $A\beta_{1\text{-}42}$ uptake and $A\beta_{1\text{-}42}$ -induced cytotoxicity were tested. In iPSC lines, fluorescent $A\beta_{1\text{-}42}$ uptake assessed by flow cytometry and cell counts showed genotype specific dose response curves. In UB068, $A\beta_{1\text{-}42}$ uptake was linear, while UB068_CHRFAM7A and UB052 demonstrated mitigated $A\beta_{1\text{-}42}$ uptake. Next, the response of MGE progenitors to donepezil and rivastigmine, (Acetylcholine Esterase Inhibitors, AChEIs) and encenicline, a selective a7 nAChR agonist, was tested. In UB068, AChEIs and encenicline decreased $A\beta_{1\text{-}42}$ uptake, $A\beta_{1\text{-}42}$ -induced cytotoxicity and apoptosis. Expression of apoptosis regulator BAX correlated with Caspase3/7 activity. In contrast, in UB068_CHRFAM7A and UB052 neurotoxicity was unchanged by AChEIs or encenicline, and donepezil treatment resulted in an increase in apoptosis. Preliminary data on potential mechanisms are discussed. The results suggest that CHRFAM7A modifies the α7 nAChR to a hypomorphic receptor for $A\beta_{1\text{–}42}$ uptake. Non-carriers of the direct allele might benefit from α7 nAChR targeting therapies. Incorporating pharmacogenetics into clinical trials may enhance signals.

P-08.4-20
Noradrenaline induces an increase in cytosolic levels of L-lactate in single neuron from the rat locus coeruleus

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Evidence suggests a correlation between cognitive decline (eg in Alzheimer's disease) and reduced number of neurons in the locus coeruleus (LC) brain nucleus (Kelly SC et al. (2017) Acta Neuropathol Commun 5, 8). The noradrenergic hypothesis implies that noradrenaline (NA) released from activated LC neurons (LCn) stimulates aerobic glycolysis in LC astrocytes which in turn produce a large amount of L-lactate (LL) that can be transported into LCn through monocarboxylate transporters, a process known as the lactate shuttle (Magistretti PJ, Allaman I (2018) Nat Rev Neurosci 19, 235–49). However, noradrenaline may also stimulate aerobic glycolysis in LCn. Therefore we have tested this hypothesis by isolating LCn neurons from rats and used FRET-based nanosensor Laconic to monitor cytosolic levels of LL in these neurons. Preliminary results revealed that an application of noradrenaline (NA, 100 uM) elicited an increase in cytosolic LL of 13 ± 3% ($P = 0.03$) in comparison with control, in 4 out of 6 cells tested. It is concluded that NA, when released from LCn may directly affect the production and/or usage of LL in LCn. LL released from LCn may then activate LCn via a yet unknown LL receptor on LCn (Tang F et al. (2014) Nat Commun 5, 3284).

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P-08.4-21
HNRNPH localisation to cytoplasmic stress granules and nuclear G4C2 foci in C9orf72 Amyotrophic lateral sclerosis

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Amyotrophic lateral sclerosis (ALS) is an incurable fatal motor neuron disease whose most common known cause is hexanucleotide (G4C2) repeat expansion in the first intron of gene C9orf72. One of the interaction partners of G4C2 expansion in nuclear G4C2 foci, found in ALS and frontotemporal dementia (FTD) patient brains [1], is heterogeneous nuclear ribonucleoprotein H (HNRNPH). We have previously demonstrated that these sense foci form paraspeckle like bodies binding also HNRNPH [2]. In the presented study we wanted to define the role of individual HNRNPH domains on its localisation into G4C2 nuclear foci and cytoplasmic stress granules (SG). We constructed a series of protein fragments based on HNRNPH1 domain structure including mutated quasi RNA-recognition motif (qRRM) domains. For this study fluorescence *in situ* hybridisation (FISH) and immunocytochemistry were used. SG were induced by sodium arsenite treatment and visualised with SG protein marker PABP. The RNA-binding domain map (RBDmap) of HNRNPH1 was constructed and a model of the HNRNPH1 binding to RNA generated with Coot and visualized with Pymol.We showed that all three individual qRRM's are sufficient for sequestration of HNRNPH1 into G_4C_2 foci. Endogenous HNRNPH exhibits nucleocytoplasmic shuttling and abundantly localizes to SGs upon arsenite treatment. Two out of three qRRM domains are required for colocalization with SG markers. The RBDmap showed insight into native HNRNPH1-RNA interactions in living cells and the binding model the possible mode of HNRNPH1 binding to specific RNAs.The results are interesting since they imply different specificities or binding activities of otherwise related domains. The nuclear foci share a group of interacting proteins with SGs and their simultaneous presence in neurons might have further pathological effects in ALS and FTD. [1] Lee et al. Cell Rep. 5 (2013) 1178–1186. [2] Bajc Česnik, Darovic, Prpar Mihevc et al. J. Cell Sci. 132 (2019).

P-08.4-22
Perturbations in the proteasome pool accompany pathology progression in FUS transgenic mice.

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The ubiquitin-proteasome system degrades most intracellular proteins and thus maintain cellular proteostasis. Different forms of proteasomes with respect to the presence of catalytic subunits and regulator complexes are known. Abnormalities of the UPS in general and proteasome function in particular are associated with neurodegenerative diseases including amyotrophic lateral sclerosis (ALS). Herein, using in vivo mouse model of FUS-mediated proteinopathy which reproduces key features of human ALS we performed in-depth characterization of proteasome pool dynamics during the pathology progression. Using qPCR and Western blot, it has been shown that the ongoing proteinopathy is accompanied by a gradual increase in expression of catalytic constitutive proteasome subunit β5 and the immunoproteasome subunits β1i, β2i and β5i. As a result, proteasomes with immune subunits constituted up to 25% of the cellular proteasome pool at the terminal symptomatic stage of the disease. Interestingly, chymotrypsin-like, caspase-like and β5i subunit-specific proteasome activities did not elevate according to the expression levels but were even decreased at the pre-symptomatic stage followed by the reversion of activity at the symptomatic stage towards levels typical for young transgenic mice and their wild-type littermates. Importantly, the reversion was associated with increased activity of constitutive and immune 26S proteasomes and proteasomes with the 11S regulator. Finally, we demonstrated that FUS-positive inclusions are colocalized with immunoproteasome subunits in motor neurons of the spinal cord. Our data indicate the upregulation of proteasomes with immune subunits in the spinal cord during the development of FUS-mediated proteinopathy. At the same time, whether it represents an adaptation or a possible pathogenic factor remains to be determined. The study was supported by the Russian Science Foundation grant #18-74-10095.

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P-08.4-23
A novel *in vitro* method for determining mitochondrial 17ß-HSD10 activity: small-molecule inhibitors screening

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Mitochondria, often called the powerhouse of the cell, contain many interesting targets for the potential treatment of Alzheimer's disease (AD). One of these targets is the multifunctional enzyme 17ß-hydroxysteroid dehydrogenase type 10 (17ß-HSD10) which catalyzes oxidation of a wide variety of substrates, including steroids, e.g. 17ß-estradiol (E2). This enzyme is overexpressed in AD and is also an interaction partner of amyloid β (Aβ), one of the main pathological hallmarks of AD. Interaction with Aβ leads to increased mitochondrial stress, apoptosis, and memory impairment. Modulation of 17ß-HSD10 and Aβ interaction and/or its enzyme activity seems to have a positive and protective effect on mitochondria and cells. The recombinant 17ß-HSD10 was expressed in *E. coli* and purified using IMAC technique. Enzyme activity was measured fluorometrically using E2 as a substrate and NAD^+ as an enzyme cofactor. This reaction is supposed to be more physiological for the estimation of the enzyme activity than the commonly used assay with acetoacetyl-CoA substrate. The activity assay was performed in a black 96-well plate at 37°C. Kinetic parameters of the enzyme were determined and the inhibition potential of selected small-molecule inhibitors was tested. Compounds with promising inhibitory potential will be further studied using specific cell models. The study was supported by Faculty of Science, University of Hradec Kralove (No. SV2104-2021, VT 2019-2021).

P-08.4-24
Application of isogenic stem cell derived motor neurons modified with genetically-encoded biosensors for Amyotrophic lateral sclerosis modeling

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Although each neurodegenerative disease has distinctive clinical features, on a molecular basis they share a similar pattern of disturbances. Oxidative stress is one of the major pathogenic mechanisms contributing to neurons' death regardless of the cause, so it presents a promising therapeutic target. Cell-based models represent a great tool for both studying the disease fundamentals and pharmaceutical screening. The genetically-encoded biosensors that allow registering a variety of molecules: from ions to enzymes can be used on such models. In the case of oxidative stress, they allow tracking changes in RedOx balance in the living cell. Here, using CRISPR/Cas9, we have generated two isogenic iPS cell lines harboring D90A and G127R mutations in the SOD1 gene which cause amyotrophic lateral sclerosis (ALS). Further, we inserted the sequences coding biosensors of cytoplasmic and mitochondrial H_2O_2 in the genome of these iPS cell lines, as well as in the genome of the control "healthy" iPS line and the iPS line obtained from the patient with the hereditary form of ALS. Notably, these insertions were made directly in the "safe-harbor" AAVS1 locus to avoid undesirable effects of random integration, and the biosensors' expression is controlled by the doxycycline-dependent promoter. We have shown that the biosensors' elements are functionally active: they were able to produce perceptible fluorescent signals and change them in response to specific stimuli. Motor neurons differentiated from these iPSCs demonstrated a natural reaction to the exogenous H_2O_2 which was detected by the biosensor. Additionally, motor neurons with mutations in SOD1 displayed a more significant reaction on the glutamate-induced stress expressed in H_2O_2 accumulation and an aberrant reaction to exogenous H_2O_2. The study was supported by the project № 0259-2021-0011 of the ICG SB RAS

P-08.4-25
Comparative salivary proteomics revealed marked changes among adult, elderly and Alzheimer individuals

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Alzheimer disease is the most worldwide common cause of dementia in the elderly, characterized by brain accumulation of misfolded proteins causing decreased cognitive functions. Diagnosis is complex and invasive, find new biomarkers is increasingly urgent. Saliva is gaining more attention as diagnostic fluid since its easy and non-stressed collection, and a comparative proteomic study revealing quali/quantitative changes can provide clues for disease biomarkers. To this aim, it is important to define a suitable control group being the variations of salivary proteome age-related. This study reports the results obtained comparing salivary proteome of Alzheimer patients, adult and elderly healthy controls, by a top-down proteomic approach LC-MS-based for protein identification and quantification. Statistical comparison performed by different tools (ANOVA and t-test) highlighted a level reducing of all the component investigated in elderly than in adult controls. Patients showed levels of peptides active in the oral homeostasis, as statherin, histatin-1 and P-B peptide, and their derivatives, higher than elderly but not than adult controls. Levels of S100A8, A9 and cystatin B, and their oxidized derivates (glutathionylated, nitrosylated and dimeric), cystatins A, α-defensins and thymosin β4 were higher in Alzheimer patients than in both the two control groups, evincing an over-expression of proteins involved in oxidative stress,

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inflammation and neuro-/antimicrobial protection. Data were also investigated by Random Forest to verify to what extent and which peptides are able to identify classes of subjects of different ages or affected by Alzheimer. P-B and P-B des1-7, histatin 5, S100A9 proteoforms, oxidized cystatins S, statherin des1-13 showed a prominent role in the discrimination of 89% of adult and elderly controls. 81% of elderly and patients were discriminated primarily by S100A9 and S100A8 proteoforms, cystatins S, α-defensins, P-B des1-5.

P-08.4-26
Mycoplasma as a trigger for the neurodegenerative process development

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Parkinson's and Alzheimer's are the most common forms of neurodegenerative disorders. One of the main events leading to neurodegenerative processes is a violation of the assembly of the tertiary structure, aggregation, and deposition of proteins, which lead to the loss of synaptic connections and the death of neurons. The involvement of brain infection (bacterial, viral and fungal) in the pathogenesis of abnormal protein accumulation is gaining more and more evidence. Various neurodegenerative disorders are associated with frequent bacterial infections, and the genus *Mycoplasma* is the most common. In this work we investigated proteomic changes occurring in neurons upon mycoplasma infection. Using the easily cultivated bacterium *Mycoplasma gallisepticum* as a model object, as well as unique cell lines of neuronal progenitors obtained from iPSCs from patients with Parkinson's disease and healthy donors, the infected cells were analyzed using a mass spectrometric approach using a quantitative shotgun analysis for Q Exactive HF-X with NanoSpray Flex II. Using the KEGG database, we found that the observed protein changes are well clustered along metabolic pathways associated with neurodegenerative diseases (Alzheimer's, Parkinson's, Huntington's), which clearly indicates that mycoplasma infection leads to the development of processes in neurons associated with neurodegeneration. In addition, we carried out experiments to analyze the direct effect of mycoplasma on the aggregation of amyloid peptides APP40 and APP42, and also evaluated the effect of mycoplasma on the phosphorylation status of tau protein. The obtained results will shed light on the mechanisms of neurodegenerative changes, and, consequently, to advance in their treatment and preventive measures. This work was supported by RSF grant №19-15-00427.

P-08.4-27
Effect of elastin-derived peptides (EDPs) and carbamylated-EDPs on vascular smooth muscle cells phenotype in relation to arterial stiffness during aging.

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Among the changes found during vascular aging, arterial stiffness represents one of the key elements in the development and/or progression of cardiovascular diseases. This phenomenon can be explained by various processes including a phenotypic switch of the smooth muscle cells constituting the vascular wall (vSMC). The role of bioactive elastin-derived peptides (EDPs) and carbamylation (i.e. a non-enzymatic post-translational modification of proteins) in the development and the evolution of certain cardiovascular diseases has been highlighted in the past few years but the combination of these two phenomena remains to be explored. The aim of this work is to study the effect of EDPs and carbamylated-EDPs on the phenotype of vSMCs. MOVAS (vSMC cell line) were treated with EDPs or carbamylated-EDPs for 24 hours before various cellular parameters were analyzed : adhesion by crystal violet staining, migration by wound healing assay, cytoskeletal reorganization by immunofluorescence, contraction by immunoblotting and cell stiffness by atomic force microscopy (AFM). The results showed that EDPs induced cytoskeletal remodeling as well as an increase in N-cadherin expression involved in morphological modifications of vSMC. The increase in GTPase RhoA activity associated with the increase in Smooth Muscle Myosin Heavy Chain expression suggests that EDPs may promote vSMC contraction as well as their adhesion and migration. Such results may be directly correlated to those obtained by AFM showing a modification of their stiffness. We also demonstrated that carbamylation of EDPs seems to limit their bioactivity. These results highlighted a possible involvement of EDPs in the behavior and stiffness of vSMC during vascular aging and a potential inhibitory effect of carbamylation reaction on their effects. *The authors marked with an asterisk equally contributed to the work.

P-08.4-28
α-Synuclein deficiency or overexpression induces neuroinflammatory responses in mice

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α-Synuclein (αSyn) is a presynaptic protein involved in Parkinson's disease (PD) pathogenesis. Point mutations or multiplications in SNCA gene encoding αSyn cause familial forms of PD. In both genetic and sporadic forms, the aggregated αSyn, among

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other cellular components, constitutes Lewy bodies, the most characteristic histopathological hallmark of PD. αSyn overexpression has been shown to drive neuroinflammatory responses, activating microglial cells and inducing the production of pro-inflammatory cytokines in patients and animal models. However, little is known about the relationship between the physiological function of αSyn and neuroinflammation. Here we investigated neuroinflammatory responses on αSyn deficiency or overexpression in a Snca knockout ($Snca^{-/-}$) mouse and the SNCA-OVX PD mouse model. We analyzed microglial phenotypes and inflammatory pathways in striatum (STR) and substantia nigra (SN), focusing on pathophysiological events in dopaminergic neurons with an axon-soma approach. Our results indicate that $Snca^{-/-}$ mice show altered microglia and astrocytes expression at 6-months-old, but not at 12-months-old. Specifically, we found a reduction in neurotoxic microglia (M1) in STR and SN and a tendency to nigral astrogliosis. Regarding SNCA-OVX mice, we observed an increased presence of M1 microglia compared to $Snca^{-/-}$ individuals in STR from 6-month-old mice. SNCA-OVX animals also exhibited activations of NF-κB and ERK1/2 signaling pathways in STR from 6-month-old animals, while $Snca^{-/-}$ mice showed the same trend without reaching statistical significance. Additional studies are needed to fully understand the role of αSyn in neuroinflammation, but our results indicate that both its deficiency and its overexpression alter the immune response in the brain.

P-08.4-29
Molecular and biochemical changes occurring in the brain after traumatic brain injury and leading to Parkinson's disease: modulation by *Hericium erinaceus* and *Coriolus versicolor*

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Traumatic brain injury (TBI) is a major health and socioeconomic problem affecting the world. This condition results from the application of external physical force to the brain which leads to transient or permanent structural and functional impairments. TBI has been shown to be a risk factor for neurodegeneration which can lead to Parkinson's disease (PD) for example. In this study, we wanted to explore the development of PD-related pathology in the context of an experimental model of TBI and the potential ability of *Coriolus versicolor* and *Hericium erinaceus* to prevent neurodegenerative processes. Traumatic brain injury was induced in mice by controlled cortical impact. Behavioral tests were performed at various times: the animals were sacrificed 30 days after the impact and the brain was processed for Western blot and immunohistochemical analyzes. After the head injury, a significant decrease in the expression of tyrosine hydroxylase and the dopamine transporter in the substantia nigra was observed, as well as significant behavioral alterations that were instead restored following daily oral treatment with *Hericium erinaceus* and *Coriolus versicolor*. Furthermore, a strong increase in neuroinflammation and oxidative stress emerged in the vehicle groups. Treatment with *Hericium erinaceus* and *Coriolus versicolor* was able to prevent both the neuroinflammatory and oxidative processes typical of PD. This study suggests that PD-related molecular events may be triggered on TBI and that nutritional fungi such as *Hericium erinaceus* and *Coriolus versicolor* may be important in redox stress response mechanisms and neuroprotection, preventing the progression of neurodegenerative diseases such as PD. *The authors marked with an asterisk equally contributed to the work.

P-08.4-30
Unravelling the mechanism of GM1-oligosaccharide neuroprotection: mitochondrial regulation

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Parkinson's disease (PD) is a neurodegenerative disorder characterized by the progressive loss of dopaminergic (DA) neurons in the brain substrantia nigra. Although its etiopathogenesis is still poorly understood, the mitochondrial (mit) dysfunction was described to have a crucial role in the exacerbation of neuronal degeneration. Hence, mit targeted protective compounds capable to minimize mit dysfunction constitute hopeful therapeutic strategies for PD. Here, we describe the properties of GM1 oligosaccharide (OligoGM1), the bioactive portion of GM1 ganglioside, that by interacting with and activating the NGF TrkA receptor at cell surface triggers crucial cellular pathways responsible for mit neuroprotection. Using proteomic and biochemical approaches, we demonstrated that OligoGM1 is able to induce the mitochondriogenesis and to enhance the mit function (Fazzari M et al. (2020) Glycoconj J 37, 293–306). Wild-type Neuro2a cells treated with OligoGM1 showed an increased number of mitochondria and, at functional level, an increased expression of mit complexes, boosted ATP levels and mit respiration. Importantly, OligoGM1 treatment determined the rescue of mit activity and respiration in a Neuro2a model of mit dysfunction. On the other hand, OligoGM1 proved to efficiently counteract the neurotoxicity associated to 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP), a PD-linked neurotoxin that acts by inhibiting the mit complex I. Specifically, OligoGM1 pretreatment strongly reduced the mit ROS overproduction and P38 MAPK hyper-phosphorylation due to MPTP exposure leading to increased cell viability and neurite network in Neuro2a cells and DA neurons with enhanced ATP levels and mit complexes expression. Collectively our data indicate that OligoGM1 is able to protect neurons possibly by via mit function restoration and oxidative stress reduction, opening a new perspective for the use of OligoGM1 in diseases where these organelles are compromised, including PD.

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P-08.4-31
GM1-oligosaccharide neuroprotective action in *in vitro* and *in vivo* models of Parkinson's Disease

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GM1 ganglioside has been considered as a master regulator of the nervous system and accumulating evidence is pointing out age-dependent GM1 deficiency as initiator of sporadic Parkinson's disease (PD), a neurodegenerative disorder characterized by the abnormal presence of α-synuclein aggregates in dopaminergic neurons (DA) of the substantia nigra and the consequent progressive neuronal death. Preclinical data have reported that GM1 administration exerts neuroprotective and neurorestorative properties, although the benefit resulting from GM1 replacement therapy is extremely limited by its amphiphilicity that prevents the BBB passage. Recently, we demonstrated in neuronal cells that the oligosaccharide portion of GM1 (OligoGM1) is the actual moiety responsible for GM1 neurotrophic properties, by the activation of the TrkA-MAPK pathway at the plasma membrane level. Thus, in this scenario we decided to evaluate the OligoGM1 neuroprotective potential using two *in vitro* PD models: i) DA neurons intoxicated with MPTP, a toxin that induces DA neurons death by affecting mitochondria and causing ROS over production and ii) α-synuclein oligomers (aSynO) injured DA neurons reproducing the essential neuropathological features of PD. By biochemical analysis, we observed that the pre-treatment of rat DA neurons with OligoGM1 significantly increases neuronal survival and preserves neurite networks affected by aSynO and by MPTP. *In vivo*, we found that following MPTP administration in mice, OligoGM1 restores nigral tyrosine hydroxylase expression reaching the healthy condition. Moreover, OligoGM1 administered to B4galnt1+/- mice, model of sporadic PD, reduce nigral αS aggregates and restore DA tyrosine hydroxylase neurons. The obtained data suggest that OligoGM1 administration protects DA neurons probably triggering a trophic signal starting at plasma membrane and implementing mitochondrial bioenergetics, reducing oxidative stress and augmenting the α-synuclein clearance.

P-08.4-32
Baikal oxyphils as a source of new antioxidants

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Microorganisms of unexplored ecosystems are the focus of great scientific interest. Lake Baikal is characterized by the low temperature of the water and high oxygen content (up to 18 mg/l). We suggested that Actinobacteria associated with Baikal organisms inhabit littoral zones with seasonal hyperoxia effect may be presented by oxyphilic forms. Also, these bacteria may produce natural products with antioxidant activity. The current study aimed to assess the influence of oxygen and ozone on morphological characteristics and actinobacteria's antioxidant potential. Actinobacteria were isolated from Baikal invertebrates and cultivated in standard conditions of natural aeration (control – 7.3 mg/l), under increased oxygen content (up to 24.3 mg/l) in the flasks, and under conditions of increased oxygen and ozone level. To assess the effects of increased concentration of oxygen and ozone on morphological characteristics of microorganisms, the heat-fixed smears were coloured by Gram-method. The composition of natural products produced by actinobacteria was evaluated using general high-resolution LCMS technique with the following dereplication analysis using the Dictionary of Natural Products database (CRC press-2019). It was shown that the elevated level of spore formation characterized actinobacteria cultivated under experimental conditions compared to natural aeration conditions. Also, novel natural products with retention time and masses 2.4 min (360.2351 Da), 2.9 min (306.6337 Da), 8.1 min (494.2271 Da), 15.3 min (751.3685 Da) were synthesized by *Streptomyces* sp. strain only under experimental conditions. Moreover, we demonstrated that *Streptomyces* sp. produce antioxidant Nocardamine only under experimental conditionals. Consequently, it was shown that oxygen influences on Lake Baikal microorganisms adapted to natural hyperoxia. The study was carried out with the main financial support of RFBR (projects 18-29-05051) and the President's grant (MK-1245.2021.1.4).

P-08.4-33
Changes in the mitochondrial network morphology in human fibroblasts with Parkinson's disease associated mutations

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Parkinson's disease (PD) is a complex disorder accompanied by impaired mitochondrial function. Cell mitochondria exist in the form of a dynamic structure – the mitochondrial network (MN). Recently, various violations of the morphology of MN (e.g. pathological changes in the length of individual mitochondria, the number of connections in MN, appearance of pathological structures) are considered as key factors in the development of neurodegenerative diseases. We analyzed some MN parameters in human fibroblasts with PD associated mutations (in genes encoding PINK1, alpha-synuclein (A53T), PINK1/Parkin) and evaluated PD phenotype using the method of automatic analysis of confocal images (staining with MitoTracker, TMRM). During image processing mitochondria were divided into 4 groups according to their Aspect Ratio (AR 1–4, 4–8, 8–12, 12–16). Statistical analysis shows that fibroblasts with PINK1 and PINK1/Parkin mutation are characterized by the small mitochondria (AR< 4), whereas the A53T mutation was characterized by the presence of longer forms and an increase in the average length of the mitochondria compared to the control. As for the MN connectivity, for PINK1 it was indistinguishable from the control, whereas for A53T and PINK1/Parkin, an increase in the number of MN connections was found. In addition, abnormal structures in the form of mitochondrial "donuts" were found in the PINK1 and PINK1/Parkin cells. We have previously shown that all these mutations lead to increase in cytosolic and mitochondrial ROS production in fibroblasts and iPS-derived neurons. In addition, these cells were more sensitive to exogenous stress, which can be correlated with characteristic changes in MN. Thus, the data obtained may be useful for understanding the fundamental mechanisms of the development of PD and the participation of MN in this process. The study was funded by RFBR grant #20-34-70074 and by the grant of the Russian

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Federation Government #075-15-2019-1877. *The authors marked with an asterisk equally contributed to the work.

P-08.4-34
Unravelling the aging process in erythrocytes from Parkinson desease patients

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Parkinson disease (PD) is a chronic progressive, age-related neurodegenerative disorder that belongs to the group of 'synucleinopathies'. The pathological features of PD suggest that diagnostic markers can be found in peripheral fluids. In this work we investigated the morphology and thermodynamic behavior of red blood cells (RBCs) derived from patients diagnosed with PD and healthy individuals along the aging process exploring differential scanning calorimetry, atomic force and optical microscopy. Analysis of the calorimetric profiles of RBCs reveal that along the aging the major structural and functional proteins (hemoglobin, the membrane spanning domain of the integral band 3 protein and peripheral band 2.1, 4.1, 4.2 proteins) of RBCs exhibit different stability against the thermal challenge in PD and healthy cells. Hemoglobin, band 3 and band 2.1, 4.1, and 4.2 proteins are thermally more stable in PD than in healthy cells. Optical microscopy data show that the biconcave shape, responsible for the unique cell deformability, is the dominating morphology type of freshly isolated healthy cells, while the crenated shape is predominant in PD cells. The cell morphology experiences ageing related alterations, reaching similar morphology for both the healthy and PD cells after 30 days of aging. The surface roughness of RBCs, that is a measure of the cell membrane skeleton integrity, has significantly lower value for PD than for fresh healthy cells and is further reduced along the aging. Our data depict a relation between the PD occurrence and the erythrocytes' aging process. Acknowledgements: This work was supported by the Bulgarian National Science Fund, grant КП-06-H31/8, Competition for financial support of basic research projects – 2019. AFM experiments were performed using Research equipment of Distributed Research Infrastructure INFRAMAT, part of Bulgarian National Roadmap for Research Infrastructures, supported by Bulgarian Ministry of Education and Science. *The authors marked with an asterisk equally contributed to the work.

P-08.4-35
The peculiar solvent accessible surface of Huntingtin

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Huntingtin is an essential protein encoded by the HTT gene, and its mutations are associated with the Huntington disease (HD), an autosomal dominant progressive neurodegenerative disorder. Taking advantage of the last cryogenic electron microscopy structure of human huntingtin, we explore with computational methods its physicochemical properties, focusing on the solvent-accessible surface of the protein, and highlighting a mix of hydrophobic and hydrophilic patterns, with the prevalence of the latter ones [1]. Huntingtin can physically interact with a large number of different proteins; we find that the number of computed interacting sites predicted by ISPRED4 (https://ispred4.biocomp.unibo.it/ispred) agrees well with the number of experimentally determined interactors. Interestingly, all the interaction sites cluster in specific zones of the protein. All the predicted interacting sites when variated may hamper functional interactions of huntingtin with other genes important for many biological processes to which it contributes and elicit different disorders. By exploring with eDGAR (https://edgar.biocomp.unibo.it) the complex space of gene-disease relations and taking advantage of the 98 gene interactors, we were able to associate huntingtin to some 43 more diseases different from HD [1]. We find that in the surface patches not containing interacting residues, we are able to allocate calcium ion-binding sites with a precision higher than 95%. Considering that calcium ions can mediate protein-membrane interactions, we propose that huntingtin–membrane interactions can also be mediated by calcium as a cofactor [1]. Our findings are justified in relation to the present knowledge of huntingtin functional annotation; hopefully, future experiments will shine a light on this aspect. [1] Babbi G et al. (2021) IJMS Molecular Neurobiology, in press

P-08.4-36
Adenosine A1 and A2A and metabotropic glutamate receptor 5 are present in blood serum and exosomes from SAMP8 mice. Potential use as blood-based biomarker for Alzheimer's disease

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Adenosine (ARs) and metabotropic glutamate receptors (mGluRs) are G-protein coupled receptors (GPCR) widely expressed in the body with different tissue-dependent biological functions. In the central nervous system, these receptors are key participants in the neurotransmission and have been described strongly altered in several neurodegenerative diseases, including Alzheimer's disease (AD). They are also altered in the brain from SAMP8 mice, an animal model of aging and AD. In the present work, it is shown the presence of both ARs and mGluRs in blood serum and derived-exosomes from SAMP8 mice as well as its possible modulation by aging. In blood serum, adenosine A1 and A2A receptors remained unaltered from 5 to 7 months of age. However, an age-related decrease in adenosine level was observed, while 5'-nucleotidase activity was not modulated. Regarding the glutamatergic system, a decrease in mGluR5 density and glutamate levels was observed in older mice which were similar to those previously described in the brain of the same animals. These GPCRs were also found in blood serum-derived exosomes, suggesting that these receptors could be released into circulation via exosomes. Altogether, we suggest that the quantification of these receptors, and their corresponding endogenous ligands, in blood serum could reflect changes taking place in

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brain and, therefore, could serve as an early biomarker for the diagnosis of AD.

P-08.4-37
Ataxia-related protein sacsin knockout disrupts the intermediate filament network in glial cells.

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Autosomal recessive spastic ataxia of Charlevoix-Saguenay (ARSACS) is a neurodegenerative disorder commonly diagnosed in infants, and characterized by progressive cerebellar ataxia, spasticity, motor sensory neuropathy and axonal-demyelination. ARSACS is caused by mutations in the SACS gene that lead to truncated or defective forms of the multidomain protein sacsin. Sacsin function has been exclusively studied on neuronal cells, where it regulates mitochondrial dynamics and regulates the polymerization of neurofilaments and vimentin. It remains unknown whether and how glial expression of mutant sacsin can contribute to ARSACS pathology. Western blot analyses and immunocytochemistry showed that sacsin is expressed in astrocytes, the major type of glia. We generated and validated a stable sacsin knockout in the C6 rat glioma cell line. Sacsin deletion in C6 cells induced an apparent depletion of mitochondria and the cytolinker protein plectin in the juxtanuclear area. The intermediate filaments GFAP, nestin and vimentin were accumulated in the same juxtanuclear area in more than 40% of $C6^{sacs-/-}$ cells, while less than 5% of control cells showed a collapsed network for these filaments. Alterations in the nuclear intermediate filament lamin B1 were also observed in sacsin-knockout cells. Interleukin 6 (IL-6) induced astrocytic differentiation of C6 cells. Treatment of wildtype and $C6^{sacs-/-}$ cells with a combination of IL-6 and soluble IL-6 receptor induced a greater increase in GFAP expression in $C6^{sacs-/-}$ cells, whereas in wildtype cells the levels of STAT3, $pSTAT3^{Y705}$ and $acSTAT3^{K49}$ were higher. Our findings provide insights into a key role for sacsin in glial cells as an organizer of intermediate filament networks and point at a possible role for astroglia in ARSACS dysfunctions.

P-08.4-38
Newly synthetized A2AAR antagonists as anti-inflammatory strategy in Parkinson's disease

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Parkinson's disease (PD) represents the second most common neurodegenerative disorder worldwide. So far, available treatments possess mere symptomatic action and severe long-term side effects, so the development of neuroprotective strategies is highly needed. It is now widely recognized that neuroinflammation is crucial in PD, especially in the disease progression. Therefore, pharmacological treatments able to modulate the pathological immune response may reveal effective in slowing disease progression. The microglial cells are the key component of the brain immune system and can be activated by adenosine through the interaction with A2AARs (A2A adenosine receptors). Indeed, the modulation of purinergic receptors has been associated with a slower degeneration of nigrostriatal dopaminergic cells in PD. Since previous findings demonstrating the ability of A2AAR antagonist 8-ethoxy-9-ethyladenine (ANR94) to protect nigrostriatal neurons from neuroinflammation in an animal model of PD (1), several ad hoc-designed A2AAR antagonists (ANR94 analogues) have been synthetized and tested in activated BV-2 microglial cells. To mimic neuroinflammation BV-2 cells were exposed to 100 ng/mL LPS or 0.5 mM MPP+ for 24 h. The potential anti-inflammatory activity of ANR94 analogues were evaluated by MTT assay and measuring the reactive oxygen species (ROS) levels by DCF-DA probe, while gene expression analyses of inflammatory cytokines such as TNFα, IL-1β and the pro-inflammatory enzymes iNOS and COX-2 were performed by RT-PCR. Interestingly, the newly synthetized compounds were more effective than the lead compound ANR94 in counteracting inflammatory damage suggesting their potential use as therapeutic agent to prevent/counteract PD. Of course, animal and clinical studies are needed to investigate their *in vivo* activity. This work was supported by MIUR-PRIN n. 20152HKF3Z and University of Camerino n. FPI000065. 1. Pinna A et al. (2005) Eur J Pharmacol 512, 157–164.

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P-08.4-39
Disruption of LONP1 mitochondrial protease initiates a stress responsive cross-talk between mitochondrial and cytoplasmic proteostatic mechanisms in *Caenorhabditis elegans* and human cancer cells

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Mitochondrial function and protein quality maintenance depend on a sophisticated network of surveillance and homeostatic mechanisms. Mitochondrial matrix protease LONP1 was shown to be an important component of this network in all species. However, the fact that lonp1 is an essential gene in both Drosophila and mammals, has made the investigation of its biological role difficult. In contrast, a lonp-1 null mutant in *C. elegans*, which we created with the help of the CRISPR/Cas9 technology, was viable and therefore used to explore the effects of loss of LONP-1 in nematode development, behavior, lifespan and stress response. The lonp-1 knockout strain exhibited developmental and lifespan defects, while it responded differently to a variety of environmental insults. In addition, we observed disturbed morphology and function of the mitochondrial network, increased ROS production and activation of the mitochondrial Unfolded Protein Response (UPR^{mt}) signaling in lonp-1 mutants. Disruption of lonp-1 also triggered induction of other key stress responsive pathways and transcription factors, involved in ROS detoxification and proteostasis. In humans, dysregulation of LONP1 activity has been associated with severe pathological conditions such as cancer. For this reason, we have investigated the effects of human LONP1 downregulation in cancer cell lines, using siRNA. We found that a number of stress-related genes associated with the Integrated Stress Response (ISR) and the UPR^{mt} were upregulated in order to meet this challenge. Taken together, the above results suggest that the absence of LONP1 initiates a cross-talk between mitochondrial and cytoplasmic protein quality surveillance mechanisms, which is essential for the viability of mutant animals or human cancer cells. *The authors marked with an asterisk equally contributed to the work.

P-08.4-40
The hepatic protein URG7 relieves tunicamycin-induced injury in SH-SY5Y neuronal cells

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Up-regulated Gene clone 7 (URG7) is a protein whose expression is upregulated in liver cells during the hepatitis B virus infection. It is located in the endoplasmic reticulum (ER), is able to inhibit apoptosis mediated by TNFα, to activate the PI3K/AKT pathway, to mitigate ER stress by modulating the expression of several Unfolded Protein Response (UPR) markers, promoting cell survival [Armentano MF et al. (2018) Biol Cell 110,147–158]. In this work, the expression of URG7 in different cell lines was preliminarily evaluated and it was found to be quite expressed in the human neuroblastoma cell line SH-SY5Y. Several pathological conditions, including neurodegenerative diseases, are known to be caused by the perturbation of proteostasis [Remondelli P et al. (2017) Front Mol Neurosci 10,1–16], giving rise to pathologies known as PMDs (Protein Misfolded Disorders). Therefore, the authors aimed to verify the possible contribution of URG7 in mitigating the alterations of proteostasis in neuronal cells. Stable overexpression of URG7 protein in SH-SY5Y cells was obtained through infection with lentiviral vectors, while ER stress was induced by using the antibiotic tunicamycin. The results showed that URG7 is still localized in the ER, its expression alone doesn't significantly alter cellular protein homeostasis and it is able to modulate the expression of some UPR markers. In fact, GRP78/BiP, crucial in the management of protein folding, was up-regulated; the proapoptotic transcription factor CHOP didn't increase its expression under ER stress conditions; the transcription factor sXBP1, that induces the transcription of ER chaperones, was moderately increased. In addition, the presence of URG7 also promoted the upregulation of p-AKT, confirming itself as a promoter of cell survival. Therefore, this work showed the activity of URG7 as ER stress reliever in a neuronal cell line and allowed to hypothesize its potential role in the context of neurodegenerative diseases.

P-08.4-41
Spilanthol-rich essential oil from *Acmella oleracea* and its nanoemulsion: anti-inflammatory strategy for the prevention of neurodegeneration.

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Neurodegenerative diseases have grown as a major health and societal challenge nowadays. These diseases share common pathological features like accumulation of misfolded proteins, mitochondrial disfunction, excitotoxicity, oxidative stress and inflammation. In the last years, neuroinflammation, caused by over-acting microglia, has emerged as a key element of the pathogenesis of these diseases. Therefore, the modulation of pro-inflammatory molecules from microglia could be a promising strategy to counteract neurodegeneration. *Acmella oleracea* (L.) is a medicinal plant whose wide use as traditional remedy is linked to its pharmacological properties, such as anti-inflammatory, antioxidant, analgesic and hepatoprotective[1]. These effects have been mostly related to its main secondary metabolite the alkylamide spilanthol. In this study, we investigated the anti-inflammatory activities of a spilanthol-rich essential oil from *A. oleracea* (AO) in BV-2 microglial cells. Cells were treated with AO, pure spilanthol(S) or a nanoemulsion (NE), (composed by AO, ethyl oleate, and polysorbate 80) for 24 h, then exposed to LPS. All the treatments significantly increased cell viability (MTT assay) in respect to cells only exposed to LPS. Interestingly, AO and NE also reduced ROS levels (DCFH-DA assay), while S had no effect on this parameter. To verify if this protection could be ascribed to an anti-inflammatory mechanism, the expression of IL-1β and TNF-α, COX-2 and iNOS was evaluated by RT-PCR. Interestingly, all the treatments reduced the

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expression of these inflammatory mediators. These results suggest AO as a potential therapeutic agent in neurodegenerative diseases thanks to its antioxidant and anti-inflammatory activities. Of note, this work also evidences a promising application of NE as a new technological formulation to further increase the potential of this essential oil. This work was supported by MIUR-PRIN n. 20152HKF3Z. 1.Matyushin AA et al. 2017 J. Pharm. Sci.9, 5.

P-08.4-42
Regulation of the lysosome-like vacuole and amino acid homeostasis in the yeast model of Niemann-Pick type C1

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Lysosome and lysosome-like vacuoles (in yeast) play crucial roles in cellular responses to nutrient availability and composition, ion homeostasis, stress resistance, and cell death. Conserved nutrient signalling pathways, including TORC1, modulate lysosomal function and autophagy. Notably, a decline in lysosomal function has been related with the progression of metabolic and age-associated diseases, including lysosomal storage disorders (LSDs). The Niemann Pick Type C (NPC) is a rare LSD characterized by an abnormal accumulation of lipids (cholesterol and sphingolipids) in the late endosomal/lysosomal network. It results from loss-of-function point mutations in NPC1 or NPC2, which are involved in lipid transport through the endocytic pathway. We have previously shown that yeast lacking Ncr1p, orthologue of human NPC1 protein, display a premature ageing phenotype associated with mitochondrial dysfunctions and accumulation of sphingolipids [1]. In this study, we performed a phosphoproteomic analysis of vacuolar membranes from wild type and ncr1Δ cells. Our results revealed changes in the levels or phosphorylation of ncr1Δ vacuolar proteins that are mostly associated with transmembrane transport (including amino acid transporters), vacuolar acidification, vesicle-mediated transport, and vesicle fusion with vacuole. Consistently, autophagy (process required for amino acid homeostasis) was impaired in ncr1Δ mutants and the supplementation of glutamate or glutamine increased their chronological lifespan and modulated TORC1 activity. These results suggest that changes in vacuolar proteins and amino acid homeostasis may contribute to the shortened lifespan in the yeast model of Niemann-Pick type C1. This work was funded by national funds through FCT - Fundação para a Ciência e a Tecnologia, I.P., under the project UIDB/04293/2020. TM (SFRH/BD/136996/2018) and CP (IF/00889/2015) are supported by FCT. [1] R. Vilaça et al. (2018) Biochim Biophys Acta Mol Basis Dis, 1864, 79–88

P-08.4-43
PPARγ antagonist – GW9662 reverse neuroprotective effect of Irbesartan in organotypic hippocampal cultures undergoing oxygen-glucose deprivation

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Ischemic stroke is the second leading cause of death in the world. Currently, the only routinely therapy applied in stroke treatment is thrombolysis with the use of a tissue plasminogen activator. The biggest disadvantage of such an approach is a very narrow therapeutic window. Since one of the stroke hallmarks is a development of destructive neuroinflammation, therapies targeting various pathways responsible for an immune response to ischemia are under consideration while thinking about new drugs. Irbesartan – antagonist of receptor of proinflammatory chemokine CCL2 is proposed to modulate neuroinflammation. Thus, the aim of this work was to answer the question, whether Irbesartan exerts its neuroprotective action through modulation of the PPARγ pathway. The main research method was rat organotypic hippocampal cultures (OHC). After 7 days of OHC, oxygen and glucose deprivation (OGD) has been performed to mimic ischemia followed by 24 hours of reoxygenation. After this time, OHC were collected for biochemical analysis as well as fixed for confocal microscopy and mechanical characterization using atomic force microscopy. OGD results in a significant increase in mortality within the examined nervous tissue. OGD induces a decrease in the stiffness (Young's modulus) of the tested OHC cultures which is associated with alteration of microtubular and actin cytoskeleton. Activation of the CCL2/CCR2 system is unfavorable in the process of ischemia and the use of Irbesartan reduced mortality and increased the stiffness of OHC undergoing OGD. The use of GW9662 on OHC treated with Irbesartan and OGD led to a significant increase in mortality and a maximum decrease in the stiffness of OHCs treated with OGD. To sum up, modulation of the PPARγ pathway by its antagonist GW9662 led to both biochemical as well as physical changes in ischemic tissue treated with Irbesartan. It confirms that the neuroprotective effect of irbesartan might be modulate by alteration of the PPARγ pathway.

Redox biology – oxidative stress signalling

P-08.5-01
Mutations found in cancer affect DPP III – KEAP1 interactions

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KEAP1 is the main cellular sensor of oxidative stress and a repressor of the transcription factor NRF2 in the KEAP1-NRF2

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signaling pathway which is regulating the antioxidative stress response. The release of NRF2 from KEAP1 and its translocation to nucleus promotes cell survival, which can be deregulated in cancer cells. It was reported that under the oxidative stress conditions dipeptidyl peptidase III (DPP III) can also competitively bind to KEAP1, inducing the release of NRF2. The interaction is mainly established by accommodation of the ETGE binding motif, located at the DPP III flexible loop, into the central pore of the 6-bladed β-propeller Kelch domain of KEAP1. Combination of different experimental and computational approaches enables the protein structure and function relationship studies. The structural study of the wild type and mutant DPP III-KEAP1 complexes has been performed by X-ray diffraction combined with MD and ASMD simulations. ITC and MST experiments enabled the binding rate determination for the proteins with mutations found in cancer cells. A number of the mutations was found in structural elements of proteins involved in the binding process. The results show that these mutations can affect the DPP III-KEAP1 binding, and consequently modulate the oxidative stress response and chemotherapy resistance in cancer cells.

P-08.5-02
The role of mitochondrial phospholipase A2γ in the regulation of cellular redox homeostasis and oxidative stress signalling

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Redox-dependent regulations play an essential role in a wide range of biological activities. Mitochondria in numerous tissues represent a primary source of superoxide and subsequent downstream oxidants, notably H_2O_2 and lipid hydroperoxides. However, the understanding of the role of mitochondrial oxidant production in pathology and normal physiology is limited. Mitochondrial calcium-independent phospholipase A2γ (iPLA2γ) belongs to a family of enzymes that participate in cellular signalling by simultaneously producing free fatty acids (FA) and lysophospholipids. Here we hypothesise that iPLA2γ plays an antioxidant role in various tissues by contributing to FA-dependent dissipation of the protonmotive force and subsequent attenuation of mitochondrial oxidant production. Following the respiration of mitochondria isolated from selected tissues of wild-type (WT) and iPLA2γ-KO mice, we demonstrated an increase in respiration following the addition of extrinsic hydroperoxides, including H_2O_2. The oxidant-induced increase in respiration was prevented by (1) R-bromoenol lactone, a selective inhibitor of iPLA2γ, (2) carboxyatractyloside, an inhibitor of adenine nucleotide translocase (ANT), and (3) thiol reducing agents. The oxidant-induced changes in respiration were absent in mitochondria isolated from iPLA2γ KO mice. To confirm our hypothesis, that the oxidant-induced increase in respiration is due to the release of FA, we have analyzed the samples obtained from the respiration assay by HPLC-MS. The data show an oxidant-dependent increase in relative concentrations mainly of docosahexaenoic, oleic, and arachidonic acid, which was prevented by R-BEL but not by ANT inhibitor. These results suggest that the oxidant-induced activity of iPLA2γ leads to a release of free fatty acids, which promote ANT-dependent H^+ transport, leading to a decrease in the mitochondrial protonmotive force and subsequent attenuation of mitochondrial superoxide production.

P-08.5-03
IGF-1 via PI3K/AKT activation promotes survival and anabolic metabolism in HEI-OC1 auditory cells

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Hearing loss is the most common sensory deficit in the human population. Mutations in the gene coding for IGF-1 cause sensorineural hearing loss in man and mice (previously published in: Rodríguez-de la Rosa L et al. (2017) Front Aging Neurosci 12,9:411). Actions of IGF-1 are mediated by binding to its high affinity transmembrane receptor, IGF1R. This interaction typically leads to the activation of the PI3K-AKT pathway and of the MAPK-ERK cascade. To gain insight into the molecular mechanisms involved in IGF-1 downstream signaling in the sensory hair cells, we have used the HEI-OC1 cell line derived from the Immortomouse™ hearing receptor (previously published in: Kalinec GM et al. (2003) Audiol Neurootol 4, 177–189), which is the cell line of choice to study the molecular steps occurring during the differentiation to outer hair cell-like cells of auditory progenitors. The study of the relative expression of genes of the IGF system by RT-qPCR showed that IGF system factors and receptors are expressed in both progenitors and differentiated auditory cells. We also studied the actions and main downstream signaling pathways of IGF-1. Apoptosis and cell viability were studied by flow cytometry and XTT assay, respectively, whilst activation of target proteins was measured by Western blotting. The consequences of blockage of IGF-1 actions were also investigated by using specific IGF1R inhibitors and XTT assay. IGF-1 increased survival, proliferation, as well as glucose metabolism and protein synthesis, whereas autophagic flux was decreased and apoptosis inhibited. Our data indicate that HEI-OC1 cells can be used as a model to understand the actions of IGF-1 in hair cells, to identify novel targets and to unravel the molecular mechanisms involved in IGF-1 deficiency-associated otic damage.

P-08.5-04
Association between oxidative stress parameters (Malondialdehyde, Superoxide dismutase, Pro-Oxidant Antioxidant Balance) and obesity in the pediatric population

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Introduction. Obesity, as one of the biggest threats to public health, is a condition that is reflected in the increased accumulation of body fat. As a complex disease, obesity can be associated with the emergence of multiple serious conditions such as oxidative stress. Oxidative stress represents an imbalance between prooxidants and antioxidants causing increased amounts of reactive oxygen and nitrogen species. The aim of this study was to investigate the association between measured oxidative stress

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parameters (MDA - lipid peroxidation marker, SOD - protects us from dangerously reactive forms of oxygen, PAB) and obesity in the pediatric population. Materials and methods. The study included 135 obese and overweight children (BMI above the 85th percentile) and 86 healthy controls (BMI less than 85th percentile) aged 2–19, who underwent an anthropometric evaluation. All subjects included in the study were recruited at the Clinical Centre University of Sarajevo and were free of evidence of chronic problems (infections, surgery, thyroid disease, polycystic ovarian syndrome), active liver and kidney damage and were not using any hormonal therapy. The oxidative stress parameters (MDA, SOD and PAB) were determined by spectrophotometric methods, according to the protocols. Results. In our study, results demonstrated a significant increase in the MDA levels in obese children compared to the healthy controls ($P < 0.001$). Also, obese children had a significant increase in the SOD levels compared to the control group ($P = 0.002$). On the other hand, results demonstrated a significant decrease in the PAB levels in obese children compared to the healthy controls ($P < 0.001$). Conclusion. Our study demonstrated that measured oxidative stress parameters show a strong association with obesity in the pediatric population. This study also suggests that adequate control of body weight could improve the quality of life and prevent possible pathological conditions caused by oxidative stress.

P-08.5-05
Mitochondria as a central hub of laser light non-specific interactions with hepatic cells

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High fluence low-power (HFLP) lasers has been recognized as a promising tool for treatment of various pathological conditions. However, the results of clinical studies are very non- consistent and controversial. In addition, the precise molecular mechanisms of laser light on cells remain elusive. In addition, HFLP laser irradiation is widely used in super-resolution localization microscopy. However, side effects of laser irradiation are largely ignored. A better understanding of the molecular mechanisms of HFLP laser-cell interaction will be an instrumental for the development of novel therapeutic modalities. Therefore, we studied sub-cellular mechanisms involved in the laser interaction with human hepatic cell lines. We compared the effects of different laser wavelengths (398nm, 505nm, 650nm) on three various human liver cancer cell lines (Huh7, HepG2, Alexander). Our results revealed mitochondria as a sub-cellular "sensor" and "effector" of laser light non-specific interactions with cells. We demonstrated that different laser wavelengths induce distinct cellular signaling and kinetic of biochemical responses. Additionally, we linked the kinetics of mitochondrial changes triggered by laser irradiation with the activity of cytochrome-c-oxidase (COX). The red laser treatment resulted in cytochrome c oxidase activation, whereas the blue laser inhibited the COX function. Contrary, HepG2 cells showed higher resistance in response to laser treatment, due to elevated level of Bcl-2 protein. Bcl-2 overexpression in Alexander and Huh7 cells confirmed cytoprotective role of this protein via stabilization of the mitochondria upon laser irradiation. Our results offer a novel insight into laser-induced cellular responses, and uncover the cell signaling pathways triggered by laser irradiation.

P-08.5-06
Brazilian bioactive compounds as inhibitors of bacterial peroxiredoxin AhpC

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The resistance of pathogenic bacterial to several antibiotic have increased all over the world in recent years, revealing the necessity of novel antibacterial compounds to medical care. Recently, some studies have shown that distinct antibiotics with different targets have the convergent ability to produce, directly or indirectly, reactive oxygen and nitrogen species (ROS and RNS), which contributes to the killing of the bacterial pathogens. ROS and RNS are also produced by host immune system to combat pathogens and are decomposed by a myriad of bacterial enzymes. Therefore, the specific inhibition of bacterial antioxidant enzymatic systems represents a promising approach to combat pathogenic bacteria. Typical 2-Cys peroxiredoxins (2-Cys Prx) are very abundant thiolic enzymes able to decompose several kinds of hydroperoxides with high second-order rate constants (10^5–$10^8 M^{-1}s^{-1}$) and, therefore, are promising targets for inhibition due to significant structural differences between eukaryotes and prokaryotes isoforms. In this work, we have isolated natural compounds from Brazilian biota and identify two compounds (here named as C3 and C5), which are able to inhibit 2-Cys Prx from bacteria (named AhpC for Alkyl hydroperoxide reductase C). By biochemical approaches we have shown that the compounds are able to inhibit intermolecular disulfide bond formation and the peroxidase activity of bacterial AhpC (IC_{50}(C3) = 0.553 $\pm$0.061μM and IC_{50}(C5) = 0.985 $\pm$0.093μM). The minimum inhibitory concentration assay revealed that C3 and C5 are toxic to cells from *Bacillus subtilis, Staphylococcus aureus* and *Staphylococcus epidermidis* (MIC_{50} ranging from 500 to 30μM). Collectively our data indicates that C3 and C5 are first compounds of the Brazilian biota identified as bacterial Prx inhibitors, and therefore, promising compounds to the treatment of infections caused by pathogenic bacteria.

P-08.5-07
Menadione-triggered cell death depends on reactive oxygen species accumulation

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Menadione (MD) is a synthetic form of vitamin K which was believed to have great potential as a food supplement and to treat various bone and vascular diseases. However, it was shown that MD can trigger different types of cell death depending on the cell type and used concentration. In our cell model, increased reactive oxygen species (ROS) production was stimulated by MD, which could be efficiently neutralized by antioxidant pretreatment. Especially two antioxidants, N-acetyl cysteine (NAC) and deferoxamine (DFO), turned out to be very effective towards MD-induced cell death, which was neither apoptotic nor necroptotic. Furthermore, our results revealed an indirect link between glutathione-depletion, lipid peroxidation, and cell death, which are all hallmarks of ferroptosis. Contrary to our expectations,

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ferrostatin-1 pre-treatment did not suppress cell death and consequently, we were not able to confirm ferroptotic cell death. We conclude that MD-induced cell death depends on ROS and free iron which can be regulated by exogenously-added antioxidants. Additionally, MD-sensitivity is cell type-dependent. In order to determine the exact mechanism of MD-triggered cell death, further investigation is needed.

P-08.5-08
Preeclampsia-like features and partial lactation failure in mice lacking cystathionine gamma-lyase

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Elevated plasma homocysteine levels are considered as a risk factor for cardiovascular diseases as well as preeclampsia-a pregnancy disorder characterized by hypertension and proteinuria. We previously generated mice lacking cystathionine gamma-lyase (Cth) as cystathioninuria models and found them to be with cystathioninemia/homocysteinemia. We investigated whether Cth-deficient ($Cth^{-/-}$) pregnant mice display any features of preeclampsia. $Cth^{-/-}$ females developed normally but showed mild hypertension (~10 mmHg systolic blood pressure elevation) in late pregnancy and mild proteinuria throughout development/pregnancy. $Cth^{-/-}$ dams had normal numbers of pups and exhibited normal maternal behavior except slightly lower breastfeeding activity. However, half of them could not raise their pups owing to defective lactation; they could produce/store the first milk in their mammary glands but not often provide milk to their pups after the first ejection. The serum oxytocin levels and oxytocin receptor expression in the mammary glands were comparable between wild-type and $Cth^{-/-}$ dams, but the contraction responses of mammary gland myoepithelial cells to oxytocin were significantly lower in $Cth^{-/-}$ dams. The contraction responses to oxytocin were lower in uteruses isolated from $Cth^{-/-}$ mice. Our results suggest that elevated homocysteine or other unknown factors in preeclampsia-like $Cth^{-/-}$ dams interfere with oxytocin that regulates milk ejection reflex.

P-08.5-09
Tumor suppressor, RUNX3, induces antioxidant regulation of gene transcription

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Runt-related transcription factor 3 (RUNX3) is feasible tumor suppressor gene since its inactivation was shown to be related to carcinogenesis. Oxidative stress by high levels of reactive oxygen species (ROS) is causally associated with carcinogenesis through DNA damages. Cellular defense against oxidative stress employs nuclear factor E2-related factor 2 (NRF2), a master regulator of a cellular antioxidants. Oncogenic K-RAS-induced ROS is actively suppressed by inducing the transcription of NRF2. However, the molecular mechanisms for the regulation of ROS-induced NRF2 transcription have been poorly studied. In this study, we show that RUNX3, a transcription factor functioning as a tumor suppressor, is required for the NRF2 transcription by oncogenic K-RAS-induced ROS and ectopic oxidative stress. Knockdown of RUNX3 or mutation of RUNX binding sites at the promoter region of NRF2 markedly reduced oxidative stress-induced NRF2 expression. Our study highlights a novel mechanism for NRF2 expression in regulating redox homeostasis, providing insights into new strategies of cancer prevention against the oxidative stress-associated carcinogenesis.

P-08.5-10
Synergy between 15-lipoxygenase and secreted PLA2 promotes inflammation by formation of TLR4 agonists from extracellular vesicles

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Damage assoiated molecular patterns (DAMPs) are endogenous ligands that induce innate immune response, thus promoting sterile inflammation. During oxidative stress, stress-derived EVs (stressEVs) were found to activate Toll-like receptor 4 (TLR4), but the activating ligands were not fully determined. Additionally, several enzymes such as 15-lipoxygenase (15-LO) and secreted phospholipase A2 (sPLA2) are induced during inflammation and were suggested to promote DAMP formation. StressEVs were produced from HEK293 exposed to 10uM A23187 and isolated with ultracentrifugation. 20:4 lysoPI was oxidized for 10 min with 15-LO. SynEVs were prepared from phospholipids (PLs), oxidized with 15-LO and hydrolyzed with sPLA2. Activity was measured by qPCR and ELISA on wt and KO cells. Ox 20:4 lysoPI was analyzed by MS. sPLA2 activity was measured in synovial fluid from patients using fluorometric assay. K/BxN serum transfer induced arthritis model on wt and TLR4 KO mice (C57Bl/6 mice) with sPLA2-IIA injection was performed. StressEVs released after oxidative stress were found to activate TLR4 with a gene profile different from agonist lipopolysaccharide. StressEVs, 15-LO oxidized synEVs, but only 15-LO oxidized lysoPLs activated cytokine expression through TLR4/MD-2. Hydroxy, hydroperoxy and keto products of 20:4 lysoPI oxidation were determined by MS and they activated the same gene pattern as stressEVs. Furthermore, sPLA2 activity, which we detected in the SF from patients, promoted formation of TLR4 agonists after 15-LO oxidation. Injection of sPLA2-IIA into mice promoted K/BxN serum induced arthritis in TLR4-dependent manner. Both 15-LO and sPLA2 are induced during inflammation, therefore these results imply the role of oxidized lysoPLs in stressEVs in promoting sterile inflammation through TLR4 signaling. The formation of TLR4 agonists is enzyme driven so it provides an opportunity for therapy without compromising innate immunity against pathogens (Ha VT. et al., PNAS 2020).

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P-08.5-11
Effects of quercetin and taxifolin on Nrf2 pathway in UVA exposed human skin cells.

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Due to changed lifestyle, human skin receives higher doses of UV radiation than before, which is linked with a more frequent incidence of various acute and chronic detrimental cutaneous effects. Predominant UVA (315–400 nm) photons attack skin chromophores that results in formation of reactive oxygen species (ROS) in epidermal keratinocytes as well as dermal fibroblasts. ROS can cause oxidative damage to DNA, lipids and proteins that affects the functions of biomolecules. Flavonoid quercetin (QE) is one of the most abundant naturally occurring polyphenols and is present in diverse vegetables, fruits, herbs or beverages. QE is a well-known antioxidant and anti-inflammatory agent. QE is used as an active component in many dermato-cosmetic preparations for photoprotective effect. However, recent reports indicate a photo-instability and phototoxic potential of QE that may increase oxidative stress caused by UVA radiation. Thus we aimed to study effects of QE and its dihydro derivative taxifolin (TA) on ROS production, GSH level, DNA single strand breaks and Nrf2 signalling pathway in adult primary human dermal fibroblasts (NHDF) and primary human epidermal keratinocytes (NHEK). Our results showed protective effect of QE at lower concentrations tested (6.25 and 12.5 μM). At the highest concentration (75 μM) QE protection was declined. TA demonstrated protection in the whole concentration range. This work was financially supported by IGA LF_2020_022 and Palacký University Olomouc RVO 61989592.

P-08.5-12
The study of the prooxidant and genetic activity of natural surface waters using lux-biosensors

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Pollution of the aquatic environment with toxic, mutagenic and carcinogenic factors poses a threat to human health. Therefore, it is relevant to study water sources near settlements for toxic and mutagenic activity. The research aim was to study the prooxidant and genotoxic activity of natural surface waters of the Almaty region of the Republic of Kazakhstan. We used genetically modified *E. coli* strains: the pCoD and pRecA promoters (genotoxicity), pKatG and pSoxS (oxidative stress), pXen7 (toxicity). Fifty two water bodies were studied. Nine water samples demonstrated an increase of bioluminescent response on the RecA biosensor. The remaining samples showed a decrease in the level of bioluminescence, which may be due to the high toxicity of the samples, which inhibits the growth of the culture. When using the ColD biosensor, a similar pattern was observed with low values of induction factor with simultaneous high values of toxicity. An increase in the bioluminescent response of the KatG biosensor was recorded in 10 sources, which indicates the presence of prooxidant substances in water. An increase in the bioluminescent response of the SoxS biosensor was recorded in 26 water samples. When studying the general (integral) toxicity using the pXen7-lux biosensor, it was found that most of the studied water samples have moderate toxic activity. A correlation analysis between toxicity indices detected on the biosensors pRecA-lux, pColD-lux, pSoxS-lux, pKatG-lux and pXen7-lux showed that the level of inhibition of bioluminescence in non-target strains statistically significantly ($P < 0.05$) correlates with data obtained on strain pXen7-lux. Thus, using a bioluminescent test, natural water sources (50% of all studied water samples) with prooxidant and genotoxic activity were identified. 92% of water samples showed toxic activity, significantly reducing the bioluminescent response of biosensors. The research was conducted within the framework of the MES project AP05130546

P-08.5-13
Role of *Stagonospora nodorum* effectors in regulation of genus Triticum wheat plants redox metabolism

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The most important virulence factors of the *Stagonospora nodorum* are multiple fungal necrotrophic effectors (NEs) that cause necrosis and/or chlorosis on wheat lines possessing dominant susceptibility genes (Snn). The pathosystem wheat–*S. nodorum* is mediated by multiple fungal necrotrophic effector–host sensitivity gene interactions that include SnToxA–Tsn1, SnTox1–Snn1, and SnTox3–Snn3 and others. In this study, combinations of different genotypes of wheat variety and fungal isolate were studied. Several *S. nodorum* isolates, characterized by presence or absence of SnToxA, SnTox1, SnTox3 effector genes expression and genetically characterized wheat samples: *Triticum timopheevii* k-58666, *T. monococcum* k-39471, *T. aestivum* variety Boevchanka (tsn1-/snn1-/snn3-), Omskaya35 (tsn1-/Snn1+/snn3-), Kazahstanskaya10 (tsn1-/Snn1+/Snn3+), Zhnitsa (Tsn1+/Snn1+/Snn3+) were used. Full compatibility reaction in combination of genotype Zhnitsa with SnB, Sn9MN (ToxA+/Tox3+) was shown. The suppression of hydrogen peroxide generation in a compatible interaction was most likely due to high activity of catalase, low activity of peroxidase and reduce of expression of genes encoding NADPH-oxidase (TRboh), anionic peroxidase (TPrx) and superoxide dismutase (TSod) at the early stage of infection (24 hours), which further led to the formation of extensive lesions. Incompatibility reaction in combinations *T. timopheevii* k-58666, *T. monococcum* k-39471, *T. aestivum* variety Boevchanka with SnB, Sn9MN (ToxA+/Tox3+), Sn4VD (toxA-/tox3-/tox1-) was shown. The increase of hydrogen peroxide generation due to alterations in redox enzymes activity and increasing expression of TRboh and TSod genes at early stage of infection, which led to the development of hypersensitivity reactions and inhibition of the pathogen mycelium growth was found in incompatible interactions. This

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work was supported by the RFBR in the framework of the research project no. 20-316-80047 and no. 18-04-00978.

P-08.5-14
Do the deletion polymorphisms of GSTM1 and GSTT1 contribute to increased risk of diabetes mellitus?

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Glutathione S transferases (GST) are important part of enzyme antioxidant defence system. Deletion polymorphisms of GSTM1 and GSTT1 may contribute to susceptibility to T2DM, because β cells express very low levels of antioxidant enzymes. Many studies have shown associations between GSTM1/GSTT1 null genotypes and DM risk. The aim of this study was to determine the distribution of GSTM1 and GSTT1 genotypes in patients with type 2 diabetes mellitus as well as in healthy subjects, and whether GSTM1 and GSTT1 gene polymorphisms represent risk factors for development of diabetes mellitus type 2, as well as to show which polymorphic variants of these genes carry the highest risk. GSTM1 and GSTT1 genotypes were determined in 170 diabetic patients and 180 age and gender-matched controls by the multiplex-PCR method. The effect of polymorphisms on the risk of diabetes was examined by logistic regression. The distribution of GSTM1 polymorphic variants was not significantly different in patients with diabetes in relation to the control group. On the other hand, the GSTT1-null genotype was significantly more frequent in the diabetic group compared to the control group ($P < 0.001$). Patients with diabetes were significantly more likely to be carriers of the combined GSTM1-active / GSTT1-null but also GSTM1-null / GSTT1-null genotype compared to the control group ($P < 0.001$). No association of GSTM1-null genotype with diabetes risk was shown, whereas GSTT1-null genotype was associated with 3-fold higher risk of disease (OR = 3.00; 95% CI = 1.90–4.72; $P < 0.001$). The risk of type 2 diabetes was statistically significantly higher in carriers of the combined genotype GSTM1-active / GSTT1-null (OR = 3.37; 95% CI = 1.80–6.33; $P < 0.001$) and GSTM1-null / GSTT1-null (OR = 2.01; 95% CI = 1.06–6.81; $P < 0.001$). These results suggest that GSTT1-null genotype may be associated with increased risk of DM type 2 development.

P-08.5-15
GSTP1 gene polymorphisms influence the seminal parameters and male infertility

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Glutathione-S-transferase 1 (GSTP1) is an important multifunctional ejaculate protein, providing protection against xenobiotic and free reactive oxygen species (ROS). ROS can reduce sperm motility and semen quality. The purpose of the research is to study the association of Ile105Val and Ala114Val polymorphisms of the GSTP1 gene with risk of pathospermia as a male infertility factor in Moscow region. Genotyping of Ile105Val and Ala114Val polymorphisms of the GSTP1 gene was performed by generated amplicons from melting curve analysis after real time PCR. The study included 138 men living in the Moscow region. Group of infertile men (n = 70) includes 26 non-obstructive azoospermic patients, 23 asthenozoospermic and 21 patients with teratospermia and 68 healthy controls with normal sperm parameters who had one child and more. The frequencies of the genotypes of the Ile105Val and Ala114Val polymorphisms of the GSTP1 gene in infertile men with the pathospermia and healthy control group have no statistically significant differences ($P > 0.05$). The frequency of the polymorphic 105Val allele in infertile patients with teratospermia is higher than in the control group ($\chi^2 = 10.004$; $P = 0.0015$). The frequency of the minor allele 114Val of the GSTP1 gene is higher in patients with teratospermia ($\chi^2 = 6.81$; $P = 0.009$) and with asthenzooospermia than in control group ($\chi^2 = 8.58$; $P = 0.003$). The 105Val and 114Val alleles of GSTP1 gene are the genetic risk factors for unexplained male infertility, especially in the group with teratospermia and asthenzooospermia. There has been considerable interest in the biological and clinical consequences of the reported GSTP1 polymorphisms in male infertility risk. Additional larger studies investigating the relationship between GSTP1 polymorphisms and risk of pathospermia are required.

P-08.5-16
Oxidative DNA damage in germ cells is regulated by the JAK/STAT pathway via the DNA damage response pathways.

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Introduction: Testicular torsion and detorsion is a urological emergency caused by the obstruction and re-flow of blood due to the twisting and untwisting of the spermatic cord explained as a testicular ischemia reperfusion injury (tIRI). Such pathological event causes oxidative stress-induced DNA damage leading to germ cell apoptosis (GCA). The aim is to investigate the internal mechanism linking the JAK/STAT signaling pathway with the DNA damage response (DDR) signaling pathways and their effect on tIRI-induced oxidative DNA damages. Methods: Male Sprague-Dawley rats (n = 36) were divided into 3 groups: sham, unilateral tIRI and tIRI+AG490 (40 mg/kg), a JAK inhibitor. The tIRI was induced by the obstruction of the spermatic cord using a surgical clamp. Spermatogenesis was evaluated using histological analysis. Apurinic/apyrimidinic sites (AP) and 8-OHdG formation were estimated using DNA damage quantification kits. Expression of the JAK/STAT pathway was assessed using immunohistochemistry and the activation of the DDR signaling pathways was detected by Western blot. Results: The presence of spermatocytes but few early spermatids during tIRI indicates damage to spermatogenesis. This was associated with a significant increase in the tIRI-induced oxidative DNA damage in the form of DNA strand breaks, formation of AP sites and 8-OHdG formation. Moreover, tIRI-induced DNA damage caused significant rise in the phosphorylation levels of the JAK2/STAT1/STAT3 proteins. Both DDR signalling pathways: ATM/CHECK2 and ATR/ CHEK1 were activated as judged by the significant overexpression of their phosphorylated forms. The tIRI-induced GCA and DNA damage was blocked by inhibition of JAK activity. Conclusions: Our findings suggest that tIRI-induced GCA and DNA damage was prompted by activation of the JAK/STAT signaling pathway, which directed the apoptosis

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decision by the activation of the ATM/CHEK2 and ATR/CHEK1 DDR signaling pathways.

P-08.5-17
Laser fluorescence spectroscopy of oxidative metabolism indicators in rat microcirculatory system under conditions of acute and chronic stress

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The oxidative metabolism (OM) of the microcirculatory system (MS) reflected by laser fluorescence spectroscopy (LFS) data on reduced nicotinamide adenine dinucleotide (NADH), oxidized flavin adenine dinucleotide (FAD) and the redox ratio (RR) of the fluorescence amplitudes of NADH and FAD. The aim of the study was to establish the dynamics of the MS OM parameters (NADH, FAD, RO) in rats under acute (AS) and chronic (CS) stress using the LFS method. LFS indicators recorded using the LASMA MC diagnostic complex (MC-III modification, LAZMA, Russia). A laser module with a radiation wavelength of 1064 nm used in the Doppler channel, and radiation sources at wavelengths of 365 and 450 nm used to initiate fluorescence. The input of the probing radiation and the reception of the secondary radiation reflected from the tissue carried out with a single fiber-optic probe with a diameter of 3 mm. The AS modeled in the forced swimming test (t of water + 22–24°C) for 1 hour. When modeling CS, hypokinesia used in fixators for 10 days for 19–20 hours per day. In AS, the normalized NADH fluorescence amplitude decreased by 34.2% ($P \leq 0.05$) and the FAD amplitude increased by 181.9% ($P \leq 0.05$) and the redox potential (RP) decreased by 75.1% ($P \leq 0.05$) in comparison with the state of the physiological norm. These data indicates a decrease in the activity of OM tissues and development of hypoxia. In CS, there is a decrease in NADH by 41.2% ($P \leq 0.05$), an increase in FAD concentration by 157.7% ($P \leq 0.05$), and a decrease in RP by 78.1% ($P \leq 0.05$) in relation to physiological rest conditions. This indicates an increase in cell demand for ATP and the predominance of oxidative phosphorylation over other processes. At the same time, RR decreased by 13.4% ($P \leq 0.05$) more intensively than at AS. Thus, changes in the OM parameters MS in rats under the conditions of AS and CS are non-specific and have the same direction, but they are more pronounced in CS due to a more intense decrease in RR.

P-08.5-18
Generating of hexose-6-phosphate dehydrogenase knock-out cellular model in human primer fibroblasts with CRISPR-Cas9 technology

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Glucocorticoid hormons are involved in the regulation of intracellular metabolic pathways, such as hepatogenic gluconeogenesis. Otherwise, the intracellular active glucocorticoid level is determined by the ER luminal 11-beta-hydroxysteroid dehydrogenase 1 (11β-HSD1). As a bidirectional enzyme, it exhibits both dehydrogenase and reductase activity, however in intact hepatocytes and fibroblast the enzyme functions primarily as a reductase, driven by the supply of its co-substrate NADPH by hexose-6-phosphate dehydrogenase (H6PD). The disturbances of glucocorticoid homeostasis have been suggested to contribute to the pathogenesis of major disorders such as obesity, metabolic syndrome, type II diabetes. Otherwise, the mutations in H6PD cause apparent cortisone reductase deficiency (ACRD). The emerging importance of these enzymes inspired us to investigate how H6PD deficiency affects various cell development and function and to create a human fibroblast cell line with targeted distruption of the implied enzyme with the efficient genome editing tool, the CRISPR-Cas9 technology. So far, we achieved a succesful transfection of the human primer fibroblast with created CRISPR plasmid construct of the H6PD guide and subsequently we selected one cell clones and sequenced the target enzyme mutation. Our aim with this cellular model is to highlight the interconnections between intracellular glucocorticoid activation and altered redox state in human fibroblast cells, and investigate the effects of H6PD deficiency on differenciation processes.

P-08.5-19
The effect of low doses of radiation and oxidized cfDNA on the transcriptional activity of genes that regulate the mitochondria work

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Mitochondrial DNA (mtDNA) is significantly more susceptible to radiation compared to genomic DNA because it hasn't got repair mechanisms that are as effective as in nuclear DNA. There is evidence that oxidative stress under the action of radiation changes the expression of genes I-V of the mitochondrial respiratory chain complexes. It was researched the transcriptional activity of genes that regulate the mitochondria work in human mesenchymal stem cells (MSC). Real-time PCR was used to determine the level expression of genes of the I-III complexes of the respiratory chain of mitochondria after exposure to radiation at a dose of 10 cGy and after adding model oxidized fragments of cfDNA to the MSC culture medium. It was shown that radiation at a dose of 10 cGy, like cfDNA, causes an increase the expression of genes of the first (genes of nuclear DNA NDUFA1, NDUFA4, NDUFA5, NDUFA10, NDUFC2, NDUFS2, NDUFS1, ACAD9 and genes of mtDNA ND4L, ND2, ND6) and third (gene of nuclear DNA UQCRH and gene of mtDNA CYB) of the mitochondrial respiratory chain complexes after 0.5–3 hours after exposure. Increased level of expression some genes persist for 24 hours. The genes of nuclear DNA SDHB, SDHA, SDHC, whose protein products regulate the work of the II complex of the respiratory chain of mitochondria in MSC, haven't changed the level expression for 24 hours after exposure to radiation at a dose of 10 cGy and after adding to the culture medium MSC model oxidized fragments cfDNA. Thus, we showed that under the action the low doses of radiation and after the addition of model oxidized fragments of cfDNA to the MSC culture medium in concentration 50 ng/ml, the expression level of a large number of genes that regulate the mitochondria work increases, and some genes have the increased

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expression after 24 hours after exposure. This work was supported by RFBR grant No 183400878.

P-08.5-20
Exhaled hydrogen peroxide as a potential marker of lower airway inflammation in neonatal calves

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Objective of this work was the comparative study of hydrogen peroxide (H_2O_2) concentration in the exhaled breath of healthy and diseased calves having acute bronchopneumonia at the age of 1–28 days. To determine the H_2O_2 concentration in the breath exhaled by calves, we used a method involving exhaled breath condensation and cooling using a special device (patent RU 134772 U1) followed by fluorometric measurement (RF-5301 PC Shimadzu, Japan) of the H_2O_2 concentration in a sample with irradiation wave of 568 nm and an emission wave of 581 nm in solution based on the Amplex Red Ultra fluorescent dye (Invitrogen, USA) containing in 1 ml the following: 20 mmol of HEPES (pH 7.4), 1 mmol of EDTA, 10 mmol of Amplex Red Ultra and 4 units of horseradish peroxidase (patent RU 2614621 C2). 15 healthy calves of Holstein breed were examined in dynamics on the 1st, 3rd, 7th, 14th, 21st and 28th days after birth and 15 animals with acute bronchopneumonia were examined on a single occasion. Bronchopneumonia in calves was diagnosed on the basis of clinical signs, results of thoracic auscultation and radiography. Samples of exhaled breath condensate (EBC) were collected from non-fed animals at 7:00 a.m. H_2O_2 concentration measured in the EBC sample was calculated per 100 l of exhaled breath. It was found that in healthy calves H_2O_2 concentration in exhaled breath was varying from 0.146 to 0.435 (average 0.270 ± 0.099, median 0.235) nmol/100 l and was not changing significantly during the first month after birth. In animals with bronchopneumonia, it raised to 0.903–1.377 (average 1.127 ± 0.197, median 1.178) nmol/100 l of exhaled breath. Results of the study suggest that the exhaled H_2O_2 is a potential marker indicating lower respiratory airway inflammation in neonatal calves. This work was financially supported by the Russian Science Foundation, Project No. 18-76-10015.

P-08.5-21
Regulation of host plant redox-status by endophytic bacteria, pathogens and pests.

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The redox status of a plant infected with a pathogen or damaged by a pest is an important indicator of a variety or species resistance. It is considered that plant develops resistance to biotic stress factor by releasing reactive oxygen species (ROS) at the site of infection, which is called an oxidative burst. It is known that endophytic bacteria of the genus *Bacillus* contribute to the formation of plant resistance to pathogens and pests through the mechanism of ISR (induced systemic resistance). ISR is provided by the accumulation of ROS, changes in gene expression, synthesis of protective proteins, increased peroxidase activity in the apoplast, strengthening of plant cell walls, etc. Bacteria of the genus *Bacillus* are often used as biocontrol agents. The effect of treatment with *Bacillus subtilis* strains 26D, 11BM and *B. thuringiensis* strains B-5689, B-6066 on the resistance of wheat to the pathogenic fungus *Stagonospora nodorum* Berk. and greenbug aphid *Schizaphis graminum* Rond. were studied. Bacteria increased plant resistance by inducing the generation of hydrogen peroxide and regulating the activity of peroxidase and catalase. In contrast, the pathogen and the pest suppressed the accumulation of hydrogen peroxide. We discovered that high concentrations of hydrogen peroxide induced the expression of PR-protein (pathogenesis-related) genes in plants, treated with bacteria, which resulted in the development of ISR, suppression in the growth of the pathogen and an increase in the mortality of the pest due to accumulation of phenolic compounds in these plants and the deposition of lignin at the sites of infection. Thus, regulation of ROS generation and the redox-status of plant play an important role in defense from pathogens and pests and are targets for endophytic bacteria, pathogens and pests to influence the host's immunity. The work was supported by grants of RFBR № 18-04-00978, № 17-29-08014.

P-08.5-22
Changing sulfhydryl groups activity in the human red blood cell membrane caused by *Macrovipera lebetina obtusa* venom

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Erythrocytes membranes are important models for biological investigations. It's interesting to study quantitative changes shulfhidril groups in erythrocyte ghosts for more clarify influence of venom mechanisms on biological membranes. SH-group oxidation of membrane proteins is known to cause considerable changes in their transport characteristics and membrane permeability. In this study the effect of the various concentrations of the *Macrovipera lebetina obtusa* (MLO) snake venom on SH-groups activity in the red blood cell membrane was investigated. The important role of SH-groups of proteins in various biochemical and physiological processes is due to their high reactivity and the variety of chemical transformations that they enter (acylation, oxidation, alkylation, the formation of mercaptides, half mercaptals, hydrogen bonds, etc.). Oxidation of thiol groups of membrane proteins also leads to defects in cell membranes. Packed red blood cells (RBCs) were obtained from Hematology Center after Prof. R. Yeolyan (Ministry of Health, Republic of Armenia). Erythrocyte membranes were prepared by the method of Dodge, Mitchell & Hanahan. The erythrocyte ghosts were visualized with ANS (8-anilino-1-naphthalene sulfonic acid) fluorescent probe. Images were collected on an epi-fluorescent microscope FM320-5M (AmScope, USA). The venom was added to the reaction mixture with a low, sublethal (0.35 mg / kg approximately 0.5 LD 50 for rat) and lethal concentration in accordance with LD 50. It was found that the number of accessible SH-groups in the erythrocyte ghosts membranes, under the influence of various concentrations of MLO venom, increases significantly at low doses and less at sublethal and lethal concentrations. Interestingly, our results suggest that both the severity of RBC membrane damage and the SH-group oxidation could be defined also by the blood group of the patient.

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P-08.5-23
Melatonin reduces oxidative damage in the myocardium of rats with type 2 diabetes mellitus

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Diabetic cardiomyopathy is a major risk factor for heart failure in individuals with diabetes. Hyperglycemia related increase in the production of reactive oxygen species or inflammation affects cardiomyocyte function and result in their death and cardiac dysfunction. Melatonin is a well-known antioxidant and free radical scavenger. The present study aimed to investigate the effect of melatonin supplementation on oxidative stress in the myocardium of rats with type 2 diabetes mellitus. Animals were divided into four experimental groups (6 animals per group). Type 2 diabetes was induced using streptozotocin (45 mg/kg, i.p.) and nicotinamide (110 mg/kg, i.p.) injections. The animal model was considered fully developed after 4 weeks. Melatonin was administered orally in drinking water (2 μg/ml; 0.2 mg/kg body weight per day) for 2 weeks. Rats treated with melatonin had lower serum concentrations of tumor necrosis factor-α (TNF-α; $P < 0.001$) as well as lower concentrations of oxidative damage biomarkers, thiobarbituric acid reactive substances (TBARS; $P < 0.001$), and advanced oxidation protein products (AOPP; $P < 0.001$) in the myocardium when compared to control. Furthermore, there was a negative correlation between serum melatonin concentration and concentrations of TBARS ($r = -0.643$, $P < 0.001$) and AOPP ($r = -0.610$, $P < 0.001$). Melatonin protects the myocardium from diabetes-induced damage and may be considered as a cardioprotective agent in diabetes.

P-08.5-24
Protective effect of metformin on diabetes-induced oxidative stress in the testes of rats with type 2 diabetes mellitus

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Metformin is widely used in the therapy of type 2 diabetes mellitus as an agent for regulating serum glucose levels. Hyperglycemia-induced overproduction of reactive oxygen species and systemic inflammation in diabetes have been reported to affect all the body systems, including testes and male fertility. The study aimed to evaluate the effect of metformin on the biomarkers of systemic inflammation and oxidative stress in the testicular tissue of rats with type 2 diabetes mellitus. Animals were divided into four groups (6 animals per group) namely control (C), control/metformin (C/M), diabetes mellitus (DM), diabetes mellitus/metformin (DM/M). Type 2 diabetes was induced using streptozotocin (45 mg/kg, i.p.) and nicotinamide (110 mg/kg, i.p.) injections. The animal model was considered fully developed after 4 weeks. Metformin was administered orally in drinking water (1mg/ml;100mg/kg body weight per day) for 2 weeks. Metformin therapy led to a reduction in the serum concentration of pro-inflammatory tumor necrosis factor-α (TNF-α; $P < 0.001$). Rats treated with metformin had lower levels of oxidative stress biomarkers, thiobarbituric acid reactive substances ($P < 0.001$), and advanced oxidation protein products ($P = 0.001$) when compared to control. Furthermore, there was a positive correlation between serum TNF-α and advanced oxidation protein products ($r = 0.457$, $P < 0.05$). Metformin protects the testes from diabetes-induced damage and may improve male reproductive health in diabetics.

P-08.5-25
TrxR/Trx/Prx system and redox-dependent changes under development of cancer drug resistance

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Among cellular redox-dependent processes TrxR/Trx/Prx system plays the significant role and may be the important part of key events in the stress response and/or resetting redox homeostasis resulted in the change of most vital functions of cells, including proliferation, differentiation, apoptosis. Here we studied changes of TrxR/Trx/Prx system as well as cellular redox state during the formation of cisplatin resistance in human ovarian carcinoma SKOV-3 cells. The development of the drug resistance found to be accompanied by the increase of genes expression not only of thioredoxin reductase isoforms (TRXDR1 and TRXRD2) but also of peroxiredoxin isoforms (PRDX1, PRDX2, PRDX6). It was detected a decrease in ROS cellular level due to enhanced expression of key antioxidant enzymes (SOD2, CAT, GPX1, HO-1) and depression of NADPH-oxidase (NOX5) gene. In addition, an increase in GSH/GSSG ratio as well as redox-dependent activity of transcription factor Nrf2 were observed in resistant SKOV3/CDDP cells. The growth of GSH/GSSG ratio due to elevation of GSH level, which needs for reduction of Prx6 oxidized form, was a result of enhancement of GSH synthesis de novo. In addition, it was established that siRNA knockdown of TRX1 increased CDDP-induced death of resistant cells that was significantly rose by additively use of siRNA knockdown of PRX6. The results testify to the important role of TrxR/Trx/Prx system in redox-dependent mechanism of cancer cell resistance to cisplatin. The publication has been supported by the RUDN University Strategic Academic Leadership Program.

P-08.5-26
Metformin ameliorates oxidative stress in renal cortex of rats with streptozotocin/nicotinamide induced diabetes

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Hyperglycemia promotes oxidative stress and the generation of reactive oxygen species, which is known to play a crucial role in the pathogenesis of diabetic nephropathy. The present study

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aimed to explore the protective effect of metformin on diabetic nephropathy in rats with streptozotocin/ nicotinamide induced diabetes mellitus. Animals were divided into four groups (6 animals/group). A rat model of type 2 diabetes mellitus was induced via a one-time application of streptozotocin (45 mg/kg, i.p.) 15 minutes after nicotinamide injection (110 mg/kg, i.p.). The animal model was considered fully developed after 4 weeks. Metformin (1mg/ml;100mg/kg body weight per day) was administered orally in drinking water for 2 weeks, and fasting blood glucose, serum insulin, serum tumor necrosis factor-α (TNF-α), as well as oxidative damage markers, thiobarbituric acid substances (TBARS), and advanced oxidation protein products levels (AOPP) in the renal cortex were examined at the end of experiment. Rats treated with metformin had lower fasting blood glucose ($P < 0.001$) as well as lower serum concentrations of TNF-α ($P < 0.001$) when compared to control. Metformin-treated rats also had lower levels of oxidative stress biomarkers, TBARS ($P < 0.001$), and AOPP ($P = 0.001$). Metformin used as the first-line therapy in type 2 diabetes mellitus exerts protective effects on oxidative stress in the renal cortex of diabetic rats and may be beneficial in diabetic kidney disease.

P-08.5-27
Interplay of potassium transporter Trk1p, antioxidants and glucose induced cell death study in *Saccharomyces cerevisiae*

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Introduction: *Saccharomyces cerevisiae* a unicellular model eukaryote can undergo apoptosis or programmed cell death (PCD). Latter a regulated suicide programme; is essential for maintenance of cells and functioning of all living systems. PCD has too been implicated in several diseases and genetic disorders. DNA damage, hydrogen peroxide, reactive oxygen species (ROS), can cause PCD. Recently it has been shown that sugars in absence of other nutrients can trigger PCD to stationary phase cells of yeast due to ROS overload as a result of hydrogen peroxide production and has been named as glucose induced cell death (GICD). K^+ efflux is also linked to PCD. Here we present the role(s) of protein kinase Hal5p; needed for plasma membrane stability for Trk1; a transporter of K^+ vis a vis GICD. Materials and Methods: Cells (wild, hal 4,5 mutants & HAL5 gene overexpressed i.e Ep) mutants were grown in YPD complete media till stationary phase 40 h. Washed cells with water, thricely and suspended in water with glucose/without in Erlenmeyer flask at 37°C on shaker (150rpm). Viability was determined by colony forming units and methylene blue staining at different intervals of time. Glutathione, externalization of phosphatidylserine (PS) using fluorescamine and trehalose were determined using DTNB by Beutler et al (1963), Sharma (2006) and Lillie & Pringle (1980) respectively. Protein estimation by Bradford assay (1976). Results and Discussion: It was observed that mutants are having diminished level of GSH than wild type and Ep strains yet GICD process was much faster in wild cells. It could be due to influx of K^+ in the Ep cells. There was not much difference between trehalose levels among cells. Exposure of PS level was 30, 20 and 15 per cent in hal 4,5 mutant, wild and Ep cells respectively. Oxidative stress has a clear role in GICD and showed that K^+ homeostasis by Hal5p plays some protective role this work.

P-08.5-28
Effect of non-oxidized cell-free DNA versus oxidized cell-free DNA on rat brain cells *in vitro* model of chronic oxidative stress

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Cell-free DNA (cfDNA) fragments accumulated in blood during oxidative stress might contain oxidized bases. Our recent study demonstrated that (1) oxidized cfDNA (ocfDNA) fragments containing 8-oxo-7,8-dihydro-2'-deoxyguanosine (8-oxodG) induce transcription factor NRF2 expression followed by anti-oxidative responses and (2) stress induce 8-oxodG accumulation in the hippocampal cells (Filev A.D. et al., 2019). The dynamics of DNA oxidation in brain cells and adaptive anti-oxidant responses of transcriptome to extra non-oxidized cfDNA (ncfDNA) vs. ocfDNA during chronic oxidative stress (COS) have not been studied. Aim: Compare effects of ocsDNA vs ncfDNA in rat cerebellar cells (RCC) in a model of COS *in vitro*. Materials and Methods: primary rat cerebellar cell culture (PCC) were exposed to sterile air atmosphere at room temperature daily for 8 min to induce COS. During exposure, ncfDNA or 3% hydrogen peroxide-treated DNA (source of ocfDNA) were added to the cells. After 1 hour, 3 days and 11 days PCC were harvested and monitored for expression of antioxidative system genes Nrf2 and Ho1 by RT-PCR and 8-oxodG-containing nuclei and Nrf2 protein expression by immunofluorescence. Results: three-phase responses to cfDNA corresponding to time points were revealed. Phase 1 (1 hr): increasing the accumulation of 8-oxodG only after ocfDNA, 2–4-fold increased Nrf2 and Ho-1gene expression ($P < 0.01$) and migration of NRF2 protein to nuclei. Phase 2 (day 3): no increase in 8-oxodG accumulation, 4–7-fold increased Ho-1 gene expression following both ncfDNA and ocfDNA actions ($P < 0.01$), migration of NRF2 protein to nuclei. Phase 3 (day 11): decreased expression of Ho-1, NRF2 protein and 8-oxodG accumulation in response mostly to ocfDNA than to ncfDNA. Conclusion: cfDNA oxidation is crucial for inducing antioxidative responses in brain cells. Prolonged action of ocfDNA might be exhaustive for cellular protective potential. Study was supported by Grant RFBR № 19-34-90072.

P-08.5-29
The effect of antioxidant cell defense system on Mn tetraphenylporphyrin/ascorbic acid treatment

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To date, the strategy for treating neoplastic diseases with reactive oxygen species is being investigated and applied. Increased levels of reactive oxygen species are observed in tumor cells and contribute to their proliferation and growth. However, a significant increase in the level of reactive oxygen species can lead to

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apoptosis. Mn^3 tetraphenylporphyrin chloride (MnClTPP) with ascorbic acid (AA) causes tumor cells death via oxidative stress. Here, we have investigated the MnClTPP/AA effect on the activity of antioxidant cell defense enzymes, namely superoxide dismutase and catalase, in order to increase the efficiency of MnClTPP/AA. It has been shown that treatment of chronic myelogenous leukemia cells (K562) with MnClTPP/AA leads to time-dependent increase in superoxide dismutase activity, while in human breast adenocarcinoma cells (MCF7), MnClTPP/AA did not cause any significant effect. On the other hand, the catalase activity remained unchanged in K562 cells during MnClTPP/AA treatment as compared with time-dependent increases of that in MCF7 cells. Comparing this observation with MTT test results of MnClTPP/AA treated cells in presence of catalase inhibitor (3-amino-1,2,4-trazole) and superoxide dismutase inhibitor (diethyldithiocarbamate), we can conclude that under inhibition conditions, the activity of antioxidant cell defense enzymes is inversely proportional to survival of tumor cells. Thus, tumor tissues with depleted antioxidant cell defense system will be more sensitive to combination of MnClTPP/AA and inhibitor.

P-08.5-30
Assesment of melatonin effect on redox balance and biochemical changes in chronically cigarette smoke-exposed rat liver tissue

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Cigarette smoke is one of the exceedingly widespread hazardous attitude along with its complex mixture of toxicants, causes cellular damage by generating free radicals. Smoke exposure enhances oxidative stress and diminishes antioxidant defence mechanisms. Particularly in recent studies are focused on its toxic effects on visceral organs alongside its major impact on the respiratory system. Melatonin, an indolic hormone derived from the amino acid tryptophan, acts as strong antioxidant and free radical scavenger and also has anti-inflammatory, and antioxidant properties. This research was carried out to examine the effects of the subcutaneous melatonin administration on oxidative liver damage caused by chronic cigarette smoke-exposure in an experimental rat model. Forty adult female Wistar-Albino rats were used as experimental subjects. The rats were randomized and divided into 4 groups including a nonsmoking control group (C), control of subcutaneous 20 mg/kg melatonin (CM) administration, long-term cigarette smoke exposed group (SE), and SE with subcutaneous 20 mg/kg melatonin administration (SM), each containing 10 rats. Chronical cigarette smoke exposure lasted 45 days of experimental period of time. Biochemical analysis was conducted on rat liver tissue homogenates. MDA, NO, AOPP, GSH, nuclear factor kappa B (NF-κB), and thioredoxin interacting protein (TXNIP) levels were measured. In this research, biomarkers, related to the cellular damage and oxidative stress were found higher (MDA, NO, AOPP, NF-κB, and TXNIP) in the SE group, whereas GSH was depleted ($P < 0.05$). The most noticeable result of melatonin treatment was found in the CM group with significant decrease of MDA and GSH levels. Cigarette smoking due to its cellular damage, deplete antioxidants and also increases oxidative stress, consequently results in inflammatory response. Conversely to cigarette smoke cellular toxicity, melatonin treatment may have regulatory effects on rat liver tissue.

P-08.5-31
Aucubin administered by either oral or parenteral route protects against cisplatin-induced acute kidney injury in mice

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Aucubin is pharmacologically active natural compound which possesses numerous beneficial properties. This study aimed to evaluate the protective effect of aucubin against cisplatin (CP)-induced acute kidney injury in mice and the mechanism of its action. Aucubin was administrated to mice orally or intraperitoneally (ip) (1.5 and 5 mg/kg) for two consecutive days, two days after ip injection of cisplatin (CP), 11 mg/kg. Treatment with aucubin by both routes of administration ameliorated histopathological changes and reduced elevated serum markers of kidney injury. CP administration increased renal expression of heme oxygenase-1 (HO-1) and 4-hydroxynonenal (4-HNE), as well as tumor necrosis factor-alpha (TNF-α), which was dose-dependently ameliorated by aucubin. Moreover, aucubin reduced increased renal expression of cleaved caspase-3 and -9 and decreased poly (ADP-ribose) polymerase (PARP) cleavage. Mechanistically, aucubin suppressed the activation of several signaling pathways involved in inflammation and apoptosis, including nuclear factor-kappa B (NF-κB), signal transducer and activator of transcription 3 (STAT3), Akt, extracellular signal-regulated kinase 1/2 (ERK1/2) and forkhead box O3a (FOXO3a). Parenteral application was marginally but statistically more effective in reducing CP-induced kidney injury than oral administration. The findings of this study suggest that aucubin acts as a protective agent against CP-induced nephrotoxicity, which should be further investigated.

P-08.5-32
Resilience and vulnerability to a stress-induced anhedonic phenotype are associated to modulation of the oxidative balance

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Stress is considered as the main environmental risk factor for the development or exacerbation of psychiatric disorders such as major depression, which affect around 10% of the world population. However, the impact of stress is highly variable, with most of the subjects exposed to it being resilient, able to positively cope with the adverse situation, and a smaller percentage being susceptible, developing psychopathologies. The molecular

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mechanisms underlying resilience and susceptibility are still elusive, nevertheless their characterization is crucial to identify new targets for a more efficient therapeutic strategy. To this aim we evaluated, at preclinical level, the involvement of oxidative balance mediators – known to be compromised in psychiatric disorders – in the differential stress response, by using the chronic mild stress (CMS) model of depression. Adult male rats were exposed to 2 weeks of CMS paradigm, and the sucrose consumption test was used to assess the insurgence of an anhedonic-like phenotype, after which the animals have been divided in vulnerable (anhedonic) animals, and resilient rats that did not develop the anhedonic phenotype. 24 hours after the last stress, we performed the molecular analyses to evaluate the gene and protein expression of oxidative balance mediators. Our study shows that chronic stress exposure has a significant impact on the balance between pro and antioxidant factors in different cerebral areas implicated in psychopathologies. Interestingly, resilient animals showed a marked antioxidant response characterized by the Nrf2 pathway activation in the prefrontal cortex. Conversely, in susceptible animals we did not found such response, and observed a region-specific increase in oxidative stress. Our results suggest the Nrf2 related antioxidant pathway as an important target to consider in developing new pharmacological therapies for stress-related disorders, as its activation could favour a positive molecular response to stress.

P-08.5-33
Parallel occurrence of reduced pyridine nucleotids and oxidized proteins in the endoplasmic reticulum lumen – a possible absence of electron transfer chains

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The endoplasmic reticulum (ER) is the major site of protein thiol oxidation during post-translational modification. Due to the dominance of oxidized proteins, the lumen of the ER is usually considered as an oxidative environment; although some processes which require reducing agents, such as NADPH, are also found here. The parallel occurrence of oxidized thiol-disulfides and reduced pyridine nucleotides may indicate that the ER lumen lacks components which connect the two systems. Our aim was to investigate the luminal presence of the thioredoxin (Trx)/thioredoxin reductase (TrxR) proteins, capable to link the protein thiol and pyridine nucleotide systems. Protein expression of Trx/TrxR isoforms was examined on subcellular fractions by Western blot analysis. TrxR activity in each organelle was measured using a colorimetric kit. The intracellular distribution of Trx/TrxR isoforms was also examined by immunofluorescent microscopy. An in silico analysis was performed to analyze the predicted localization of each isoform. We showed that the specific activity of TrxR in the ER is around zero (0.02 U/mg $\pm$ 0.01), while we measured higher activities in the cytoplasm (1.26 U/mg $\pm$ 0.11) and mitochondria (1.57 U/mg $\pm$ 0.19). Analysis of rat liver subcellular fractions revealed that the two isoforms of Trx, and the three isoforms of TrxR are not expressed in the ER. Immunofluorescent analysis confirmed that Trx and TrxR isoforms did not show colocalization with ER-specific marker Grp94. In silico prediction analysis also predicted a very low probability of luminal localization for each isoform (0–5%). Our results show that none of the components of the Trx/TrxR system is expressed in the ER lumen. The absence of this electron transfer chain may explain the uncoupling of redox systems in the lumen, allowing the parallel presence of a reduced pyridine nucleotide pool and oxidized proteins.

P-08.5-34
Oxidative stress and antioxidant defense response of a yeast cell model after treatment with metal ions from orthodontic alloys

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In the oral cavity, fixed orthodontic appliances are subjected to corrosion leading to the release of metal ions, a potential inductor of reactive oxygen species formation (ROS). This study aimed to simulate the effect that the metal ions of stainless steel, cobalt-chromium, nickel-titanium and β-titanium orthodontic alloys can exert on the yeast cell model. Concentrations of 1 μM, 10 μM, 100 μM and 1000 μM of four orthodontic alloy types were proposed to obtain metal ion mixtures that were used for yeast *Saccharomyces cerevisiae* exposure. After 24 hour exposure, the ROS level and activity of antioxidant enzymes such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), and thioredoxin peroxidase (TrxR) were determined. Additionally, oxidative damages of proteins were evaluated by measuring protein carbonyls content. The considered parameters of exposed yeast cells were compared with those of untreated yeast cells. Metal ion concentrations of 1000 μM triggered an overproduction of ROS and a versatile antioxidant defense was observed. Although lower metal ion concentrations did not induce oxidative stress, antioxidant enzyme activity was higher compared to the control. The occurrence of oxidative stress corresponded with the level of oxidative damage of proteins. The results show that depending on the type of orthodontic alloy, as low as 1000 μM concentrations of released metal ions can lead to harmful oxidative reaction formation, which the antioxidant defense response is unable to cope with.

P-08.5-35
The deletions of sulfur metabolism genes in *Drosophila melanogaster* cause a delay in ovarian maturation.

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Gasotransmitter hydrogen sulfide (H2S) plays an important regulatory role in various processes providing a strong cytoprotective effect. In eukaryotes, H2S is synthesized in the cells mostly through the reverse transsulfuration pathway exploring cystathionine B-synthase (CBS) and cystathionine y-lyase (CSE). The transsulfuration pathway includes the conversion of homocysteine to cysteine following the breakdown of methionine. Previously, mutant *Drosophila melanogaster* strains with single and double deletions of the CBS and CSE genes were obtained using the CRISPR/Cas9 technique. We demonstrated that deletion of

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the CBS gene and, to a greater extent, double deletion cause a delay in ovarian development and oocyte maturation. The delay in oocyte maturation due to CBS gene deletion reaches 2 days, double deletion results in 3 days delays. In flies with double deletion, significant differences in the size of the ovaries and the level of ovariole maturation are observed after 5 days of development. At the same time, single deletion of the CSE gene does not exhibit any significant influence on ovary development. Interestingly, deletion of the CBS gene significantly reduces the fertility and reproductive age of the females. Immunofluorescence staining of DNA and F-actin revealed the death of different phases of egg chambers in ovarioles in the flies with deletions (the maximal number of dead chambers was detected in double transformants). Besides, significant changes in the spatial structure of ovarioles at the early stages of development were detected in the flies with deletions. We also observed elongation of the overall fly development time mainly in the larval stage in the case of CBS and double transformants.

P-08.5-36
Intracellular damage of mcm5S2U-tRNA induced by oxidative stress

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The 5-substituted 2-thiouridines (R5S2U) present in the first position of specific tRNAs ($tRNA^{Lys}$, $tRNA^{Glu}$, $tRNA^{Gln}$) are important for proper decoding of genetic information in protein biosynthesis. We have already proved in in vitro experiments that a 2-thiouridine (S2U) nucleoside present in an oligoribonucleotide, in oxidizing environment is desulfured, i.e. the sulfur atom is either replaced by an oxygen atom to form the corresponding uridine (R5U), or is removed from the molecule to form 4-pyrimidinone riboside (R5H2U).[1,2] The formation of H2U must be considered a damage, because the tRNAs containing this desulfured unit cannot properly recognize appropriate codons, so the reading of genetic information is impaired. It was interesting to learn whether similar oxidative stress transformations of R5S2U occur in cells. Experiments carried out in yeast or human cancer cells exposed to H_2O_2, $NaAsO_2$ or NaClO confirmed oxidant-concentration-dependent loss of mcm5S2U in $tRNA^{Glu}$. LC-MS/MS analysis confirmed the presence of mcm5H2U and mcm5U in the mixture of tRNA-derived nucleosides. Acknowledgements for the financial support from The National Science Centre in Poland [UMO-2016/23/B/NZ1/02316] to M.S. References [1] Sochacka et al., Chem. Commun. 47, 4914, 2011. [2] Sierant et al. ChemBiochem 19, 687, 2018.

P-08.5-37
Combined effects of radiation and immobilization stress on lipid peroxidation

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The aim of the study was to study and evaluate the effect of a sublethal dose of gamma radiation (6 Gy) on the parameters of lipoperoxidation in immunocompetent organs and cells against the background of immobilization stress. The study was conducted on 40 male Wistar rats divided into 4 groups: group I – intact; group II — exposed to immobilization stress after 25 hours; group III — exposed to gamma radiation; and group IV – exposed to combined exposure (immobilization stress and gamma radiation). To reproduce emotional stress, experimental animals were kept in a special device, where the animals were motionless for 6 hours, under bright lights. They were removed from the experiment 25 hours after stress, by decapitation on the background of light ether anesthesia. Animals of groups III and IV were irradiated on the TERAGAM Co60 radiotherapy unit ("ISOTREND spol. s.r.o.", Czech Republic) once, 6 Gy. Before irradiation was carried out topographic and dosimetry preparation of experimental animals to irradiation: an object placed on the table isocentric therapy x-ray simulator "Tigah" (Czech Republic), which designs and parameters corresponds to the therapeutic table of gamma irradiator. Ionizing radiation combined with immobilization stress has a more pronounced effect than with separate action. The combined effects of immobilization stress and ionizing radiation led to an increase in the level of DC and MDA, which lead to the development of double oxidative stress in the studied objects [1]. Previously published in: 1. Okassova AK et al. (2020) Bulletin of North Kazakhstan University named after Manash Kozybayev. Issue No. 3 (48), 76–82.

P-08.5-38
Different Nrf2 activity in brain tissue as part of endogenous neuroprotection against I/R injury

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In order to give new insight into naturally occurring adaptation mechanisms responsible for brain protection against stresses we investigate the transcription factor Nuclear Factor-E2-Related Factor 2 (Nrf2), genes/proteins under Nrf2 regulation and their metabolic pathways. Nrf2 is the "master regulator" of more than 400 genes encoding the antioxidant, cytoprotective and anti-inflammatory proteins. Pharmacological activation of Nrf2 is beneficial in the treatment of neurodegenerative diseases e.g. multiple sclerosis. An example of endogenous neuroprotection is the resistance to ischemia-reperfusion (IR) injury of the abdominal region of hippocampus (CA2-4,DG) vs. its dorsal, ischemia-vulnerable region (CA1), well known phenomena remaining unresolved. Here, using gerbil model of 5-minute bilateral ligation of carotid arteries followed by 15, 30, 60, 120 minutes, and 24, 36, 48 and 72 hours of reperfusion we show that the Nrf2 activity is higher in CA2-4,DG vs. CA1 already in controls, and that IR results in a brief and short Nrf2 activation in CA1 and delayed, prolonged activation in CA2-4,DG. What is more, the level of heme oxygenase-1 (Nrf2-downstream effector) is much greater in CA2-4,DG

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than in CA1 in controls and this dependence persists after IR, confirming the higher activity of Nrf2 in CA2-4,DG. We also see upregulation of glutamate cysteine ligase (GCL, another Nrf2 regulated protein), modifier subunit in CA2-4,DG which had similar pattern as HO1. Interestingly, immunoreactivity of GCL catalytic subunit do not differ between CA1 and CA2-4,DG in controls. However, for all these proteins, significant peak in CA2-4,DG is seen 72h post IR. It is consistent with Nrf2 increase in CA2-4,DG. These data show that CA2-4,DG is naturally rich with Nrf2 and its effectors, which can be responsible for endogenous resistance in this region by activating protective genes. Further studies are carried out to evaluated molecular mechanisms of this phenomena.

P-08.5-39
Lactate supplementation mitigates obesity in high-fat diet-fed mice

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In the last two centuries, lactate was considered only a byproduct of anaerobic metabolism causing muscle pain after physical exercise. However, the last decade of research revealed that it has a number of signalling and metabolic functions mediated by its specific G protein-coupled receptor, GPR81, and by mitochondrial retrograde signalling. Lactate plays previously unexpected roles in memory formation, humoral signalling, inflammation, tissue repair, and also metabolic homeostasis of adipose tissue. Together with butyrate and other bacterial metabolites, lactate is a member of an emerging group of nutraceuticals called postbiotics. Here, we prepared a postbiotic formula releasing lactate in the gut and examined its effect on the adipose tissue of high-fat diet (HFD) fed C57BL6/J mice. Three weeks of daily supplementation with 2 % formulated lactate in the diet resulted in a significant decrease in body weight compared to HFD only (56 ± 4 g versus 61 ± 2 g, $P < 0.03$). Interestingly, no change in the amount of feed consumed by mice was observed (3.2 ± 0.2 g versus 3.1 ± 0.3 g, $P < 0.03$). While fat tissues shrank significantly (4.0 ± 0.5 g versus 5.2 ± 0.5 g, $P < 0.03$ for visceral fat; 2.6 ± 0.2 g versus 3.0 ± 0.4 g, $P < 0.03$ for subcutaneous fat), the weight of heart (0.17 ± 0.01 g versus 0.16 ± 0.01 g), kidney (0.39 ± 0.03 g versus 0.40 ± 0.19 g), and gastrocnemius muscle (0.48 ± 0.05 g versus 0.50 ± 0.06 g) remained unchanged. In parallel, a drop in insulin and leptin levels was observed in lactate+HFD fed mice compared to HFD only (26 ± 8 ng.ml^{-1} versus 33 ± 17 ng.ml^{-1}, $P < 0.03$ for insulin; 580 ± 310 pg.ml^{-1} versus 1080 ± 380 pg.ml^{-1}, $P < 0.03$ for leptin). No signs of pathological changes in the liver and gastrointestinal tract of lactate-fed mice were observed. In conclusion, the lactate-releasing postbiotic formula is a promising cure for obesity.

P-08.5-40
H2S donors for photo-polymerization damage protection in 3D-stem cell culture systems

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Photopolymerized hydrogels are ideal materials to improve stem-cell based tissue regeneration but photopolymerization may itself cause cell damage requiring conjugation between proteins and synthetic polymers, photo-initiators and UV exposure to generate free radicals for monomers crosslink, all processes whose effects on cells are not yet clarified. The latest discovered gasotransmitter H_2S protects cells from oxidative damage acting as a scavenger molecule and inducing the expression of antioxidant proteins. The ability of a phytochemical slow H_2S-donor, that we named GSGa [Buhyan A. et al. (2015) Molecules 20,1,1731-50], to prevent photo-polymerization (PhP) damage was here evaluated using an enzymatic molecular model and a 3D stem cell culture system based on PEG-fibrinogen hydrogel (PFHy) [Mauretti A. et al. (2016) Macromol Biosci.16:847-58].The decrease of the activity of a recombinant cyanide: thiosulfate sulfurtransferase (TST,EC. 2.8.1.1) in the presence of PEGDa and UV exposure was inhibited by the presence of GSGa, demonstrating the protective effect of GSGa from PhP damage on the enzyme. We also demonstrated that GSGa was able to up-regulate the expression of Antioxidant Responsive Elements (ARE)-controlled enzymes in mesenchymal stem cells (MSC) and human dermal fibroblasts (NHDF). The up-regulation of the expression of both NAD(P)H -quinone 1-dehydrogenase (NQO1)[Di Giovanni E. et al. (2020) Int JMolSci 21,5] and heme oxygenase-1 (HO1) after three days of GSGa treatment was proved by western blotting analysis. The effects of the MSC preconditioning with GSGa before embedding into 3D-PFHy scaffold were assessed by cell viability assays and fluorescence microscopy, demonstrating a protective effect of the GSGa cell preconditioning from PhP damage. These findings pave the way to the use of slow H_2S-releasing agents in cells pretreatment for tissue engineering applications, such as 3D-bioprinting and tissue repairing based on stem cell-delivery systems.

P-08.5-41
Crosstalk between long-term sub-lethal oxidative stress and inflammation as potential drivers for age-related retinal degeneration

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Age-related retinal degenerations, including age-related macular degeneration (AMD), are caused by the loss of retinal pigmented epithelial (RPE) cells and photoreceptors. The pathogenesis of AMD is deeply linked to the aging process and involves both oxidative stress and inflammatory responses. However, the molecular mechanisms contributing to the shift from healthy aging to AMD are still poorly understood. Since RPE cells in

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the retina are chronically exposed to a pro-oxidant microenvironment throughout life, we simulated *in vivo* conditions by growing ARPE-19 cells in the presence of 10μM H_2O_2 for several passages. This long-term oxidative insult induced senescence in ARPE-19 cells without affecting cell proliferation. A dysregulated expression in proteins involved in antioxidant response, mitochondrial homeostasis, and extracellular matrix organization has been revealed by global proteomic analysis. The analyses of mitochondrial functionality showed increased mitochondrial biogenesis and ATP generation and improved response to oxidative stress. The latter, however, was linked to NF-κB rather than Nrf2 activation. NF-κB hyperactivation also resulted in increased pro-inflammatory cytokines expression and inflammasome activation. Moreover, in response to additional pro-inflammatory insults, senescent ARPE-19 cells underwent an exaggerated inflammatory reaction. Our results indicate senescence as an important link between chronic oxidative insult and detrimental chronic inflammation, with possible future repercussions for therapeutic interventions. Previously published in: Macchioni L. et al. (2020) Antioxidants 29;10:25. *The authors marked with an asterisk equally contributed to the work.

P-08.5-42
Analysis of 8-hydroxy-2′-deoxyguanosine (8-OHdG) and 2′-deoxyguanosine (2-dG) by LC–MS/MS

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Free radicals are chemical products that occur with endogenous/exogenous agents during metabolic processes. These products interact with biomolecules such as DNA, proteins, lipids, carbohydrates in cells, resulting in oxidative damage. Reactive oxygen species cause the formation of more than 20 oxidative base damage products in DNA. The most common of these damaged bases is 8-hydroxy-2'-deoxyguanosine (8-OHdG), which is a highly sensitive marker of oxidative DNA damage. The OH radical reacts with the carbon atoms at the 4, 5 and 8 positions of the guanine and forms the DNA product radicals. The adduct radical (C8-OH) formed by the addition of the OH- radical to C-8,and loses an electron and proton and oxidizes to 8-OHGua. This, increases the tendency to mutate by causing conversion from G-C to A-T during DNA replication. Therefore, 8-OHdG and related molecules measurement are accepted as a direct indicator of oxidative damage in DNA and is applied as the most commonly used method in determining oxidative DNA damage. There are various methods used in the measurement of oxidative DNA damage. We modified a new ultra-high performance liquid chromatography/triple quadrupole mass spectrometry (UHPLC-MS/MS) method for 8-hydroxy-2'-deoxyguanosine (8-OHdG) and 2'-deoxyguanosine (2-dG). The analysis was carried out on a ACE-C18 (2.1x50mm, 5μm) reversed phase analytical column using gradient elution mode. Detection was performed using multiple reaction monitoring in electrospray ionization mode at m/z 284.1→167.9 and 139.8 for 8-OHdG, m/z 268→152.1 and 135.1 for 2-dG. The calibration curve for 8-OhdG and 2-dG method was between 1.25–80 ng/mL concentration range, linear and R^2 0.999. The limits of quantification (LOQ) were 2.27 ng/ml for 8-OhdG and for 2-dG. %RSD for all analytes ranged from 0.5–8% for intra-day and 1.7– 17.6% for interday experiments. A simple LC-MS/MS method has been developed and validated for measuring 8-OhdG and 2-dG.

P-08.5-43
Hidrox® ameliorates biochemical alterations in rats with deep endometriosis

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Endometriosis is a gynecological and painful condition affecting women in reproductive age. It is characterized by dysfunctional endometrium-like implants outside of the uterine cavity. The purpose of this study was to evaluate the effects of Hidrox®, an aqueous extract of olive pulp containing hydroxytyrosol, on endometriotic lesions associated pro-oxidative alterations and pain like behaviors. Endometriosis was induced by intraperitoneal injection of uterine fragments and Hidrox® was administer daily. At the end of the 14-day treatment, behavioral alterations were assessed and hippocampal tissues were collected. Laparotomy was performed and the endometrial implants were harvested for histological and biochemical analysis. Hidrox® treatment reduced endometriotic implant area, diameter and volumes. Vehicle treated rats showed lesional fibrosis, epithelial-mesenchymal transition and fibroblast-myofibroblast transdifferentiation, angiogenesis and prooxidative alterations in the peritoneal cavity. Hidrox® treatment reduced the aniline blue stained area, a-sma, CD34 and VEGF positive expressions. Moreover, it reduced myeloperoxidase activity and lipid peroxidation and increased SOD activity and glutathione levels in the endometrial explants. Hidrox® administration also reduced for peripheral and visceral sensibility as showed by the behavioral tests (open field test, hot plate test, elevated plus maze test and acetic acid-induced abdominal contractions). Animals treated with Hidrox® also showed reduced astrocytes and microglia activation and brain oxidative status restoring BDNF protein expression and increasing Nfr2 nuclear translocation. In conclusion Hidrox® displayed potential ameliorative effects on endometriotic implants and related pain-induced behaviors due to its potent antioxidative properties. *The authors marked with an asterisk equally contributed to the work.

P-08.5-44
Flavonoid nobiletin's significance in development of ischemic pre-conditioning phenomena

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Ischemic preconditioning is a phenomenon whereby brief episode(s) of sublethal ischemia protect the heart against sustained ischemia-reperfusion injury. Plant derived polyphenolic compounds with neuroprotective effects, like nobiletin, attracts lots of attention as promising candidates for pharmacologic preconditioning. We studied the effect of nobiletin and 3-nitropropionic acid (3-NPA) on differentiated PC12 cell survival, mitochondrial membrane potential, oxygen consumption, ROS and RNS production, mitochondrial calcium and ATP production. Besides, estimated their effect on apoptosis biomarkers. We have found that preincubation with nobiletin and 3-NPA increases cell

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viability in hypoxic conditions. Nobiletin, like 3-NPA prevents increase of calcium levels in both hypoxia and oxygen-glucose deprivation condition. Pre-incubation with nobiletin eliminates the effect of hypoxia-induced decrease of ATP production, 10^{-5}M concentration induces transient hyperpolarization of mitochondrial membrane which is followed by mild depolarization and significantly reduces the enhanced generation of hypoxia-induced reactive oxygen and nitrogen species. Furthermore, we found that both concentrations of nobiletin significantly reduced hypoxia induced apoptotic biomarkers. We propose that nobiletin induces chemical preconditioning and thus increases cell survival under ischemic condition. Besides, transient hyperpolarization of the mitochondrial membrane caused by nobiletin is considered to be one of the key mechanisms of chemical preconditioning, which enables adaptive processes in the mitochondria and the whole cell. Furthermore, significantly reduced hypoxia induced apoptotic biomarkers can be a result of preconditioning mechanism activation. This research was supported by Shota Rustaveli National Science Foundation of Georgia (SRNSFG), grant number YS-18-1159.

P-08.5-45
The effects of the acclimation and measurement temperature on antioxidant enzyme activity in Baikal endemic amphipods

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Baikal amphipods are active in the winter season and a number of species are characterized by winter breeding season. Being cold-adapted ectotherms, they may have enzymes that stay active when the ambient temperature falls. The aim of our research was to estimate the effects of the acclimation temperatures (AT) and measurement temperatures (MT) on antioxidant enzymes activity of Baikal endemic amphipods. The amphipods *Eulimnogammarus verrucosus* and *E. cyaneus* were collected from Lake Baikal (near Listvyanka, southern Baikal) and acclimated to 10°C (sampling temperature). In 2 days, the control groups of the amphipods were fixed in liquid nitrogen, and the remaining animals were acclimated to 1.5, 6 and 12°C. The amphipods were exposed to different AT for 21 days and fixed in liquid nitrogen. The activities of peroxidase (POD), catalase (CAT) and glutathione S-transferase (GST) were measured spectrophotometrically at 1.5, 6 and 12°C. The results were analyzed with two-way ANOVA. We found that both AT and MT affected the activities of POD and GST in *E. verrucosus*. At the same time, CAT activity did not change in response to studied temperatures in this psychrophilic species. It probably has cold-adapted isoforms of CAT, which enable maintenance of the necessary enzyme activity at low positive temperatures. In the case of *E. cyaneus*, AT influenced the activities of POD and CAT. The activity of GST was influenced by AT and MT. Overall, these data show that the antioxidant enzymes activities in *E. cyaneus* are more susceptible to the effects of low temperatures. A possible explanation is that this species inhabits the shallow littoral zone, where the change of seasons directly affects the thermal regime and the range of temperatures is wide (from 1-1.5°C to 20°C). Support by the Ministry of Science and Higher Education of the Russian Federation (grant Goszadanie FZZE-2020-0026), DAAD (grant Goszadanie: application 2301-21), RSF (20-64-46003). *The authors marked with an asterisk equally contributed to the work.

P-08.5-46
Binding of ceruloplasmin to myeloperoxidase and following inhibition of myeloperoxidase enzymatic activity under *in vitro* hyperglycemic conditions

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Chronic hyperglycemia (HG) in diabetes mellitus (DM) is associated with oxidative/halogenative stress mediated by modification of a number of antioxidant enzymes and increased neutrophil production of reactive oxygen and halogen species. We have recently shown that as HG progresses in type 2 DM patients, the specific activity of the plasma protein ceruloplasmin (CP) decreases. CP displays several antioxidant activities, including the suppression of neutrophil respiratory burst through superoxide dismutase activity and the inhibition of the neutrophil pro-oxidant enzyme myeloperoxidase (MPO) through forming a complex with it. The aim of this work was to investigate MPO binding with CP modified under *in vitro* conditions mimicking HG and to evaluate the accompanying changes in MPO activity. MPO activity was measured by oxidation of the fluorogenic substrate Amplex Red. CP binding to MPO was determined using a solid-phase assay in which polystyrene plate-bound MPO captured biotinylated CP that then was detected using streptavidin conjugated to alkaline phosphatase. Biotinylated CP (3 μM) was modified at 37°C by glucose for 7 days or methylglyoxal (MG, the main product of non-enzymatic glucose oxidation) for 1 day. The biotin tag retained bound to modified CP. CP modification by increasing the glucose (5, 15, 30 mM) or MG (5, 10, 30 mM) concentration resulted in a progressive (i) enhancement of fluorescence from Schiff bases (350 nm exc./430 nm em.) by 8, 31, 73-fold and 16, 41, 81-fold for glucose case or MG case, respectively; (ii) decrease in CP oxidase activity towards p-phenylenediamine by 22, 54, 76% and 33, 65, 86%; (iii) reduction in CP's capability to bind to MPO by 32, 61, 81% and 45, 68, 88% as well as inhibition of MPO by 40, 68, 88% and 57, 78, 91%. Thus, HG-induced modification of CP can exacerbate the development of oxidative/halogenative stress in DM patients. The study was supported by the Russian Science Foundation grant No. 20-15-00390.

P-08.5-47
Role of OGG1 in cerulein induced acute pancreatitis

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Acute pancreatitis (AP) is one of the most common diseases in gastroenterology. It is an inflammatory disorder of the exocrine pancreas with a high mortality. The severity of AP depends largely on the balance between different forms of cell death, especially apoptosis and necrosis. Oxidative stress and redox homeostasis are also involved in the early stage of AP. Various reactive oxygen species (ROS) oxidize guanine in DNA resulting in the formation of 7,8-dihydro-8-oxoguanine (8-oxoG). As the

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most predominant DNA oxidative lesion in the genome, 8-oxoG is recognized and repaired by 8-oxoguanine DNA glycosylase1 (OGG1) during the base excision repair (BER) pathway. The guanine oxidation product has been proposed to be more than just a lesion to be repaired but to also serve as an inflammatory signaling molecule via OGG1 and NF-kappaB activation. Our aim was to investigate the contribution of this pathway to AP. AP was induced in mice be repeated injections of cerulein. TH5487, a selective active-site inhibitor of OGG1, known to hamper OGG1-DNA interactions at guanine-rich promoters of proinflammatory genes was used. Our results show that TH5487 significantly attenuated leukocyte infiltration (decreased tissue myeloperoxidase level), edema, NF-kappaB-dependent inflammatory cytokine levels (IL6, IL1beta expression) and prevented cell injury as shown by decreased serum amylase and lipase levels. Our data suggest that OGG1 could be essential for NF-kappaB dependent gene expression in AP and OGG1 inhibition can alleviate inflammation in this disease. Acknowledgements: LV received funding from the National Research, Development and Innovation Office grants GINOP-2.3.2-15-2016-00020 TUMORDNS", GINOP-2.3.2-15-2016-00048-STAYALIVE, OTKA K132193.

P-08.5-48
Contribution of flavourings to e-cigarette toxicity

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E-Cigarette (EC) consumption has been exponentially increased over the recent years due to the possibility of flavouring personalization and to the idea that ECs are less harmful than conventional cigarettes. Although carcinogens appear to be reduced or eliminated, EC smoke is able to induce oxidative stress and DNA damage probably due to molecules generated during the e-liquid heating. E-liquid contains a vehicle-humectant, such as propylene glycol, and/or vegetable glycerin along with nicotine and a mix of flavouring chemicals. These flavourings are permitted for ingestion as their use through the digestive tract is well documented, however little information is available about their effects on the inhalation exposure. Therefore, the cytotoxicity of the aromatic component was assessed treating bronchial cells (BEAS-2B) with EC smoke extracted from nicotine-free e-liquids containing the commonly used flavourings of different chemical classes: aldehydes, alcohols and esters. Differences among extracts and exposure timespan (24h or 72h) were observed. In particular, after 24h exposure, only ethyl vanillin and ethyl maltol decreased cell viability, while apoptosis was increased by all extracts with the exception of vanillin. Moreover, mortality was not affected after 24h exposure, while it was increased by three flavorings at 72h. These changes could be caused by an increase of ROS, more pronounced in mitochondria compared to cytosol, in particular with ethyl vanillin and ethyl maltol, vanillin and ethyl butyrate extracts. While maltol, menthol, benzyl alcohol and ethyl acetate extracts did not impair the oxidative balance after both acute and chronic exposure. In conclusion, although flavourings were strongly less toxic than tobacco cigarette smoke, the aromatic component differentially contributed to the EC toxicity by modulating cellular redox status and impairing cell viability, in particular after 72h of exposure.

P-08.5-49
Calcium dependent activation of mitochondrial ROS production in neurons and astrocytes

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Mitochondria is an organelle involved in various vitally essential processes in the cell. Besides their main function – ATP production – mitochondria play a key role of in calcium signaling, reactive oxygen species production and cell death regulation. Although all these processes are described in detail, the mechanism of their interaction during the propagation of an intracellular signal remain unclear. Using live cell imaging in primary coculture of neurons and astrocytes we studied the interaction of ROS and mitochondrial calcium uptake. We have found that addition of ATP or L-Glu triggered the calcium signal in astrocytes and neurons that initiate calcium uptake by mitochondria. Elevation of mitochondrial calcium stimulated an increase in the rate of ROS in mitochondria of neurons ($P = 0.035$) and astrocytes ($P < 0.01$). $Ca2^{+}$-ionophore ionomycin is also induced activation of ROS production in mitochondria. $Ca2^{+}$-induced increase of ROS production was dependent on the presence of uncoupler FCCP or inhibitor of mitochondrial complex I rotenone. Thus, mitochondrial calcium uptake in neurons and astrocytes in response to physiological stimulus can induce ROS production in mitochondria which is dependent of calcium on mitochondrial respiration. Activation of mitochondrial ROS production can play important role in physiology and development of pathology. *The authors marked with an asterisk equally contributed to the work.

P-08.5-50
Beneficial effects of tricetin in cerulein induced acute pancreatitis

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Acute pancreatitis (AP) is one of the most common gastrointestinal diseases in developed societies. The disease has a high (30-40%) mortality due to the lack of efficient therapies. In AP, digestive pancreatic enzymes are prematurely activated in the acinar cells leading to tissue damage. Our aim was to examine the possible beneficial effect of the flavonoid compound tricetin in a mouse model of cerulein-induced AP (8 injections at hourly intervals, 50μg/kg body weight). *In vivo* data were complemented with cell-based (*in vitro*) experiments performed with cerulein-treated (100nM) isolated acinar cells. Intraperitoneal injection of tricetin (10mg/kg body weight) reduced cerulein-induced acinar cell damage as reflected by serum markers of acinar injury (lipase and amylase activities). Tricetin also reduced granulocyte infiltration of the pancreas as indicated by tissue myeloperoxidase assays. Moreover, histological evaluation of H&E stained pancreas sections showed lower disease score in the pancreata of tricetin-treated animals compared to the cerulein group. *In vitro*, tricetin suppressed the expression of the inflammatory cytokines (IL1-beta, IL6) and matrix metalloproteinase 2. Furthermore, cerulein-induced necrotic cell death was suppressed by the flavonoid.

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Our data suggest that tricetin may be an effective treatment option for AP. Acknowledgement: LV received funding from the National Research, Development and Innovation Office grants GINOP-2.3.2-15-2016-00020 TUMORDNS", GINOP-2.3.2-15-2016-00048-STAYALIVE, OTKA K132193.

P-08.5-51
Time-dependent recruitment of different mechanisms of ROS formation during SW872 cell adipocyte differentiation

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Accumulating evidence indicates that obesity represents a state of chronic oxidative stress. Enhanced formation of reactive oxygen species (ROS) apparently ensues at the early stages of adipogenesis, to regulate differentiation of pre-adipocytes to mature adipocytes and maintenance of specific metabolic functions in differentiated adipocytes. These processes, mostly studied in mouse cells, remain poorly understood and require further attention in relevant human cellular models. We initially characterised at both the morphological and biochemical levels the different phases of adipogenesis in SW872 cells. Next, we focused on ROS formation and obtained evidence for an early (day 3) activation of NADPH oxidase(s) in the absence of concomitant mitochondrial ROS production. There was no evidence of ROS formation at day 6, instead observed at day 10, with the involvement of both NADPH oxidase and mitochondria. Early ROS formation was associated to the progression of differentiation and activation of the Nrf2 antioxidant response, leading to increased GSH levels. Late mitochondrial ROS formation was instead detected only in a subpopulation of cells and was likely mediated by their high lipid content. We also obtained evidence of mitochondrial dysfunction associated with reduced mitochondrial GSH content and oxidation of both cardiolipin and thioredoxin 2. In conclusion, we herein provide a detailed morphological and biochemical characterization of the adipogenic differentiation of SW872 human cells associated with the discontinuous involvement of NADPH oxidase in ROS formation. In addition, mitochondrial ROS formation was restricted to the late phase, and possibly causally linked to mitochondrial dysfunction. *The authors marked with an asterisk equally contributed to the work.

P-08.5-52
Muscari comosum L.: the ancient wild plant protagonist of the Mediterranean diet and its effects on liver cancer cells.

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Among the wild plants that grow throughout the Mediterranean area including Basilicata, particular attention was paid to *Leopoldia comosa* L. (syn. *Muscari comosum* L.), a spontaneous plant belonging to the Asparagaceae family known for its therapeutic effect known in folk medicine [1]. We investigated the effects on HepG2 hepatoma cells of *Muscari comosum* aqueous and methanol extracts. The metabolomics analysis performed by NMR suggested that *M. comosum* extracts modify the intra- and extracellular metabolic profile. Moreover, the extracts exhibited concentration/solvent dependent radical scavenging activity, as well as promotion of Nrf2 expression which, in turn and in different way, modulated the expression level of downstream target genes SOD-2, GPX-1. Several ATP binding cassette (ABC) transporters are Nrf2 targets. The extracts promote the expression of ABCC6 and ABCG2, two ATP dependent transporters involved in chemotherapy, multidrug resistance and also responsible for glutathione transport [2]. The increase in ROS is responsible for blocking the cell cycle and the reduction of cell viability. ROS levels increased at higher concentration of the extracts. Depending on the extract, $p21^{cip1/waf1}$ expression level increased in p53-independent or -dependent manner. Over-expression of p53 protein plays a key role in activating the intrinsic pathway of apoptosis. Treatment with *M. comosum* extracts increased the expression level of cytochrome c as well as downregulated GRP78/BiP and upregulated CHOP, involved in the unfolded protein response (URP) and, consequently, in ER-stress. Previously published in: [1] Hadjichambis Ach et al. (2008) Int. J. Food Sci. Nutr. 59, 383–414. [2] Giglio F et al. (2021) Molecules, 26, 416.

The circadian clock and disease

P-08.6-01
Transcriptome landscape of HepG2 cells with deleted late genes of cholesterol synthesis: focus on RORC and the circadian clock

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Cholesterol synthesis is a metabolic pathway with many steps and still open mysteries. Evidence suggests that cholesterol intermediates between zymosterol and desmosterol may serve as ligands of nuclear receptors from ROR (RAR Related Orphan Receptor) family. These sterols are thought to be RORC agonists, regulating the expression of genes involved in lipid and glucose metabolism, as well as in the circadian clock genes. RORs are part of stabilizing loop of circadian clock and we hypothesize that the activation of RORC with non-polar sterols fine-tunes the expression of clock genes. To investigate the downstream biological roles of the different cholesterol intermediates, we used the CRISPR-Cas9 on human HepG2 cells. We produced cell lines, each with a knockout (KO) of a different gene from the late part of cholesterol synthesis (CYP51, DHCR24, SC5DL and HSD17B7). Each cell line accumulates the upstream sterols and lacks intermediates downstream of the deleted enzyme. Transcriptome changes in KO cell lines were evaluated using microarrays and Nanopore sequencing and differentially expressed genes and pathways assessed using KEGG and TF enrichment analysis. Analysis revealed 102 differentially expressed genes altered in all KO cells, mostly associated with impaired cholesterol synthesis and related metabolic pathways. RORC target genes were altered to varying degrees, with the highest number of upregulation in SC5D (23) and HSD17B7 (62) KO cells. This suggests RORC activation through accumulated zymostenol and zymosterol.

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Each KO cell line differentially expressed a number of genes, indicating the specific signalling functions of the accumulated sterols which are still under investigation. The role of individual sterols from cholesterol synthesis in RORC-mediated fine tuning of the clock genes, the circadian phase and the period, is in progress.

P-08.6-02
Effects of DNA damage on the cell cycle through the circadian clock

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Most cancer treatment induces DNA damage to eliminate highly proliferative cells. This process, however, also affects healthy cells creating numerous side effects. Cells respond differently to DNA damage depending on where they are in their cell cycle at the time of the treatment. Intriguingly, circadian rhythms provide temporal information to the cell cycle machinery to control the timing of cell divisions. Personalized chronotherapy based on this: the time of the treatment based on the patient's natural daily rhythm. To understand the impact of DNA damage in altering the timing of cell divisions via circadian rhythms, we built a complex mathematical model and performed in-silico experiments for the proposed system that integrates circadian rhythms, cell cycle, and DNA damage response in *Neurospora crassa*. We simulated the perturbation of the circadian clock and the cell cycle upon DNA damage and analysed the consequences of this perturbation on the following cycles using phase-response curves. We can observe that in the subjective day the phase shift is near to 0, but in the subjective night, high phase advance appears. We analysed the behaviour of the cell cycle, where the strength of connection between the cell cycle and circadian rhythm and the cell cycle time was noisy. We followed the number of nuclei in mitosis at each timepoint after perturbation. We observed, that it matters, when during the day perturbation upon DNA damage occurs. This consequence may have relevance to cancer treatment as well. Depending on the timing of the induction of DNA damage, the healthy cells react differently: they can continue their normal division or they can be perturbed as a side effect of the treatment.

P-08.6-03
Effects of various conditions related to circadian rhythm disturbances on plasma and erythrocyte lipids: a peroxisomal perspective

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Circadian rhythm disorders are associated with chronic diseases including metabolic syndrome, diabetes and cardiovasculer diseases. Lipidomics studies revealed alterations of some plasma phosholipid levels in some circadian perturbations including plasmalogens which are partly synthesized in peroxisomes. It was aimed to investigate effects of various conditions known to cause circadian rhythm disturbances (i.e. calorie restriction, time-restricted feeding, constant light exposure) on various peroxisomal parameters and to compare those effects with that of fenofibrate, a PPAR-α agonist, in rats. Plasmalogens and some fatty acids in erythrocyte lysates were analyzed by GC. Peroxisomal metabolites including very long chain fatty acids as well as phytanic and pristanic acids in plasma were measured by GC-MS. Peroxisomal activation was evaluated by catalase immunohistochemistry in liver sections. Among the conditions of circadian disturbances tested, calorie restriction, known to be associated with both central and peripheral clock machinery, resulted in more pronounced alterations in peroxisomal parameters analysed compared to control group. Fenofibrate treatment yielded lower level of plasma phytanic acid concentration implying higher peroxisomal α-oxidation rate. However both calorie restriction and fenofibrate treatment exhibited lower plasmalogen, DHA and arachidonic acid contents of erythrocyte lysates. Shared effects of conditions associated with circadian rhythm disturbances and peroxisomal induction by fenofibrate on erythrocyte membrane lipids might indicate a link between them.

P-08.6-04
Escherichia coli affects expression of circadian clock genes in human hepatoma cells

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The mammalian circadian clock is important for homeostasis, as is the intestinal microbiota. Recent research indicated that dysbiosis can lead to an altered circadian clock. However, the role of *Escherichia coli*, the important facultative anaerobe in the gut, in influencing the host's circadian clock has yet to be defined. Therefore, we investigated the effect of *E. coli* on the host circadian rhythm in an established stable co-culture with human liver cells HepG2. Two genotypically different *E. coli* strains from the collection of faecal *E. coli* strains from healthy individuals were selected for the assays. The obtained results showed that the

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bacterial strains had different impacts on the HepG2 cells circadian clock genes. The performed study hence revealed that the *E. coli* genetic background is important for the bacterial effect on the circadian clock genes indicating possible future use of different probiotic *E. coli* strains to influence the host circadian clock.

P-08.6-05
Behavior and redox regulation are different in per01 flies after psychostimulant administration

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Studies in Drosophila identified a group of circadian genes as regulators of behavioral sensitization, a simple form of drug-induced neuronal plasticity that develops after repeated exposures to psychostimulants. Recent studies have shown the importance of redox modulation in the cellular signaling and neuronal plasticity. Circadian gene period (per) regulates cocaine-induced neuronal plasticity in flies and mammals. Here we investigate if per regulates drug-induced changes in the redox status, as it is known that there is a mutual regulation and interaction between redox metabolism and expression of circadian genes. In our experiments we correlate the deficits that per01 shows in the drug-induced behavior with the activity of antioxidant enzymes superoxid dismutase (SOD) and catalase (CAT) after the administration of volatilized cocaine (vCOC). We show that unlike wild type (wt) flies per01 males: do not develop locomotor sensitization to (vCOC) or volatilized methamphetamine in the FlyBong test, and show no preferential consumption of methampetamine laced food in the FlyCafe test. However, we can rescue locomotor sensitization to vCOC by feeding flies quercetin, a plant flavonoid with antioxidant property, suggesting that redox signaling is involved in the regulation of cocaine-induced neuronal plasticity. In per01 flies CAT and SOD enzymes are significantly less active after one or two doses of vCOC compared to wt flies, but surprisingly, the amount of hydrogen peroxides is also lower. These results show that cocaine modulates endogenous antioxidant enzymes and that this modulation is different in per01 flies. The discrepancy between the antioxidant enzyme activity and the amount of hydrogen peroxide suggests that per01 flies use different or additional antioxidant defense molecules after cocaine administration. Filošević A et al. (2018) Front Mol Neurosci. 5;11:25. Rigo F et al. (2020) Addict Biol. 24:e12963. *The authors marked with an asterisk equally contributed to the work.

Structural and functional glycobiology

P-09.1-01
Heparin-binding activity of extracellular vesicles from human seminal plasma

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Prostasomes are extracellular vesicles (EVs) originating from the prostate and abundantly present in human seminal plasma (SP). They have intrinsically heterogeneous molecular composition and ultrastructure. Surface glycans specifically contribute to their properties in terms of marking distinct prostasome populations and were, also, found to change during different physiological conditions. In this study, heparin-binding activity is aimed as an adjunct parameter to additionally annotate prostasome populations already established on the basis of a specific pattern of glycosylated markers. Heparin-binding proteins (HBP) are found on different membranous structures, but not studied in the context of association with prostasomes. Resolving this issue could be also of biomedical importance since HBP may attribute prostasomes their various biological activities some of which are known to correlate with fertility status. Heparin-affinity chromatography was probed for separation of prostasomes isolated by ultracentrifugation and gel filtration, from normozoospermic- (sPro-N) and oligozoospermic- (sPro-O) men. EVs recovered in non-bound and bound fractions were examined by transmission electron microscopy and characterized according to total protein/glycoprotein composition. Both sPro-N and sPro-O consisted of one major and at least three minor charge-resolved EVs populations differing in the presentation of mannosylated and sialylated glycans. Prostasomal patterns of glycoproteins and tetraspanin markers remained mainly in the non-bound fraction, whereas heparin-binding activity was annotated to vesicles exhibiting different morphology and low activity of surface-associated gamma-glutamyl transferase. Addressing both glycosylated and matching carbohydrate-binding molecules on prostasomal surface is of general importance since they make the first contact with interacting molecules during exerting putative role as a communication tool in reproduction-related processes.

P-09.1-02
Identification of the main prostate specific antigen glycoforms in aggressive prostate cancer

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Prostate cancer (PCa) is the most common cancer and the second cause of cancer death in men. Serum levels of the glycoprotein prostate specific antigen (PSA) have been used for PCa detection since 1994. However, PSA levels may also rise in other prostate pathologies. This lack of specificity prompted a research to

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develop new non-invasive biomarkers to reduce PCa overdiagnosis and overtreatment. Study of PSA glycosylation has been emerged as a very promising field. In particular, an increase in the percentage of α2,3-linked sialic acid (SA) of PSA glycoforms are indicative of aggressive PCa. Nevertheless, specific PSA glycoforms that are either increased or decreased in aggressive PCa have not been described yet. Thus, we aim to identify the main PSA glycan structures from high-risk PCa patients that differ from healthy controls. For that, 6 serum samples from aggressive PCa patients with high levels of PSA (>300 ng/ml) were obtained from the Hospital Dr. J. Trueta and PSA purified from healthy individuals' seminal plasma was used as a control. PSA was immunoprecipitated and α2,3/α2,6-SA glycoforms were separated by SNA-lectin affinity chromatography. The PSA collected fractions were immunoprecipitated and resolved on SDS-PAGE. PSA bands were excised and digested with PNGaseF. The obtained N-glycans were analysed by Hydrophilic Liquid Chromatography combined with exoglycosidase digestions and glycan differences between healthy and aggressive PCa patients were determined. PSA sialylated glycoforms containing a GalNAc residue were increased in high-risk PCa, whereas the disialylated core fucosylated biantennary structures with α2,6-SA, which are the major PSA glycoforms from healthy individuals, were markedly reduced in aggressive PCa. These results have shown that changes in the proportions of these specific PSA glycoforms are associated with aggressive PCa, and could be used as templates to develop specific methodologies for their detection.

P-09.1-03
Knock-down of alpha2,3-sialyltransferases in pancreatic cancer cells and its effect on EGFR glycosylation

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Pancreatic ductal adenocarcinoma (PDA) is the third leading cause of cancer mortality and presents a dreadful prognostic, mainly due to its delayed diagnosis and its resistance to therapies. Epidermal growth factor receptor (EGFR) participates in the regulation of cancer cells and it is highly glycosylated. During the neoplastic process, EGFR glycan structures can undergo several modifications that can alter its activity and signalling. We aim to analyse the glycosylation pattern of EGFR in BxPC-3 and Capan-1 PDA cells deficient in α2,3-sialyltransferases (STs) ST3GAL3 and ST3GAL4 and to determine the influence of such changes in the activation and signalling of EGFR. First, EGFR levels were evaluated by flow cytometry and western blot (WB) in the knock-down and control PDA cells. The receptor was immunoprecipitated from cell lysates and EGFR glycosylation was examined by WB with lectins and antibodies against glycan structures. The activation of EGFR and its downstream signalling pathways after treatment with EGF was determined by WB analyses of the phosphorylation levels of EGFR specific residues and of MAPK and AKT proteins. EGFR expression level of BxPC-3 and Capan-1 cell lines did not change between STs knocked-down cells and control cells, but a decrease in the Sialyl-Lewis x levels of EGFR of the silenced cell lines vs control ones was found, in agreement with the cell surface glycosylation changes. After EGF stimulation, specific EGFR residues, involved in the proliferation signalling pathways and internalization of the receptor, showed higher level of phosphorylation in BxPC-3 and Capan-1 silenced cells. Additionally, there was an increase in the phosphorylation level of AKT in the silenced cell lines. Altogether, these results suggest that silencing of ST3GAL3 and ST3GAL4 in PDA cells alters the phosphorylation pattern of the receptor that regulates proliferation and internalization pathways, and could influence the effect of EGFR-targeted drug therapies.

P-09.1-04
Glycogen molecules structure in hepatocytes of starved and fed rats

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Glycogen is a source of energy in many organs and tissues and its content is especially high in skeletal muscles and the liver, where it is stored in the form of β-particles. Glycogen is traditionally referred to as a polysaccharide, however β-particles contain numerous proteins. The presence of proteins in β-particles underlies a high heterogeneity of glucose residues. It has been shown that glycogen in tissues is represented by two fractions differing in their solubility in trichloracetic acid (TCA). Studies of the content of acid-labile fraction (LF) and acid-stable fraction (SF) have showed considerable differences in their dynamics during various physiological and pathological conditions. However, there have been no attempts to study the spatial structure of β-particles in regard to the data on the content of glycogen fractions. Thus, the aim of this study was to obtain new data about the structure of glycogen molecules in individual hepatocytes of starved and fed rats employing the fluorescent variant of PAS reaction. PAS staining of the smears of isolated hepatocytes oxidised with KIO_4 solution in HNO_3 with such dyes as Au-SO_2 or EtBr-SO_2 with different spectral characteristics allowed us to determine the content of the LF and the SF separately as well as the total content of glycogen (LF + SF). Data obtained indicate that an increase in the glycogen content in hepatocytes of fed rats if compared with starved ones is accompanied by changes in the spatial structure of its molecules including a greater number of chains and tiers filled with glucose residues as well as an increased amount of proteins associated with glucose residues at the surface of the molecules. Our data indicate that the external tier of glycogen molecules in hepatocytes is never completely filled with glucose residues and always contains glucose residues associated with proteins. We also showed that both synthesis and degradation of glycogen may occur in the same liver cell.

P-09.1-05
Molecular properties of the bacterial levan's – promising sorbents of heavy metals

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Molecular characteristics and mechanisms of heavy metals sorption by biocomposites based on natural and modified levan - fructane exopolysaccharide (EPS) synthesized by levansaccharases of

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bacteria were studied. The general properties of levan EPS include: the ability to form a flexible film, renewable, biocompatibility, biodegradability, and environmental safety. When *Azotobacter vinelandii* D-08 was cultivated on a sucrose medium, the maximum yield of levan was 16 g/l on third days of growth. On molasses media, the yield of EPS increased significantly and reached a maximum to second days (25.6 g/l). The HPLC method shows that levan from the sucrose medium contained high-molecular (600–700 kDa), two medium-molecular (210 kDa and 190 kDa), and low-molecular (less than 25 kDa) fractions. Levan isolated from molasses medium had high-molecular (more than 1000 kDa), medium-molecular (300 kDa) and three low-molecular (less than 25 kDa) fractions. Levan was modified using the TEMPO-oxidation method. In the IR spectrum of the modified levan, a peak corresponding to the carboxylate group was registered at 1620 cm^{-1}. A hydrogel based on polyvinyl alcohol, as well as native and modified Levan was obtained. The hydrogel was used for adsorption of zinc ions from solutions. The efficiency of adsorption by the gel based on modified levan was on 1.5 times higher than by the control gel based on native levan. This may be due to the formation of ionic bonds between metals and carboxyl groups of modified levan. Native levan with the hydroxyl groups of worse binds ions. In addition, the modification of the polysaccharide reduced the adsorption time for zinc ions from three days to 10 minutes. Thus, the gel based on modified Levan is a more effective adsorbent for zinc ions, which can be used for the development of biocomposites-polysorbents with different applications. This work was supported by the RFBR (Russia), project 18-29-05054. *The authors marked with an asterisk equally contributed to the work.

P-09.1-06
Expression of glycan biosynthetic enzymes and glycan-binding proteins in *Ixodes ricinus* tick life stages

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Pathogens transmitted by the castor bean tick (*Ixodes ricinus*) are an emerging threat to public health. Glycoproteins and lectins are known to play a role in tick innate immunity and, additionally, pathogens' interactions with glycoproteins enable colonization of both the tick vector and mammalian host. Thus, the understanding of glycosylation in different life stages could shed light on the tick-pathogen interactions. In this study, we identify transcripts involved in glycan biosynthesis as well as transcripts of lectins and other genes containing sugar binding domain (SBD) in transcriptomes derived from different life-stages of the *I. ricinus*. We searched the tick stage specific transcriptome assemblies using candidate sequences for the selected SBD proteins and glycosylating enzymes from related arthropod species as query. We identified enzymes underlying the process of N-glycan sialylation which is otherwise attributed to the glycosylation specific for complex glycans in vertebrate lineage. Moreover, we found some of these transcripts specific to early stages of tick development. Thus we suggest their role in the mechanism of molecular mimicry in tick and we make conclusions on their significance for tick development. We also found *I. ricinus* homologues of lectins integrated in an innate immune network and recognizing specific glycan structures on the surface of invading pathogens as described in other blood feeding arthropods. Collectively, our work extends the knowledge background about the role of carbohydrate-mediated interactions in the tick innate immunity being challenged by the tick parasitic life style and we also provide more insight into the role of glycosylation in a tick life cycle and development.

P-09.1-07
Characterization of mesothelin glycosylation: mesothelin core-fucosylation level as a biomarker for pancreatic cancer diagnosis.

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Pancreatic cancer (PaC) is the third most common cause of cancer death, as most PaC cases are not curable when detected. Glycoproteomics stands as an approach for finding novel biomarkers based on the analysis of glycan determinants on specific glycoproteins. In this study, we have focused on mesothelin, a glycoprotein with three N-glycosylation sites, neo-expressed in PaC and present in the bloodstream. Mesothelin showed expression in all the PaC cell lines analysed (both in cell lysates and conditioned media) by western blot (WB), and in most of the PaC tissues (58–77%) by immunohistochemistry and WB, respectively. Glycosylation analysis by mass spectrometry (UPLC-ESI-QTOF) of immunoprecipitated mesothelin from PaC samples revealed a total N-glycan site occupancy. Moreover, hydrophilic interaction liquid chromatography (HILIC-UPLC) combined with exoglycosidase digestions of mesothelin N-glycans, and western blot on immunopurified mesothelin using specific lectins mainly showed sialylated complex-type N-glycans, with differential expression of core fucosylation, branching and bisecting GlcNAc. We further developed an ELISA/ELLA assay to specifically quantify core-fucosylation levels on mesothelin, finding lower levels on PaC against ovarian cancer cell lines. Next, this assay was applied to a cohort of 33 serum samples (15 PaC of different staging, 11 chronic pancreatitis and 7 healthy controls). Mesothelin core-fucosylation was under-expressed in PaC patients when compared with the other control groups. Thus, we have described, for the first time, the structure of mesothelin N-glycans. In addition, we have developed an assay to specifically measure blood levels of mesothelin-core fucosylated glycoforms, which has showed significant differences between PaC patients and control groups. The mesothelin core-fucosylation degree should be assessed in a larger cohort of samples to evaluate its potential as a biomarker, alone or in combination with other biomarkers.

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Biomembranes and lipid mediators

P-09.2-01
Autophagy-driven lipid droplet formation protects cancer cells from nutrient stress

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Lipids are stored in cells mainly in the form of triacylglycerols (TAGs) and sterol esters in organelles named lipid droplets (LDs). Initially considered only inert fat reservoirs, LDs are now emerging as major players in the cellular stress response. In cancer cells, LD formation is induced in response to various kinds of stress, including nutrient deprivation or excess. Recent studies suggest a complex association between LDs and autophagy, whereby autophagy may either contribute to LD biogenesis or breakdown. We aim to uncover the importance of autophagy for LD metabolism and for the resistance of cancer cells to nutrient stress. We found here that LDs are dynamically formed and broken down in HeLa cervical cancer cells depending on the severity and length of nutrient stress. Intriguingly, LD biogenesis is upregulated within hours of short-term, severe starvation, whereas a milder nutrient deficiency stimulates LD breakdown. Using inhibitors of diacylglycerol acyltransferase (DGAT) 1 and 2 enzymes, which are necessary for TAG synthesis and LD formation, we show that the observed increased levels of LDs are a consequence of activation of LD biogenesis. Furthermore, we found that autophagic flux is significantly elevated in acutely starved HeLa cells and that blocking autophagy leads to inhibition of LD biogenesis. Importantly, both DGAT-mediated LD synthesis and autophagy were essential for HeLa cell survival during acute starvation. Surprisingly, we found that increased DGAT-dependent LD accumulation occurs also after a prolonged (5-day) serum deprivation. However, blocking autophagy led to a further increase in LD content, indicating that it participates in the breakdown of LDs (via "lipophagy") under these conditions. Altogether, our results suggest that, depending on the specific nutrient stress conditions, autophagy drives LD biogenesis and/or LD breakdown and that this relationship is essential for the protection of cancer cells against metabolic stress. *The authors marked with an asterisk equally contributed to the work.

P-09.2-02
Store-operated and receptor-activated calcium entry in rat podocytes with type 2 diabetes

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One of the most common and severe complication of diabetes mellitus is diabetic nephropathy. It is characterized by proteinuria developed mainly due to the changes of podocyte number and disturbances of podocyte structure. Recent data suggests that altered store-operated calcium entry is associated with diabetic complications. The main focus of our current study was the influence of type 2 diabetes on calcium signaling in isolated rat glomerular podocytes. To induce type 2 diabetes Wistar rats were fed with a high-fat diet for 15 weeks with a one-time injection of 20 mg/kg streptozotocin after 9 weeks. This protocol resulted in impaired glucose tolerance in rats, increased glucose level, increased renal/creatinine clearance. For current studies we isolated rat glomeruli by standard sieving protocol and plated them on glass coverslips coated with poly-Lysine. Glomeruli were loaded with 2 μM fura-2 AM calcium indicator. Calcium imaging demonstrated that podocytes in glomeruli from diabetic rats have lower level of store-operated $Ca2^+$ entry induced by 2 μM thapsigargin application (ΔF: 0.49 ± 0.03 vs. 0.38 ± 0.02 a.u. for control and diabetic rats, respectively). At the same time calcium basal level and receptor-activated calcium entry induced by angiotensin II were unchanged (Fb: 0.61 ± 0.01vs. 0.6 ± 0.01 and ΔF: 0.13 ± 0.01 vs 0.16 ± 0.01 for control and diabetic rats, respectively). Thus our results demonstrate that type 2 diabetes could impair $Ca2^+$ signaling in podocytes. This could be one of the step towards disturbances in glomerular filtration barrier and developments of diabetic nephropathy. This work was supported by the RSF №19-14-00114.

P-09.2-03
Role of transmembrane domain in alkali-sensing insulin receptor-related receptor phosphorylation

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The insulin receptor (IR) family consists of IR, insulin-like growth factor receptor (IGF-IR), and insulin receptor-related receptor (IRR). All three receptors are highly homologous receptor tyrosine kinases (RTK) with a single transmembrane (TM) segment. RTK are known as key regulators of critical cellular processes. IRR functions as an extracellular alkali sensor involved in the regulation of the acid–base balance. The qualitative analysis of IRR/IR chimeras showed involvement of several extracellular domains in IRR alkali sensing and our recent SAXS analysis of soluble IRR ectodomain revealed asymmetrical drop-like shape of IRR ectodomain wherein two IRR TM domains are located close to each other. These data raise the question of the role of TM domains in IRR activation by alkali. In order to study the role of the TM-domain in pH-dependent activation of the receptor IRR, we obtained several mutants of IRR containing single- and double-point mutations in TM region based on the recently described oncogenic mutations in human ErbB2 receptor. Mutations P928A, T927I-G930I, F942I-F943I, did not affect the character of the receptor activation, but double substitution V929E-G930R led to strong basal receptor phosphorylation at pH 7.4. The similar picture we observed for double mutant A938E-A939R. Mutant A938E-A939R was phosphorylated at neutral pH and retained pH sensitive property. For the

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correct interpretation of these data, we characterized the spatial structure of IRR TM domain embedded into membrane mimicking micelles using high-resolution NMR and carried out the molecular modeling of the TM domain dimerization with some mutations in a lipid bilayer, revealing a pivotal role of the TM domain conformation in molecular mechanism of the IRR activation. The reported study was funded by RFBR grants № 20-04-00880, 20-04-00959, 19-04-01042, 19-34-51034 and by Russian Science Foundation (project № 19-74-30014). *The authors marked with an asterisk equally contributed to the work.

P-09.2-04
Peptide amyloid-beta affects the amount and rate of ATP production by human mitochondria according to luciferin-luciferase assay

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The ATP amount and rate of ATP production by human mitochondria are decreased in presence of Aβ(1–42) peptide, which is known that involved in pathogenesis of Alzheimer's disease. The 1st experimental group of SH-SY5Y human neuroblastoma cell line were cultivated 24h (~90% confluence) with 200 nM Aβ monomerased by standard procedure (Jao 1997, Džinić 2018), 2nd group (control) of cells had no additives, and the 3d group contained 0.1% DMSO (this amount of DMSO were added to the 1st group with Aβ). Mitochondria were isolated by standard procedure (Martin 1998, Daum 1982). To isolated mitochondria were added substrates of Complex I, II and IV and corresponded inhibitors of OxPhos Complexes – in separate experiments to highlight the changes of activity of each Complex induced by Aβ. Immediately before the measurements of luminescence to mitochondria were added ADP and luciferase-luciferin mixture (Ugarova et al. Patent RU 2420594, 2011). The luminescence (RLU) depending on time was plotted for each experimental group. The maximum values of the first derivatives of the left sides of the obtained bell shaped curves (the rise of the ATP production) were compared (the rate of ATP production). Also were compared the the peak heights (corresponded to the amount of the produced ATP). The presence of DMSO led to the slight lowering of ATP production by human mitochondria – amount on 9.3% and rate on 6.6% if compare with the control group (mean value for the Complexes I, II, IV). Cultivation of cells with Aβ makes mitochondria to produce 26.3% lower ATP (if compare with the group treated with DMSO) with 48.3% lower rate. Widely used the methods which include the disruption of cells or mitochondria to measure the total amount of ATP molecules in the lysates (Drew 2003, Gao 2009, Lim 2011). But we suppose that the plotting the level of luminescence (ATP concentration) in time can give more information. This work is supported by 20-015-00526 RFBR. *The authors marked with an asterisk equally contributed to the work.

P-09.2-05
Affine separation exosomes and influenza A virus virions from infected cells.

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Exosomes are extracellular membrane vesicles with a diameter of 40–100 nm. They are a part of the cell secretome and they have a great significance in intercellular communication. Exosomes transport a wide range of biologically active molecules, including lipids, proteins, mRNA, and microRNA. The transfer of these molecules to the recipient cells can regulate the cell's normal functionality, but also contribute to the pathogenesis of many diseases. In this investigation we compared the efficiency of three different methods for exosome isolation from the cell culture medium: 1) ultracentrifugation, 2) concentration by tangential flow filtration followed by gel-filtration, 3) exosome precipitation by a commercial reagent. Using transmission electron microscopy, Western blot, and dynamic light scattering, it was shown that the gel-filtration of a pre-concentrated sample is the optimal method for isolating exosomes from the culture medium. This approach provides a significantly higher purity of exosome preparations compared to the rest methods. To study exosomes from influenza A virus-infected cells, additional separation of the exosomes themselves and the virus particles is required. For this task, affinity chromatography using monoclonal antibodies to influenza A virus hemagglutinin was applied. The effectiveness of this method was evaluated by electron microscopy and electrophoretic analysis of the obtained chromatographic fractions. Thus, we have developed and optimized approaches for the isolation of exosomes from the culture medium and the separation of exosomes and virions after IAV infection. The obtained highly purified preparations of cellular exosomes are suitable for further investigation of their proteome and transcriptome, as well as the possible involvement of exosomes from infected cells in the disease pathogenesis. The study was supported by RSF project 20-15-00228.

P-09.2-06
How lipids affect the degradation of food allergens

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Bet v 1 homologues and LTPs are the key plant allergens which bind and transfer lipid ligands due to the presence of an internal hydrophobic cavity. Recent data show that protein-ligand interactions affect their properties and allergenic potential including the degradation in human gastrointestinal tract. But much is still unclear. To clarify the situation, we investigated the effect of lipids on the degradation of soy Gly m 4 and peanut Ara h 9 as model proteins. Various fatty acids (FAs) and lysolipids were

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used as possible ligands of these allergens. Simulation of gastrointestinal digestion of the allergens *in vitro* was performed. Gly m 4 showed a high susceptibility to pepsin while Ara h 9 degradation occurred only with the subsequent action of a mixture of trypsin and α-chymotrypsin. Both allergens bound all ligands with different efficiencies in experiments with TNS displacement. The presence of FAs and lysolipids to varying degrees increased resistance of Gly m 4 and Ara h 9 to pepsin or trypsin/α-chymotrypsin degradation, respectively. Analysis of the spatial structures of digestive enzymes revealed the presence of hydrophobic areas on their surface. Bioinformatic approach demonstrated a possible interaction of some lipids with active centers of trypsin and α-chymotrypsin, but not pepsin. All proteases bound lipids with different efficiencies *in vitro*. Some FAs and lysolipids inhibited the cleavage of non-lipid-binding proteins (cytochrome c and α-casein) and chromogenic substrates by digestive enzymes. Thus, in human gastrointestinal tract lipids from digestive juices or foods may bind to lipid-binding allergens changing their structure and digestibility. On the other hand, lipids may interact to digestive enzymes reducing their activity. Therefore, lipids may affect the degradation of not only lipid-binding food allergens and these effects depend on their physicochemical properties, concentration and behavior in physiological solutions. The work was supported by the Russian Science Foundation (project no. 20-45-05002).

P-09.2-07
Influence of mitochondrial pore modulators (cyclosporine A, anthimicin A) on oxidative processes in the wheat seedlings (*Triticum aestivum* L)

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Programmed cell death plays a significant role in plant development and aging programmers, in response to environmental stresses, in hypersensitive reactions, in defense against pathogen attack. Mitochondria have an important function in programmed cell death (PCD) in plants as well as animals. Cyclosporin A (CsA) is a well-known inhibitor of the opening of the mitochondrial permeability transition pore (PTP), inducing mitochondrial Ca release, and apoptosis in mammalian cells. Antimycin A (AA) is a fairly potent mETC inhibitor, which disrupts the electron flow from cytochrome b to cytochrome c1 in complex III (cytochrome c oxidoreductase) and is a modulator of the alternative respiratory pathway (AP) in plants. The impact of cyclosporin A and antimycin A on the rate of superoxide anion ($O2^{\cdot-}$) generation, membrane permeability, and MDA production was studied in the first leaves and coleoptiles of wheat seedlings (*Triticum aestivum* L.) as model systems of developing and senescent organs. The $O2^{-}$ generating rate was determined by nitroblue tetrazolium (NBT) staining, with some modifications. Permeability of the cell membranes was measured by detecting electrolyte leakage (EL), MDA content was determined by the thiobarbituric acid (TBA) reaction. We show that CsA, an inhibitor of the permeability transition pore of animal mitochondria, strongly decreases the reactive oxygen species production in investigated organs of plants, stabilizes the cell membranes, and inhibits the programmed cell death, DNA fragmentation in first leaves and coleoptiles. But antimycin A significant increase in the $O2^{\cdot-}$ generating rate in wheat seedlings depending on different stages of development and membrane permeability and MDA production increased under AA treatment depending on the stage of development. The role of the alternative respiratory pathway (AP) in oxygen species production in plants is discussed. *The authors marked with an asterisk equally contributed to the work.

Lipidomics

P-09.3-01
Ancestral paternal exposure to high-fat diets causes testicular metabolic and functional disturbances in mice up to two generations

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The onset of overweight and type 2 diabetes occurs at ever younger age, raising concerns regarding the metabolic health of the offspring due to possible transgenerational effects of high-fat diet (HFD). Herein, we describe the effects of HFD, even if transient, in glucose homeostasis and in testicular metabolome, lipidome and function of the offspring (sons and grandsons). 3 groups of 12 mice/each were randomly assigned to distinct diet regimen after weaning: CTRL–standard chow; HFD–high-fat diet; HFDt–HFD for 60 days, then replaced by standard chow. Animals had unrestricted access to water and food until sacrifice, at 200 days post-weaning. Body weight was monitored. Testis, serum and sperm were collected after sacrifice. Glucose homeostasis was assessed according to HOMA2 metrics. Sperm quality, testicular metabolome (^{1}H-NMR) and lipidome (GC-MS) were characterized. At 120 days post-weaning, males were mated with normoponderal females (1:1) to obtain the F_1 generation (sons), later used to obtain the F_2 generation (grandsons), in the same conditions. The descendants were all fed with standard chow and procedures were repeated. No evidence of metabolic syndrome was found in sons and grandsons of HFD-fed mice. However, the grandsons of mice fed with HFD, even transiently, had lower sperm concentration and higher prevalence of sperm defects than the grandsons of mice fed with standard chow. Using sPLS-DA, we found distinct testicular metabolome and lipidome profiles between the 3 diet regimens, notably in the diet-challenged mice and their grandsons. The differences are mostly explained by metabolites related to energy production and lipid remodelling (glutamine, acetate, inosine) and, in grandsons of lifelong HFD mice, to an increased proportion of ω6 fatty acids.

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Therefore, ancestral HFD causes testicular metabolic signatures in the progeny up to two generations which are correlated to testicular dysfunction, expressed as poorer sperm parameters.

P-09.3-02
Lipidomic analysis of different morphological forms of *Mycoplasma hominis*

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Mycoplasmas are parasites of almost all living organisms. They are small, wall lacking bacteria with reduced genome and deficiency of many metabolic pathways. *Mycoplasma hominis* is considered to live in the urogenital tract of human and characterize by "fried egg" colonies on a solid culture medium. Recently, the unusual morphological form of mycoplasma was discovered. *M. hominis* forming non-typical, micro colonies was isolated from clinical samples of the serum of patients with inflammatory diseases of the urogenital tract. These colonies are tiny propeller-shaped colonies and resistant to antibiotics against classical form of *M. hominis*. These differences from usual colony of mycoplasma may occur due to changes in cell surface. The aim of this work is lipidomic analysis of the *M. hominis* cytoplasmic membrane for the classical cultivated form and a new discovered potentially persistent in the host organism. Studying the lipid composition was made for the laboratory strain *M. hominis* H-34 in both morphological forms obtained in vitro. Lipids was extracted using MTBE-methanol method and studied by LC-MS analysis. As the result, we obtained a lipidomic profiling for both morphological form of *M. hominis* and determined the difference in lipid compositions between them. Mycoplasma is known to be unable to synthesize fatty acids and takes them from the host or culture medium. The major lipid differences between morphological forms are in glycerolipids and glycerophospholipids classes that involved in lipid bilayer formation and metabolism and responsible for host-pathogen interactions. This work was supported by Russian Science Foundation 19-75-10124 «Mycoplasma hominis adaptation mechanisms to new niches in the host organism and the formation of its resistant persistent form».

P-09.3-03
Toxicity of trans fatty acids (TFAs) and its correlation with ceramide and diglyceride accumulation

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Detrimental effects of excessive free fatty acids (FAs) on various cell types have been intensively studied in the past decade. This lipotoxicity is largely due to an increased intracellular acyl-CoA supply and the consequent accumulation of biosynthetic lipid intermediates, such as ceramides and diglycerides (DG). The deleterious effects of saturated palmitate (16:0) exceed those of cis-unsaturated oleate (18:1 cis-Δ9), moreover simultaneous addition of oleate can lessen palmitate toxicity. While this effect of oleate is widely investigated, very little data is available on the cell damages caused by elaidate (18:1 trans-Δ9) and vaccenate (18:1 trans-Δ11), although the potential health effects of these dietary trans FAs (TFAs) received great attention. In this study, we aimed to investigate the effects of these four FAs alone (Sarnyai F et al. (2019) Food Chem Toxicol. 124, 324–335) or in combination treatments, at high concentrations (250–500 μM) on cell viability, apoptosis, ER stress, JNK phosphorylation and autophagy, in RINm5F insulinoma cells. FA profile and ceramide and DG contents of the cells were determined by using GC-FID and HPLC-MS/MS analysis. We observed a marked toxicity of palmitate, while the three unsaturated FAs (UFA) were found to be scarcely toxic. Palmitate also caused a several fold increase in both ceramide and DG levels, while much smaller elevations were induced by the UFAs. Palmitate toxicity and palmitate-induced ceramide and DG build-up were attenuated by a simultaneous addition of either of the UFAs. It is noteworthy, however, that incorporation of TFAs in ceramides was markedly more pronounced than that of oleate, and discernable differences between TFAs and oleate could be observed at the co-treatments, too. Our findings do not support a short term toxicity of TFAs in insulinoma cells; nevertheless, they revealed some metabolic characteristics that might underlie a long term toxicity and hence deserve further investigation.

P-09.3-04
The molecular crosstalk between diminished cholesterol synthesis and chronic liver disease in mice and humans

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Non-alcoholic fatty liver disease (NAFLD) is a complex liver condition starting with metabolic alterations and simple steatosis that could progress to advanced stages, such as non-alcoholic steatohepatitis, fibrosis, cirrhosis and even hepatocellular carcinoma. We describe a new molecular mechanism initiated by disrupted late cholesterol synthesis leading to fibrosis and

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hepatocarcinogenesis. We used the mouse model with hepatocyte deletion of lanosterol 14α-demethylase (CYP51) ($H^{Cyp51-/-}$), which is involved in cholesterol biosynthesis. Disturbance of cholesterol metabolism in progressed chronic liver disease in 4 different genetic mouse models (Glmp, Rbpj, Ikbkg and Abcb4) and also in humans was identified by functional comparative analysis of relevant public gene expression datasets and compared to findings in the $H^{Cyp51-/-}$ model. Analyses revealed that the disturbed hepatocyte cholesterol homeostasis caused liver injuries with sustained inflammation, fibrosis, changed lipid plasma parameters and a widespread oval cell response, which progressed towards tumorigenesis. Furthermore, these metabolic liver injuries caused pronounce liver damage in female mice confirming sex-specific mechanisms in development of liver disease. The network-based analyses revealed common transcriptome signatures between the models with high similarity to human NAFLD, even though different genes in selected models are knocked-out, and different pathways in liver chronic pathologies were initiated. Comparison of transcriptome data between the models, completed with genome-scale metabolic models (GEMs) also revealed new insights in understanding of the complexity of this disease and its progression. All mouse models show common metabolic rearrangements, with negatively enriched metabolic processes (including cholesterol and bile acid synthesis) and up-regulated cancer transcriptional regulators (JUND, cETS1 and SP1) that can be closely associated with mechanism of NAFLD induced tumorigenesis *The authors marked with an asterisk equally contributed to the work.

P-09.3-05
Palmitate inactivates insulin signaling but stimulates basal glucose uptake in 3T3-L1 adipocytes

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Obesity is the major risk factor for T2DM, which is thought to be due to increased levels of circulating free fatty acids (FFA). FFA interfere with insulin signaling in hepatic and muscle cells leading to insulin resistance and hyperglycemia. However, the short-term effects of increased FFA on insulin-sensitive adipocytes are less clear, given the notion that FFA-induced insulin resistance in adipose tissue may involve the long-term latent inflammation. To address this issue, we treated mature 3T3-L1 adipocytes with increased concentrations of the typical FFA, palmitate (0.3-1 mM), and measured 2-deoxyglucose (2-DG) uptake both under normal (5 mM), and hyperglycemic (28 mM of glucose) conditions in the absence and presence of insulin. To monitor insulin cascade and AMPK activities, phosphorylation levels of critical components of insulin signaling (IRS, Akt, AS160) and acetyl-CoA carboxylase (ACC) were determined in parallel by western blots. We found that under all experimental conditions palmitate dose-dependently increased basal 2-DG uptake, whereas the insulin-stimulated uptake was only significantly reduced by high palmitate (1 mM), which is typical for severe diabetic conditions. Yet, the unstimulated uptake remained high despite a complete loss of the insulin cascade and AMPK activities, and unaltered levels of Glut-1 and Glut-4 expression. These results argue against the hypothesis of reciprocal use of the energy substrates (glucose vs. FFA) as the mechanism of insulin resistance development in fat cells, like it was originally proposed by P.Randle for muscle cells. Similarly, that FFA inactivate insulin signaling and AMPK in adipocytes may not account for inability of fat cells to accumulate glucose per se. These results will be added by and further examined against the ability of palmitate to recruit and/or activate glucose transporters and unexpectedly involve the eNOS/NO pathway to regulate adipose glucose uptake. Supported by RFBR grant #17-04-02225.

P-09.3-06
Serum free fatty acids and bilirubin concentration in Type 2 diabetic subjects from Bosnia and Herzegovina

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Serum bilirubin concentrations show antilipolytic effect on free fatty acids metabolism (FFA), and there are indications that FFA levels interfere with bilirubin metabolism. Recent studies have reported that bilirubin is reversibly associated with increased adiposity and impaired insulin sensitivity caused by elevated FFA levels and that increased concentrations of these bioactive lipid metabolites are significantly associated with the risk of developing Type 2 Diabetes (T2D). The aim of this study was to examine the potential association of serum concentrations of various/different types of FFA (saturated, SFA and unsaturated, UFA) and bilirubin with T2D.This study included 145 participants, of which 54 were controls, 55 T2D patients, 12 prediabetics, and 24 were non-treated/newly diagnosed with T2D, both gender. Bilirubin level was measured with the vanadate oxidation method, using the chemical analyzer VITROS 350, while FFA levels were determined by gas chromatography analysis. Our results showed a significant difference in levels of glucose, glycated hemoglobin (HbA1c), bilirubin, lipid profile (cholesterol, triglycerides, HDL, LDL), myristic acid (C14:0) and gamma-linolenic acid (C18:3) between all study groups ($P < 0.05$). There was a positive correlation between the levels of bilirubin and triglycerides ($P < 0.01$). Interestingly, our results demonstrated a strong negative association between serum levels of bilirubin and myristic acid, C14:0, ($P < 0.001$) and positive association of bilirubin with dihomo-gamma-linolenic acid (C20:3) as well as docosapentaenoic acid (C22:5) ($P < 0.05$) in all participants. It appears that elevated concentrations of bilirubin and FFA of different chain length and saturation are associated with the T2D traits and could be used as potential T2D biomarkers and therapeutic targets.

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P-09.3-07
Role of cholesterol in COVID-19 disease and in the SARS-CoV-2 spike interaction

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Facing SARS-CoV-2 requires rapid development of effective diagnostic and antiviral therapeutic agents. Changes in lipid metabolism have been previously reported during viral infections (e.g. HBV, HCV, HIV), where a specific lipid profile of the host could serve as a biomarker. Besides being a fundamental lipid component of vertebrate cell membranes and participating in numerous physiological processes, cholesterol is being recognized as a molecule involved in regulating SARS-CoV-2 entry. Generally, higher membrane cholesterol coincides with higher efficiency of coronavirus entry, while disruption of cholesterol-enriched lipid rafts by lipid-lowering treatment affects their infectivity. Moreover, studies on COVID-19 patients show decreased levels of serum total cholesterol, HDL- and LDL-cholesterol, but the role of cellular cholesterol synthesis has not yet been thoroughly investigated. Herein we focus on serum cholesterol and its intermediates in patients hospitalized due to COVID-19. Samples were taken at three-time points during hospitalization, to contrast the disease severity in individual patient. We aim to evaluate whether the sterol-related biomarkers can be used for the prediction of the disease progression or outcome and whether the cholesterol and its sterol intermediates affect the entry of SARS-CoV-2. Initial results indicate that cholesterol precursors (i.e. lathosterol) correlate with COVID-19 severity in the Slovenian cohort. The analysis of the targeted cholesterol-related RNAs from patients' serum is in progress. The role of cholesterol and other sterols in SARS-CoV-2 spike protein interaction is studied in immortal cell lines with impaired cholesterol synthesis. Uncovering to which extend the blood and membrane-bound cholesterol imbalance relates to COVID-19 pathology will give valuable insight also regarding the suitability of treatment of patients with lipid-lowering drugs.

P-09.3-08
Mechanisms of glaucoma: the insights from lipidomic studies

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Glaucoma is a severe ocular disease associated with abnormalities in aqueous humor (AH) circulation and an increase in intraocular pressure (IOP) leading to damage to retinal ganglion cells, progressive optical neuropathy, and loss of vision. The common idiopathic form of the disease is primary open-angle glaucoma (POAG) characterized by complex pathophysiological mechanisms involving oxidative stress, excitotoxicity, and other factors, which can contribute to IOP increase and ganglion cell death. In this study, we report novel insights into the pathogenesis of POAG gained from targeted lipidomic studies focusing on lipid mediators – omega-3 and omega-6 polyunsaturated fatty acids, and their derivatives oxylipins. Previous studies indicated that some of these compounds (prostaglandins) regulate AH outflow, and therefore can be employed for the treatment of glaucoma. Using biological samples collected from a cohort of POAG patients and healthy individuals, we examined alterations in the content of polyunsaturated fatty acids and oxylipins in AH accompanying IOP growth, and progression of the disease. A total of 19 signaling lipids were identified, including three polyunsaturated fatty acids, fourteen oxylipins, and two phospholipid derivatives. Despite the variety of the identified mediators, the POAG-related alterations were provided by a small set of these compounds, the pattern of which pointed to oxidative stress as a critical pathological factor in POAG. Interestingly, similar lipidomic alterations were found in tear fluid of the same patients indicating a diagnostic value of its content in glaucoma, especially since this eye liquid can be collected using a non-invasive procedure. This study was supported by the Russian Science Foundation (Project no. 21-15-00123).

P-09.3-09
Investigation of o-alkyl/o-alkenyl glycerolipid ratios in erythrocyte lysates of patients with obstructive sleep apnea

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Vulnerabilities of plasmalogens in erythrocyte membrane to hypoxic conditions and alteration induced by sleep restriction make them potential biomarkers for the evaluation of sleep apnea related sleep disorders. To investigate and to compare ratios of alkyl/alkenyl glycerolipids of erythrocyte membranes (representing plasmanyl/plasmenyl plasmalogens) between controls and patients with sleep apnea and to evaluate possible differences, if any, which could be considered as a diagnostic tool. The patients underwent polysomnography and categorized according to the severity of sleep apnea using Apnea-Hypopnea Index (AHI). Phospholipids in erythrocyte lysates were hydrolyzed by phospholipase C and the acylgroups were saponified. Then the O-alkyl/O-alkenyl glycerolipids were separated by thin-layer chromatography (TLC). Densitometric image analyses were performed on the lipid spots of TLC plates and the ratios were determined. No significant correlationwas observedbetween the alkyl/alkenyl glycerolipid ratios of the eryhtrocyte lysates of sleep apnea patients and that of controls. Results of this study warrants reinvestigation of alkyl/alkenyl glycerolipid ratios in erythrocytes of patients with sleep apnea, with each subjects sampled before and after the treatment of sleep apnea to better understand the potential of this ratio as a diagnostic tool.

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P-09.3-10
Alterations in lipid mediators of inflammation associated with glaucomatous damage in a rabbit model

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Ocular inflammation may contribute to the pathogenesis of a number of blind-causing retinal disorders, including age-related macular degeneration and diabetic retinopathy, and may play a role in glaucoma. It can manifest in the aqueous humor (AH) as alterations in the content lipid mediators regulating inflammation and resolution pathways as well as neuronal survival. In this study, a model of glaucoma was induced in rabbits and the developing pathological process was characterized on clinical and electrophysiological levels as well as by lipidomic analysis of AH. Chronic intraocular hypertension was stimulated by single or double (with an interval of one week) injections of 2% methylcellulose in the anterior chamber. The intraocular pressure peaked at 4–5 hours after each injection and remained elevated for at least ten days yielding glaucoma-like neuronal damage as evidenced by electroretinography. The acute increase in intraocular pressure was accompanied by hyperemia of the precorneal vessels and corneal edema. The use of targeted lipidomic technique employing ultra-performance liquid chromatography-tandem mass spectrometry allowed identifying patterns of signaling lipids of AH, including polyunsaturated fatty acids, oxylipins, and phospholipid derivatives. The revealed glaucoma-related alterations in AH lipidome were indicative of oxidative stress and apparently low-grade ocular inflammation. Taken together, these findings provide a rationale for trialing a combination of antioxidant and anti-inflammatory therapies as a prospective route in the complex treatment of glaucoma. This study was supported by the Russian Science Foundation (Project no. 21-15-00123).

P-09.3-11
Development of a laser dissection-coupled quantitative microlipidomic method

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Lipid metabolic reprogramming is a newly recognized hallmark of malignancy. Most normal cells build up their membranes from dietary lipids. In contrast, cancer cells re-activate the de novo lipogenesis, which is also promoted by oncogenic signaling. Thus, the lipid metabolic network is flexible and tuned to fulfil the requirements of cancer cell. Tumors resemble complex organs, consisting of tumor cells and highly heterogeneous host-derived stroma. Because the communication between tumor and stromal cells influence the aggressiveness and metastatic potential of tumors, the exploration of tumor heterogeneity is of great interest but also a great challenge. Currently available data on lipid metabolic reprogramming derive from measurements conducted in 2D cell cultures (unable to mimic metabolic zonation) or they examine relatively large tumor pieces (low spatial resolution). Mass spectrometry imaging techniques are capable of high spatial resolution but do not provide quantitative data and have limited lipidome coverage. Here, we present the development of a novel laser dissection-coupled microlipidomic method including preparation of parallel native and hematoxylin-eosin-stained cryosections from spheroids and tumor samples, AI-driven co-registration of stained and autofluorescence images, laser dissection of marked regions (100–150 cells), microextraction and mass spectrometry analysis. By using this workflow, we could identify a radial gradient in the lipidomic profile of 3D spheroids, whereas the reproducibility of matching areas from parallel sections was excellent. We could also dissect different tumor regions (cancer and stromal cells) from mouse allografts and human tumors. In summary, the novel approach ensures high throughput, semi-automatic analysis of the lipidome, provides quantitative data with broad-range coverage and good spatial resolution, thereby enabling an unbiased, hypothesis-generating research. Funding: 2020-1.1.2-PIACI-KFI-2020-00079

P-09.3-12
Metformin exerts dual effect on pancreatic β-cells through downregulation of stearoyl Co-A desaturase 1

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Progressive loss of pancreatic β-cell function which results from lipotoxicity is central to the development of obesity related type

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2 diabetes (T2D). Stearoyl-CoA desaturase 1 (SCD1) is a pivotal enzyme involved in saturated fatty acids (FAs) metabolism that exhibits a protective role against lipotoxicity in pancreatic β-cells. SCD1 deficiency leads to accumulation of toxic lipid species, diminished insulin secretion and β-cell exhaustion. Our recent research showed that AMP-activated protein kinase (AMPK) mediates some of the effects of SCD1 in pancreatic β-cells, thus indicating SCD1 as a potential target for metformin - antidiabetic drug, which is also a potent activator of AMPK. Therefore, the present study aims to determine whether metformin affects FAs metabolism and insulin secretion in pancreatic β-cells in SCD1-dependent manner. The experiments were performed in INS-1E pancreatic β-cell line, treated independently or in combination with metformin and palmitate (16:0). Assessment of overall lipidome showed, that treatment of pancreatic β-cells with metformin can prevent these cells from lipid accumulation by downregulation of lipogenesis. Such an effect was linked to decreased expression of SCD1 and sterol regulatory element-binding protein 1 (SREBP-1). Moreover, metformin partially restored insulin secretion in β-cells overexposed on 16:0. However, in control conditions, metformin treatment led to impairment of the secretory capacity of β-cells, that was related to SCD1 deficiency. Dysfunction of pancreatic β-cells after metformin treatment was also followed by changes in abundance of the transcription factors involved in maintenance of β-cell identity such as PDX1, FOXO1, ISL1. Altogether, these findings provide additional mechanistic insight toward better understanding the pleiotropic actions of metformin and its role in regulation of pancreatic β-cell functioning. This work was supported by National Science Center grant UMO-2017/27/N/NZ3/01987.

Posters – Education

Educational technology and e-learning

P-E-01-1
The effect of portfolios on medical students' academic achievement and attitudes toward endocrine disruptors course

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Portfolio is an assessment method for both the teacher and the student, including the student's researches, photographs, pictures, performance assignments, projects, examples of activities and evaluation papers. Although it was used as an alternative measurement and evaluation tool in the past, it was later used as an instructional material and teaching method. The aim this study was to examine the effect of portfolios on students' academic achievement and attitudes toward endocrine disruptors chemicals course of undergraduate medical students. The study was conducted with 63 third year students (22 of students studying in Turkish language and 41 of students studying in English language) at Faculty of Medicine of a state university in fall semester of 2020–2021 academic year. A pre-test post-test single group research design was used to investigate the effect of portfolios on students' academic achievement and attitudes toward endocrine disruptors chemicals course. Also open-ended questions were used to determine students' ideas on the use of portfolios in class. The implementation was continued for 14 weeks. Data were analyzed by performing dependent sample t-test and descriptive statistics. The results showed that there was a statistically significant increase in the post-test achievement and attitude scores of the students compared to their pre-test scores. Also, students had positive opinion on the use of portfolios in lecture. Students responses to the open-ended questions showed that students enjoyed using portfolios; they could think over retrospectively what they had learned and they have improved their writing skills. These results are important for the transformation of knowledge into daily life and lifelong continual behavior. Portfolios can be used to increase the academic success and attitude of university students. When we take into account the pandemic and online education, the portfolio study is the bridge between us and students.

P-E-01-2
Remote teaching for small project-based classes: case reports

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Running advanced-level courses for a limited number of students involves making a lot of individual assignments, therefore to transfer such courses online rapidly appeared to be a much bigger issue compared to lecture-based courses. We would like to share our experience of how we managed to organize remote work in three different sets of classes for small groups of senior students, which we have been running for several years in different institutions, during COVID-19 pandemic. The first case is a two-term-long set of educational modules which we conduct for fourth-grade bachelor students. This set includes introductory bioinformatics, biostatistics, population genetics and biotechnology, and two main types of assignments in these modules are small consequent projects (either computational or in the wet lab) and colloquia. The second case is a statistics class in a one-year-long educational program in bioinformatics; small projects are a big part of this class as well. The third case is a biotechnology class for first-year master students, in which we had to cancel wet lab practices. While organizing remote work for these three classes, we tried different elements of the "flipped classroom" approach, either successfully or not. The main issue was to engage students to participate in discussions actively, even though students said they liked webinars and oral colloquia more than written assignments. However, students preferred to run their computational projects remotely. Interestingly, some of the techniques that we had to use during remote teaching seem to be applicable and useful for offline classes, too. *The authors marked with an asterisk equally contributed to the work.

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Innovations and good practices

P-E-02-1
Combining spaced learning and team-based learning to promote students motivation, involvement and satisfaction – a pilot study

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Spaced learning (SL) and team-based learning (TBL) approaches were combined as teaching/learning (T/L) strategies in the discipline of Integrative Biochemistry in the Biomedical Sciences Bachelor at University of Aveiro, as a pilot project. I decided to follow the trend of passing from the strict memorization of contents, a normal strategy used in a biochemistry theoretical discipline, for an active T/L strategy. This change is essentially influenced by the growing amount of information available online, which leaves more space for knowledge application activities. The main objective of the innovative pilot project was to promote motivation, involvement and student satisfaction while learning, in a 3-hour class with theoretical schooling, through the fulfillment of the following goals: implement active learning strategies; combine SL and TBL; develop collaborative and interdisciplinary practices among a group of colleagues; collect and analyze evidence on the pedagogical practices and share the results with the academic community. Briefly, a typical class comprised several stages: students had previous access to the bibliography of the contents covered during class; in class, students took individual questionnaire (IQ), followed by group questionnaire (HQ). These activities were followed by an interval. After, they applied the learned contents to a practical case and a second break follows (physical activity). Finally, there was a content review and clarification of doubts. Different colleagues collaborate with the physical activities and the introduction of questionnaires on the online platform, management, and treatment of the results. The main lessons learned from the pilot to improve the designed T/L strategies, according to student and teacher feedback, were the need to: prepare more concise content for the preclass study; optimize the time spent on carrying out IQ and HQ; change the assessment mode; listen to students in the development of the discipline.

P-E-02-2
An approach to scientific dissemination: from the academic laboratory to the society

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In the last years, European research and innovation projects (e.g. Horizon 2020) make the results dissemination to the public an important goal to be taken into consideration. A good communication outline can increase the impact of the scientific results as it leads to public awareness and it enhances the visibility of the whole research programme. Our group belongs to "Universidad Complutense de Madrid" and works for the development of advanced microalgae technologies for a circular economy. In the last two years, we have developed different initiatives for spreading our results with bacteria and our work at the University to different social sectors: from social networks (for instance Instagram and YouTube channels) to practical workshops to pregraduate students and interactive talks to non-scientific people in events such as The Science Week, AULA and The European Night of Researchers. During the 2020, all our activities were adapted to be on-line. We present in this work these initiatives and the pros and cons we have faced in these kind of dissemination activities. We concluded that activities which contained a guided practical learning were more accepted and enjoyed in all age ranges than those that were based only in the state of art and theoretical concepts.

P-E-02-3
Boosting the learning of biotechnology by concept-based teaching

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The most frequent method of students' teaching is based on study subject/courses . Each course is focused at a different field of knowledge e.g. biochemistry, molecular biology, biophysics. In this traditional teaching method, students are assessed in each course separately. An alternative method of students' teaching is based on "big ideas" and is called concept-based teaching. In this teaching strategy, courses are built around particular topics and combine knowledge from different fields traditionally taught at separated courses. We adopt concept-based teaching strategy and have entirely reorganized the first level study (Bachelor's degree) program. Instead of conventional teaching (subject oriented), we prepared new innovative program, based on the six main ideas – Thematic Modules, one for each semester. In each Module, students focus on acquiring knowledge and practical skills aimed at understanding and combining facts into a coherent logical whole concerning a specific issue, e.g. Biomolecules, Unicellular organisms, or Biotechnology in medicine. Progress in learning is assessed during the semester, (small pieces of material), and at the end of the Module, during the examination session with only one exam (the so-called integrating exam), covering material from the whole Module. After the first year of operation of new studies program, we observed that more students achieved learning outcomes, as well as obtained better grades in material related to biophysics and biochemistry of biomolecules, which were for many students difficult to achieve within traditional teaching. Because the student's overall progression is assessed, without breaking down into subjects, found in traditional teaching, the students can make the most of their strengths to overcome weaknesses in certain areas. More reliable conclusions of the functioning of the new teaching system will be determined after its longer operation, however the preliminary data are very encouraging. *The authors marked with an asterisk equally contributed to the work.

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