THE PROGRAMME AND ABSTRACT BOOK

XVth INTERNATIONAL CONFERENCE OF THE LITHUANIAN BIOCHEMICAL SOCIETY

DUBINGIAI, JUNE 26-29, 2018

Welcome word

Dear Colleagues, Members of the Lithuanian Biochemical Society and Guests,

On behalf of the conference organizing committee I would like to welcome you to the XVth International Conference of the Lithuanian Biochemical Society (LBS).

The LBS unites biomedical and life sciences researchers and students, from academia and industry. With the emergence of the new Life Sciences Center, as of March 15, 2016, recently established in Vilnius, and together with the rapidly growing Biotech industry, the society occupies an important role among the scientific societies in Lithuania. Currently there are over 300 members of the LBS.

This year, for the first time, under the initiative of the Federation of the European Biochemical Societies (FEBS), the conference is being organized together with the Latvian and Estonian Biochemical societies whose representatives are in the organizing committee and have significantly contributed to the scientific program.

We are delighted to welcome you in Dubingiai, a beautiful place in Lithuania, and hope that you will enjoy both the scientific sessions and the cultural and social program.

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Organizers thank the sponsors for the generous support of the conference:

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Lithuanian Biochemical Society AWARDS 2018

Sofija Kanopkaitė – *the past long-time president of the LBS who put great efforts to build the society.*

Sofija Kanopkaitė – *ilgametė LBD pirmininkė, daug jėgų atidavusi draugijos veiklai.*

Jurgis Kadziauskas – *long-time professor of biochemistry, an author of classical „Biochemistry" textbook in Lithuanian.*

Jurgis Kadziauskas – *ilgametis biochemijos dėstytojas parašęs klasika tapusį lietuvišką vadovėlį "Biochemija".*

Awards Ceremony will be held on Thursday, June 28, 2018 at 13:40.

Best Oral and Poster Presentation AWARDS

Prizes will be awarded for:

The Best Young Researcher Oral Presentation

1st place: the sum of € 100, a certificate and sponsors gifts.
2nd place: the sum of € 60, a certificate and sponsors gifts.
3rd place: the sum of € 40, a certificate and sponsors gifts.

The Best Poster Presentation:

1st place: the sum of € 100, a certificate and sponsors gifts.
2nd place: the sum of € 60, a certificate and sponsors gifts.
3rd place : the sum of € 40, a certificate and sponsors gifts.

Winners will be selected by voting – please vote by placing your provided card to the voting box.

Awards Ceremony will be held on Friday, June 29, 2018 at 14:20.
Do not forget to vote before this time and attend the ceremony!

Programme of the XVth International Conference of the LBS

TIME	Tuesday, June 26, Young Researcher Day
10:00	REGISTRATION
Session moderators **Vaida Paketurytė, Lina Aitmanaitė**	
11:00	***Vaida Paketurytė.*** Intrinsic thermodynamics of o-S-substituted benzenesulfonamide binding to Carbonic Anhydrases
11:15	***Aivaras Vaškevičius.*** Synthesis of 3,4,5-trisubstituted-2,6-difluorobenzenesulfonamides as selective inhibitors of human carbonic anhydrase IX
11:30	***Žilvinas Dapkūnas.*** High-cell density Escherichia coli fermentation monitoring based on culture fluorescence
11:45	***Raminta Mineikaitė.*** The landscape of small non-coding RNAs in probiotic lactic acid bacteria Lactobacillus casei
12:00	LUNCH
Session moderators **Mihails Sisovs, Priit Eek**	
13:00	***Aistė Imbrasaitė.*** Characterization of monoclonal antibodies against human carbonic anhydrase IX
13:15	***Martynas Simanavičius.*** The evidence of hepatitis E virus infection in wild rats from Lithuania
13:30	***Indrė Valiulytė.*** The role of Sema3C and R745A mutant on cell motility and microcapillary formation
13:45	***Dukas Jurėnas.*** Acetyltransferase toxins – activity, neutralization, transcriptional autoregulation and how they are linked together
14:00	***Priit Eek.*** Structure and regulation of arachidonate 11R-lipoxygenase
14:15	***Greta Streleckienė.*** miR-20b acts as potential oncogenic miRNA in gastric cancer derived cell lines by targeting phosphatase and tensin homolog (PTEN) and thioredoxin-interacting protein (TXNIP) genes
14:30	***Ugne Gyvyte***. Small RNA sequencing-based miRNA profiling and functional analysis in gastrointestinal stromal tumors (GISTs)
14:45	***Rūta Urbanavičiūtė.*** Astrocytoma specific blood serum protein markers
15:00	***Viktorija Kurbatska.*** Development of the screening assay for selection of novel small-molecule inhibitors of sortase A enzyme
15:15	***Mihails Šišovs.*** Investigations of single-stranded RNA bacteriophages virus-like particles and design of novel vaccines
15:30	COFFEE BREAK (sponsors area)
16:00	**FEBS Chair of the Working Group on Integration Lecture** ***Jerka Dumic.*** Challenges in Personalized Medicine: Science, Clinics, Society and Education
16:30	Free time
19:00	DINNER
20:00 – 21:30	OPENING CEREMONY

TIME	Wednesday, June 27, LBS Conference
8:00	REGISTRATION

Session moderator **Daumantas Matulis**	
9:00	OPENING SPEECHES
9:15	**FEBS National Lecture** ***Martin Bachmann.*** Therapeutic vaccination against chronic diseases based on virus-like particles.
10:00	***Kaspars Tārs.*** Virus-like particles of ssRNA phages: tools in vaccine development against Lyme disease
10:25	***Jānis Kloviņš.*** Alterations in DNA methylation and RNA expression from peripheral blood cells in humans treated with metformin.
10:50	***Zane Kalnina.*** Relevance of tumour-associated autoantibodies in early detection and prognosis of cancer
11:15	***Vladimir Sirotkin.*** Preferential solvation and hydration of proteins in water-organic mixtures
11:40	***Mikhail Kurbat.*** Adenosine and cyclic adenosine monophosphate (cAMP) as diagnostic indicators for drug-induced liver disease (DILI) in HIV-infected patients
12:00	LUNCH
Session moderator **Jānis Kloviņš**	
13:00	***Vyacheslav Yurchenko.*** Evolution of dsRNA viruses in protists
13:40	***Vytautė Starkuvienė.*** Functional interplay of gene and pseudogene in cell
14:10	***Artūras Jakubauskas.*** Markers for prediction of early complications after unrelated haematopoietic stem cell transplantation
14:40	***Saulius Serva.*** Yeast viruses – friends or foes?
15:30	COFFEE BREAK (sponsors area)
16:00	**Poster session I**
Session moderator **Kaspars Tārs**	
17:00	***Urmas Arumäe.*** Molecular and cellular mechanisms of CDNF action
17:35	***Vello Tougu.*** In vitro fibrillization of Alzheimer's amyloid-β peptide
18:00	***Peep Palumaa.*** Cellular copper metabolism in health and disease
18:25	***Anthony Watts.*** Multiscale spectroscopies for understanding GPCR mediated cell signalling
19:00	DINNER

TIME	**Thursday, June 28, LBS Conference**
8:00	REGISTRATION
Session moderator **Ago Rinken**	
9:00	***Urtė Neniškytė.*** Phospholipid scrambling as a signal of synapti pruning in developing brain
9:45	***Vilmantė Borutaitė.*** Mechanisms of neuronal loss in Alzheimer's disease models
10:15	***Andrius Kaselis.*** PNS regeneration in 2D and 3D models
10:35	***Ago Rinken.*** Fluorescence anisotropy assay as a novel approach to characterize ligand binding dynamics to G protein coupled receptors
11:05	***Martti Tolvanen.*** Studies of the extracellular carbonic anhydrase subfamily: CA VI, IX, XII, and XIV
11:35	***Mart Loog.*** Synthetic biology: cell factories for future biotechnology
12:00	LUNCH

Session moderator **Peep Palumaa**	
13:00	***Alessandro Prinetti.*** Biochemical approaches for the study of organization and biological functions of lipid rafts
13:40	**LBS AWARDS CEREMONY**
13:55	**LBS GENERAL ASSEMBLY**
15:25	COFFEE BREAK (sponsors area)
15:45	**Poster session II**
Session moderator **Aurelija Žvirblienė**	
17:00	***Natascha Brinskelle-Schmal.*** Branched DNA technology: Great tool to measure RNA in different platforms
17:25	***Juozas Šiurkus.*** Disposable Bioprocesses for Production of High Quality Recombinant Proteins
17:45	***Piotr Tarnowski.*** When Protein Quality Matters
18:05	***Renata Gronczewska.*** BLI systems for regulated environments – providing data integrity of label-free biomolecular binding
19:00	GALA DINNER
20:00	CULTURAL PROGRAM
22:00	DJ (22:00-0:00)

TIME	**Friday, June 29, LBS Conference**
8:00	REGISTRATION
Session moderator **Saulius Serva**	
9:20	***Reza Zolfaghari Emameh.*** The targeting of β-carbonic anhydrase from Ascaris lumbricoides for treatment of ascariasis
9:45	***Gintautas Tamulaitis.*** Type III CRISPR-Cas immunity
10:15	***Miglė Tomkuvienė.*** A doubly methylated base N4,5-dimethylcytosine in DNA and its repair
10:45	***Violeta Šaltenienė.*** MicroRNA as biomarkers for non-invasive diagnostics of precancerous and cancerous colon diseases
11:15	***Jurgita Skiecevičienė.*** Standing and active human gut microbiome in pediatric and adult IBD
11:35	***Marius Dagys.*** Amperometric bioelectrocatalytic systems: new technologies and their applications
12:00	LUNCH
Session moderator **Jaunius Urbonavičius**	
13:00	***Sonata Jarmalaitė.*** The role of liquid biopsy in cancer management
13:20	***Jaunius Urbonavičius.*** Discovery of novel bacterial genes encoding the enzymes acting on modified uracil/uridine derivatives and their use for gene therapy in cancer treatment
13:40	***Vida Mildažienė.*** Plant response to seed treatment with cold plasma and electromagnetic field involves changes in seed ROS production, phytohormone amount and protein expression
14:00	***Paulius Gibieža.*** The study of post-mitotic midbody and factors controlling cell division
14:20	STUDENT ORAL AND POSTER PRESENTATION AWARDS
14:30	CONFERENCE CLOSURE

ABSTRACTS

FEBS Chair of the Working Group on Integration lecture

Challenges in Personalized Medicine: Science, Clinics, Society and Education

Jerka Dumic

Department of Biochemistry and Molecular Biology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

The lecture will be held on Thuesday, June 26, 2018 at 16:00

FEBS NATIONAL LECTURE

This year we have FEBS national lecture given by **Prof. Martin Bachmann** (Switzeland).

Vaccination against chronic diseases using virus-like particles

Martin F Bachmann

University of Bern; University of Oxford

Researchers working on the development of vaccines face an inherent dilemma: to maximize immunogenicity without compromising safety and tolerability. Early vaccines often induced long-lived protective immune responses, but tolerability was a major problem. Newer vaccines have very few side effects but can be of limited immunogenicity. One way to tackle this problem is to design vaccines that have all the properties of pathogens with the exception of causing disease. Key features of pathogens can be mimicked by virus-like particle (VLP) based delivery systems. Here we discuss the use of immunologically optimized VLPs for the generation of therapeutic vaccines.

The lecture will be held on Wednesday, June 27, 2018 at 9:15.

June 26, Young Researcher Day
Oral presentations

Intrinsic thermodynamics of o-S-substituted benzenesulfonamide binding to Carbonic Anhydrases

Vaida Paketurytė, Alberta Jankūnaitė, Alexey Smirnov, Audrius Zakšauskas, Asta Zubrienė, Daumantas Matulis

Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Vilnius University, Saulėtekio al. 7, Vilnius LT-10257, Lithuania; E-mail: vaidapaketuryte@gmail.com

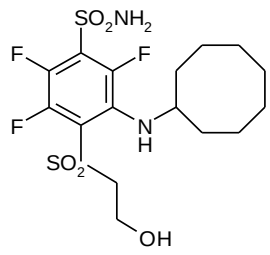
Lead compounds that bind target proteins are usually discovered by screening random chemical libraries. However, the goal of rational drug design is to be able to understand how a compound would recognize the target protein and what determines the binding affinity. To contribute towards this goal, we have chosen a group of twelve related human proteins – catalytically active carbonic anhydrases (CA) participating in many physiological processes. Inhibition of one or several CA isoforms has pharmacological applications in the treatment of numerous diseases. However, the high similarity within the active sites of CAs complicates the task to design a compound, which would have high selectivity and affinity for a single target CA isoform, such as cancer-related CA IX.
Benzenesulfonamide is the most common scaffold used to design CA inhibitors. We have synthesized a series of structurally similar o-substituted benzenesulfonamides bearing S in various oxidation states. The sulfonamide-pharmacophoric group binds to the Zn(II) in the active site of CAs while the second substituent guides the affinity and recognition of some isoforms thus determining their binding selectivity. Compound observed affinities to CA isoforms were determined by the fluorescent thermal shift assay and for selected compounds confirmed by isothermal titration calorimetry, and stopped-flow enzymatic inhibition assay.
Furthermore, it was important to calculate the intrinsic binding parameters that are independent of pH and buffer. They were calculated to represent the binding between the negatively charged sulfonamide ($R\text{-}SO_2NH^-$) and the CA with electrostatically neutral water molecule bound to the Zn(II) ($CA\text{-}Zn(II)\text{-}H_2O$). The correlation of intrinsic affinities with compound chemical structures could be valuable in further design of novel and more specific compounds.

Synthesis of 3,4,5-trisubstituted-2,6-difluorobenzenesulfonamides as selective inhibitors of human Carbonic anhydrase IX

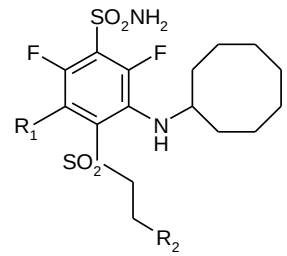
Aivaras Vaškevičius, Virginija Dudutienė, Asta Zubrienė, Daumantas Matulis

Institute of Biotechnology, Vilnius University, Vilnius, Lithuania; E-mail: virginija.dudutiene@bti.vu.lt

There are twelve catalitically active carbonic anhydrase (CA) isoforms in human body. Their malfunction or overexpression causes numerous diseases including cancer. CA IX shows limited expression in normal tissues but is significantly up-regulated in a variety of tumours. Inhibition of CA IX is one of possible ways to affect cancer growing. Compound VD11-4-2 was synthesized by our group and showed exceptionally strong binding to CA IX as confirmed by the enzymatic inhibition and binding assays. However, it's selectivity towards CA IX still can be improved. VD11-4-2 was modified by adding second functional group at *meta* position and/or substituting hydroxyl group at *para* position. All new compounds with *meta* substituents showed increased selectivity towards CA IX and exhibited up to 90 pM binding affinities.



VD11-4-2



High-cell density *Escherichia coli* fermentation monitoring based on culture fluorescence

Žilvinas Dapkūnas[1]; Aurimas Baranauskas[2]; Gintautas Žvirblis[1]

[1] Vilnius University, Life Sciences Center, Institute of Biotechnology; [2] UAB Profarma;

High-Cell Density fermentation control method was investigated. It uses on-line culture and dye for measuring membrane potential fluorescence to optimize productivity of Escherichia coli BL21(DE3) and Tuner(DE3) strains that express recombinant human growth hormone. Using culture and specific dye fluorescence to measure on-line cell mass and cell membrane potential, glucose feeding rates were determined. Under these conditions, commercially viable amounts of growth hormone were obtained accumulated as inclusion bodies inside the cells. This on-line fluorescence evaluation provides an efficient and reproducible tests of monitoring Escherichia coli fermentation behavior.

The landscape of small non-coding RNAs in probiotic lactic acid bacteria *Lactobacillus casei*

Raminta Mineikaitė[1], Milda Mickutė[1], Sigita Grigaitytė[1], Kotryna Kvederavičiūtė[2], Giedrius Vilkaitis[1]

[1] Department of Biological DNA Modification, Institute of Biotechnology, Vilnius University, Vilnius, Lithuania; [2] MAP Kinase Resource, Bioinformatics, Bern, Switzerland
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Non-invasive and non-pathogenic *Lactobacillus casei* bacteria have been used in food industry for a long time. In recent years much interest has been shown in *L. casei* potential in medicine as probiotics and live drug delivery tools. In order to achieve effective application of these probiotics it is of utmost importance to understand how *L. casei* adapt to constantly changing environmental conditions in human gastrointestinal tract. It is now known that not only proteins but also ~50-500 nt bacterial small non-coding RNAs (sRNAs) control many cellular processes. sRNAs are now considered major post-transcriptional regulators of gene expression. This study covers identification and analysis of *Lactobacillus casei* BL23 sRNAs. A new protocol for ~50-500 nt *L. casei* sRNA library preparation for *Illumina*™ sequencing was designed in order to obtain deep insight in novel sRNAs. After sequencing bioinformatic analysis was performed and potential sRNAs were identified. To avoid mistakes, putative sRNAs were manually inspected. 304 potential bacterial small non-coding RNAs were identified. The putative sRNAs were analyzed in detail and categorized into four groups: intergenic, antisense, 3′-UTR and 5′-UTR derived. The majority (27 out of 30) of selected newly identified sRNAs were experimentally verified by Northern hybridization: 18 out of 18 intergenic, 3/4 antisense, 3/3 5′-UTR and 3/5 3′-UTR derived sRNAs. The results show that bioinformatic analysis used to identify novel sRNAs is reliable and accurate. It is now known that sRNA molecules serve a wide range of regulatory functions in prokaryotes at almost every aspect of the cell′s life. Therefore the identified *L. casei* sRNA network will be used for further studies to explore gene expression regulatory systems in lactic acid bacteria. Newly discovered sRNAs will be helpful in understanding how bacteria adapt to constantly changing environmental conditions.
The work was supported by a grant from the Research Council of Lithuania MIP-059/2015.

Characterization of monoclonal antibodies against human Carbonic anhydrase IX

Aistė Imbrasaitė, Aušra Vaitiekaitė, Dovilė Stravinskienė, Jurgita Matulienė and Aurelija Žvirblienė

Vilnius University Life Sciences Center Institute of Biotechnology, Saulėtekio al. 7, Vilnius, Lithuania

Carbonic anhydrase IX (CA IX) is a transmembrane protein that catalyzes the reversible hydration of carbon dioxide and plays an important role in the regulation of intracellular pH favoring tumor cell growth and survival. This protein has N-terminal proteoglycan-like (PG) domain and is being recognized as a potential biomarker for different tumors. Monoclonal antibodies (MAbs) are widely used in diagnostics and treatment of many diseases including some types of cancer (breast cancer, non-Hodgkin's lymphoma).
The aim of this research was to characterize the MAbs against recombinant CA IX using various immunoassays.
In total, 14 MAbs against recombinant CA IX were generated by hybridoma technology. Eight MAbs recognised the catalytic domain of recombinant CA IX, other 6 MAbs were specific to the PG domain of CA IX. The MAb of clone H7 was shown to recognise the linear epitope of CA IX by Western blot. It was demonstrated that MAb of clone H7 is suitable to isolate CA IX from CA IX-expressing tumor cell lysates by an immunoprecipitation method. Three antibodies (clones A3, H7 and F4) were shown to recognise the native CA IX protein on the cell surface by flow cytometry and were used to investigate CA IX expression in tumor cells. It was demonstrated that the expression level of CA IX is strongly upregulated by hypoxia in several tumor cell lines (A549, HeLa, U87, Ca Ski, COLO, A431, MDA-MB-231 and MCF-7).
In conclusion, the newly developed MAbs against CA IX are promising tools for the analysis of biological samples for CA IX expression.

The evidence of hepatitis E virus infection in wild rats from Lithuania

Martynas Simanavicius1; Karolina Juskaite[1]; Arune Verbickaite[1]; Marius Jasiulionis[2]; Paulius Lukas Tamosiunas[1]; Rasa Petraityte-Burneikiene[1]; Aurelija Zvirbliene[1]; Rainer G. Ulrich[3], Indre Kucinskaite-Kodze[1]

[1] Vilnius University, Life Sciences Center, Institute of Biotechnology; [2] Nature Research Centre, Laboratory of Mammalian Ecology; [3] Friedrich-Loeffler-Institut, Federal Research Institute for Animal Health, Institute of Novel and Emerging Infectious Diseases, and German Center for Infection Research (DZIF)

Rat hepatitis E virus (HEV) is an orthohepevirus which is related to other HEV found in humans and other mammals. It was first identified in Norway rats (Rattus norvegicus) from Germany in 2010, then it was detected in Black rats (Rattus rattus) and Norway rats from USA, China, Indonesia, Vietnam and many European countries. In this study, we describe molecular and serological investigations of Black and Norway rats trapped in Lithuania for infections with rat HEV and human HEV genotypes 1-4. Rat HEV-specific real-time reverse transcription PCR (RT-qPCR) analysis of rat liver samples revealed the presence of rat HEV. In contrast, a RT-qPCR specific for HEV genotypes 1–4 did not reveal any positive samples. A nested broad spectrum RT-PCR was used for a confirmation of rat HEV infection with a subsequent sequencing of the amplified rat HEV genomic fragment. Phylogenetic analysis revealed a clustering of all newly identified rat HEV sequences with Norway rat-derived rat HEV sequences from Germany and a Black rat-derived sequence from Indonesia within the species Orthohepevirus C. An indirect ELISA using a yeast-expressed truncated rat HEV capsid protein variant revealed even more seropositive than RT-qPCR positive samples indicating a high rate of rat HEV circulation in the rat population examined. In conclusion, the current investigation confirms rat HEV infections in Norway and Black rats in Lithuania and the non-persistent nature of infection.

The role of Sema3C and R745A mutant on cell motility and microcapillary formation

Indrė Valiulytė1; Viktorija Preitakaitė[2]; Giedrius Steponaitis[1]; Arūnas Kazlauskas[1]

[1] Institute of Neuroscience, Lithuanian University of Health Sciences; [2] Faculty of Medicine, Lithuanian University of Health Sciences

Introduction: Class 3 semaphorins (Sema3) are characterized as axon guidance factors involved in tumor angiogenesis by interacting with the VEGF signaling pathway. Sema3 proteins convey their regulatory signals by binding to receptors neuropilins and plexins, which are located on the effector cell. This process is regulated by furin endoproteinases that cleave RXRR motifs in the Plexin-Sema-Ig domain and C-terminal basic domain of Sema3. Therefore, the aim of this study is to examine the role of Sema3C and R745A mutant, which has a point mutation at the basic domain at the hypothetical furin recognition site 742RNRR745, on microcapillary formation by human umbilical vein endothelial cells (HUVEC) in vitro angiogenesis assay.
Material and methods: Bicistronic expression vectors encoding Sema3A, Sema3F, Sema3C, mutant Sema3C (R745A) and a yellow fluorescent protein Venus separated by IRES2 element were constructed and used for transfection of the human embryonic kidney cells 293FT. The expression of semaphorins in 293FT cells and secretion to cell medium was confirmed by reverse transcription-PCR (RT-PCR) and Western-blot analysis, respectively. For microcapillary formation assay, HUVEC cells were suspended in medium from transfected 293FT cells containing secreted semaphorins and seeded on 96-well plate coated with Geltrex. Similarly, the semaphorin-enriched media was used in cell migration assay, which was performed on 96-well Oris Cell Migration Assay plate. After 16 and 24 hours, the microcapillary structures and migration area were captured with a microscope and analyzed with ImageJ Angiogenesis analyzer program. Results were statistically evaluated by the two-tailed Student's t-test.
Results: RT-PCR and Western-blot analyses showed that Sema3A, Sema3F, Sema3C and Sema3C (R745A) were synthesized in transfected 293FT cells and secreted in cell medium. The microcapillary tube formation in vitro assay revealed that Sema3C significantly inhibited the formation of a tube-like HUVEC network ($p<0.05$), whereas this activity by Sema3C has been lost upon R745A mutation. The migration assay showed that Sema3C protein strongly induced HUVEC cell migration ($p<0.01$), whereas effects of mutant Sema3C (R745A) was similar to control.
Conclusions: Our research showed that Sema3C inhibits angiogenesis process in an in vitro system, and this inhibitory effect is lost upon mutation at the Sema3C basic domain within putative furin cleavage site 742RNRR745, indicating that this site is essential for the Sema3 biological activity.

Acetyltransferase toxins – activity, neutralization, transcriptional autoregulation and how they are linked together

Dukas Jurėnas; Laurence Van Meldren, Abel Garcia-Pino

Université Libre de Bruxelles, Département de Biologie Moléculaire

Bacteria constantly encounter conflicts at cellular as well as genomic level. They have evolved various systems that include toxins that are active against intruders but also those that kill their own progeny as a result of genomic conflict. Most simple but yet elegant systems are comprised only of toxin and antitoxin – i.e. TA systems. They reside primarily on mobile genetic elements and are active against progeny that have lost genes encoding TAs, as well as in some cases against intruding phages, or as a result of environmental stresses. We have recently discovered a novel class of toxins comprising acetyltransferase activity that targets tRNAs and acetylates their cargo amino acids. We have demonstrated that this activity leads to efficient translation inhibition. Our examples show that this family of toxins have diverged in the specificity towards different tRNAs. We provide model of target binding and recognition by AtaT acetyltransferase toxin which shows necessity of dimeric form of the enzyme. We demonstrate that the AtaR antitoxin interacts physically with the AtaT toxin and prevents its dimerization, tRNA binding and correct placement of the acetyl-CoenzymeA in

the active site. This neutralization involves two different folds of AtaR antitoxin in the same complex. Intriguingly, this unique toxin-antitoxin complex formed during the protein synthesis from the operon cannot be later restored from free dimerized toxins and antitoxins. Only this unique complex efficiently binds DNA and represses the transcription of the ataR-ataT toxin-antitoxin operon. Slight changes in toxin-antitoxin ratios lead to de-repression of ataR-ataT operon. We demonstrate each step of this intricate regulation at the atomic resolution supported by in vivo experiments and capture the first molecular level example of how operon arrangement serves regulation of TA systems.

Structure and regulation of arachidonate 11*R*-lipoxygenase

Priit Eek, Ivar Järving, Nigulas Samel

Department of Chemistry and Biotechnology, Tallinn University of Technology, Estonia

Lipoxygenases (LOXs) are a diverse family of peripheral membrane proteins. Although they all catalyze the peroxidation of polyunsaturated fatty acids, homologs can vary not only in terms of substrate selectivity or catalytic specificity, but also in regulatory aspects (1). While many LOXs are stimulated by Ca^{2+}-induced membrane association, the arachidonate 11*R*-LOX from the Arctic coral *Gersemia fruticosa* is characterized by strict requirement for Ca^{2+} and lipid membranes for any catalytic activity (2).

We crystallized recombinant 11*R*-LOX and solved its structure to a 2.47-Å resolution (3). The overall structure matches the canonical framework of animal LOXs with an N-terminal β-sandwich called the PLAT domain, and a larger, mostly α-helical C-terminal catalytic domain. The crystals were obtained in the absence of Ca^{2+}, so the PLAT domain loops that compose the putative Ca^{2+}-binding sites are not aligned as in known PLAT:Ca^{2+} complexes, indicating notable conformational changes upon Ca^{2+} binding. Furthermore, the active site is completely enclosed as the putative portal is blocked by a short α2 helix and the adjoining portion of the chain, meaning that here, too, conformational changes are necessary. Conserved interactions were found between the PLAT domain in the vicinity of Ca^{2+}-binding sites and the N-terminal side of the α2 region in the catalytic domain, namely Trp^{107}–Lys^{172} and Arg^{106}–Asp^{173}, that are crucial for proper enzymatic activity and also for structural stability (4). Since 11*R*-LOX is a dimer in solution, we also investigated the quaternary structure of the protein by small-angle X-ray scattering, chemical cross-linking, and mutagenesis experiments. In the determined dimer assembly, the catalytic domains associate by their PDZ-like subdomains and the C-terminal sides of the α2 regions (5). In sum, the results suggest that the closed, serpentine-like conformation of the α2 region may be imposed by stabilizing interactions from both interdomain connections on one side, and protein dimerization on the other.

Human ALOX5 is a key enzyme in the production of leukotrienes—potent inflammatory mediators—making ALOX5 a prominent biological target. Like 11*R*-LOX, it is a Ca^{2+}-induced enzyme featuring a similar fragmented α2 helix (6). As ALOX5 bears the greatest similarity to 11*R*-LOX among mammalian LOXs, the regulatory mechanisms of the two enzymes are likely comparable. ALOX12 and ALOX15 also participate in inflammatory processes in humans. Strikingly, both can dimerize, and the interfaces of their assemblies have been narrowed down to the same region of the protein surface as in 11*R*-LOX (7). While human LOXs tend to be relatively unstable and difficult to handle, 11*R*-LOX is a remarkably stable enzyme facilitating extensive structural studies. As a result, 11*R*-LOX makes an exceptional source of information regarding the dynamic structure of LOXs.

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miR-20b acts as potential oncogenic miRNA in gastric cancer derived cell lines by targeting phosphatase and tensin homolog (PTEN) and thioredoxin-interacting protein (TXNIP) genes

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Recently discovered microRNAs (miRNAs) post-transcriptionally regulate gene expression and play important role in a variety of processes. The aim of a study was to examine hsa-miR-20b-5p (miR-20b) function in gastric carcinogenesis by experimentally determining miRNA target-genes and impact to physiological cell processes in vitro and in vivo.
After bioinformatics analysis five potential target genes were selected for miR-20b (EREG, FAT4, IRF1, TXNIP, PTEN) and analyzed for expression changes in mRNA and protein levels after transfection with miR-20b inhibitor. Cell viability was assessed using a MTT assay, colony formation and proliferation was evaluated using clonogenic assay, and cell migration was determined using wound healing assay. INS-GAS mice model was used to evaluate the miR-20b alterations in vivo following H.pylori infection with follow up to 50 weeks.
Inhibition of miR-20b expression significantly increased IRF1, PTEN, TXNIP genes expression level in AGS (p=0.008, p=0.021, p=0.046, respectively) and MKN28 cell line (p=0.003, p=0.005, p=0.0004, respectively) 24 h after transfection. IRF1, TXNIP genes expression remained increased 48 h post-transfection in AGS cell line (p=0.001, p=0.006, respectively), while only TXNIP - in MKN28 cell line (p=0.001). Secondly, miRNA target-genes were analyzed for protein expression changes. Analysis revealed that PTEN protein expression statistically significantly increased in AGS cell culture (p= 0.020) and TXNIP protein expression increased in MKN28 72 h after transfection (p=0.036). Finally, miR-20b inhibition reduced cell viability in AGS cell line (p= 0.029). Furthermore, number of colonies reduced dramatically in both cell lines (p=0.0002, p= 0.021; AGS and MKN28 respectively) compared to cells transfected with control miRNA. INS-GAS mice showed gender specific miR-20b expression pattern following H.pylori infection. Only male mice showed significantly higher miR-20b expression for all time points (p=0.0285). There was a stepwise increase in miR-20b expression during the different time points from 12 to 50 weeks with highest difference at 50 weeks (p=0.0033).

Our data shows that miR-20b-5p may target PTEN and TXNIP and play an important role in gastric carcinogenesis by mediating cell viability and colony formation in GC-derived AGS and MKN28 cell cultures. The data from INS-GAS mice experiments support the role of miR-20b in GC in vivo.

Small RNA sequencing-based miRNA profiling and functional analysis in gastrointestinal stromal tumors (GISTs)

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MicroRNAs (miRNAs) are a class of small non-coding RNAs involved in post-transcriptional regulation of gene expression. Deregulated miRNA profiles and their contribution to carcinogenesis has been observed in different types of cancer. However, their involvement in pathogenesis of rare gastrointestinal stromal tumors (GISTs) is not yet fully understood. Therefore, the aim of this study was to determine highly deregulated miRNAs in GIST and to investigate their possible involvement in GIST pathogenesis.
MiRNA expression profile was determined using small RNA-seq approach in paired tumor and adjacent tissue samples of 15 GIST patients and validated by Taq-Man low-density array in a larger sample group of 40 patients. Potential targets of deregulated miRNAs were predicted using TargetScan Release 7.1. Further transfections with miRNA mimics and functional analysis was performed in GIST-T1 cell line. Changes in target gene expression were detected using TaqMan primers and probes, protein expression analysis was performed using Western Blot technique. Alterations in cell viability and migration rates were evaluated by MTT and Wound Healing Assays. Statistical analysis was performed using the computing environment R.
Sequencing data showed distinct miRNA expression profiles between tumor and adjacent tissue samples. In a further validation step, expression levels of 19 miRNAs showed significant differential expression in the same direction as in the sequencing data (Bonf. adj. $p < 0.01$; $FC > 2$). MiRNAs, potentially targeting genes involved in cancer associated signaling pathways, were selected (hsa-miR-375, hsa-miR-200b-3p, hsa-miR-490-3p) for target gene expression and functional analysis in GIST-T1 cell line. Increased amounts of hsa-miR-375 significantly reduced expression of its predicted target gene KIT ($FC = 1.8$, $p < 0.05$), however KIT protein expression remained unchanged. Overexpression of hsa-miR-200b-3p significantly reduced EGFR and ETV1 gene expression ($FC = 0.7$ and $FC = 0.81$, respectively, $p < 0.05$) and significantly reduced levels of EGFR protein ($FC = 0.55$, $p < 0.05$). Analysis of physiological changes of GIST-T1 cells revealed that only hsa-miR-375 significantly reduced both viability and migration rate of GIST-T1 cells, while hsa-miR-200b-3p significantly lowered cell migration rate and showed slight, but not significant impact on cell viability.
MiRNAs show distinct expression profiles in GIST tumor and adjacent tissues. Analysis in GIST-T1 cell line showed that miRNA hsa-miR-375 potentially targets known GIST associated oncogene KIT and therefore affects cell viability and motility. Another miRNA hsa-miR-200b-3p might be involved in GIST pathogenesis by targeting oncogenes EGFR and ETV1. Therefore, miRNAs hsa-miR-375 and hsa-miR-200b-3p should be further investigated as a potential components of GIST pathogenesis and a promising tools for targeted therapy in GIST.

Astrocytoma specific blood serum protein markers

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In a period of 30 years the survival rate of patients diagnosed with cancer has improved around 20% however, the increase of survival rate of patients diagnosed with brain tumours is barely noticeable and is only about 1%. The most malignant and the most common brain tumour in adults is grade IV Astrocytoma, also called Glioblastoma (GBM). GBM patients have the poorest overall survival as compared to other primary brain tumours and less than 5% of patients survive 5 years after diagnosis. Astrocytomas are often considered to be the most difficult tumours to treat because to their heterogeneity at the cellular and molecular levels and also because of late diagnosis at the advanced tumour stages. Thus, it is essential to recognize Astrocytomas heterogeneity to improve the accuracy of disease prognostics and diagnostics as well as to improve current treatment decisions.
To achieve this goal our group attempted to composed Astrocytoma specific blood serum protein detection method. 10 proteins (AREG, MMP-2, PAI-1, NCAM-1, TGFBeta1, OPN, IGF-1, ANG-1, IP-10 and TIMP-1) which play important role in tumour development were selected for the analysis applying commercially available custom design ELISA-based multiplex protein array. Target-proteins levels of grade II-IV Astrocytoma patients' blood serum were measured before (S1) and after (S2) tumour resection. A control specimens - healthy (with no indications of CNS or other type of cancer and/or inflammation) volunteers' blood serum samples were used. RT-q-PCR technique was applied to measure mRNA level of AREG, MMP-2, PAI-1, NCAM-1, TGFBeta1, OPN, IGF-1, ANG-1, TIMP-1, GFAP and CHI3L1 genes in Astrocytoma patients' tumour tissue samples.
Individual analysis of serum protein levels revealed that TGFBeta1, IGF-1 and ANG-1 protein levels statistically significantly differed between GBM patient S1 serum and healthy control groups indicating the potential of the protein markers for early GBM diagnostic. Other promising proteins such as TIMP-1, NCAM and OPN showed different blood serum levels between Astrocytoma malignancy grades and/or between serum S1 and S2 groups. Gene mRNA expression analysis showed that the level of - PAI-1, NCAM-1, TGFBeta1, IGF-1, TIMP-1, GFAP and CHI3L1 genes significantly differed between Astrocytoma grades. The significance of these proteins needs to be further characterized.

Development of the screening assay for selection of novel small-molecule inhibitors of Sortase A enzyme

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Gram-positive pathogenic bacteria display virulence, adhesion-associated proteins and enzymes on their surface that may interact with host cells and tissues and play a role in pathogenesis. Sortase A enzyme (SrtA) is an important virulence factor as it is responsible for anchoring LPXTG-containing proteins to the cell wall peptidoglycan that may lead to severe human infections. Nowadays, keeping in mind the growing antibiotic resistance epidemic, sortase appears to be a promising target for identifying novel inhibitors that could be of general use in therapeutics against Gram-positive bacteria that pose a serious healthcare threat. In this work we produced truncated forms of the sortase A from several microorganisms (Staphylococcus aureus, Listeria monocytogenes, Bacillus anthracis) representing catalytic domains of the enzyme. We expressed recombinant soluble proteins with hexahistidine tag in E.coli cells and purified them by metal affinity chromatography. The proteins are enzymatically active as they cleaved Dabcyl - LPETG – Edans substrate in Fluorescence Resonance Energy Transfer (FRET) assay revealing the fluorescent signal that could be measured to study sortase activity. The aim of this work was to use recombinant

SrtA proteins in FRET assay based high-throughput screening of the chemical library comprising of 8000 compounds in order to discover novel small-molecule sortase A inhibitors. We also produced non-tagged recombinant SrtA proteins that could be used in the enzyme structure and enzyme-inhibitor interaction studies using nuclear magnetic resonance spectroscopy (NMR).

Investigations of single-stranded RNA bacteriophages virus-like particles and design of novel vaccines

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Small RNA bacteriophages are viruses with a simple genome and structure, their particles have small size and icosahedral symmetry. They have been extensively studied as the models of viral structure, evolution, assembly and translation processes. ssRNA viruses derived virus-like particles, produced by the means of bacterial expression system, are promising candidates for vaccine development and targeted drug delivery containers. Several ssRNA phages VLP vaccine candidates have already reached clinical trials.
Current research studies newly explored ssRNA phage VLPs in terms of their production, assembly, stability and structure. At present, we managed to express nearly 100 of coat proteins, the majority of whom assemble in VLPs. We have also crystallized and solved structure for several of them. An ultimate goal is to detect suitable VLP for vaccine construction and create potential VLP vaccines against immunologically significant antigens like B. burgdorferi CspZ protein or influenza virus haemagglutinin conservative domain.

FEBS Chair of the Working Group on Integration lecture

Challenges in Personalized Medicine: Science, Clinics, Society and Education

Jerka Dumic

Department of Biochemistry and Molecular Biology, Faculty of Pharmacy and Biochemistry, University of Zagreb, Zagreb, Croatia

June 27, LBS Conference
Oral presentations

FEBS National Lecture
Vaccination against chronic diseases using virus-like particles

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Researchers working on the development of vaccines face an inherent dilemma: to maximize immunogenicity without compromising safety and tolerability. Early vaccines often induced long-lived protective immune responses, but tolerability was a major problem. Newer vaccines have very few side effects but can be of limited immunogenicity. One way to tackle this problem is to design vaccines that have all the properties of pathogens with the exception of causing disease. Key features of pathogens can be mimicked by virus-like particle (VLP) based delivery systems. Here we discuss the use of immunologically optimized VLPs for the generation of therapeutic vaccines.

Virus-like particles of ssRNA phages: tools in vaccine development against Lyme disease

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RNA phages, belonging to family Leviviridae are among the simplest known viruses with about 3500-4200 bases long single-stranded RNA genome, packaged in a T=3 protein shell. For decades, ssRNA phages have been used as simple models to study various problems in molecular biology, such as translation repression, virus evolution and virus assembly. ssRNA phages have also found a number of applications – notably, their virus-like particles (VLPs) are being used in vaccine development. Recently, we have produced, purified and characterized more than 60 different VLPs of ssRNA phages, identified in sequencing of various metagenomes. We have used modified VLPs of ssRNA phages AP205 and Qβ to construct vaccine candidates against Lyme borreliosis, which is the prevalent zoonotic disease in Northern hemisphere with more than 300,000 cases reported annually. We have attached recombinant surface proteins from Lyme disease causing bacteria Borrelia burgdorferi to the surface of VLPs by both genetic fusion and chemical coupling methods. Further, we demonstrated that serums, obtained from immunization of mice with modified VLPs displayed potent bactericidal activity. We have also checked the protectivity of some of our vaccine candidates in mice models and confirmed that bacterial burden is indeed significantly decreased in animals, which were vaccinated prior to infection.

Alterations in DNA methylation and RNA expression from peripheral blood cells in humans treated with metformin.

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Metformin is a widely prescribed antihyperglycaemic agent that has been also associated with multiple therapeutic effects in various diseases, including several types of malignancies. There is growing evidence regarding the contribution of the epigenetic mechanisms in reaching metformin's therapeutic goals, however, the effect of metformin on human cells in vivo is not comprehensively studied. The aim of our study was to examine metformin induced alterations of DNA methylation and RNA expression profiles in white blood cells of healthy volunteers, employing a longitudinal study design. 12 healthy metformin naïve individuals where enrolled in the study. Global DNA methylation pattern was estimated at baseline, 10 hours and 7 days after the start of metformin administration. The whole-genome DNA methylation analysis revealed differentially methylated CpGs at 11 genes, while differentially expressed RNA was observed for 596 genes. Several differentially methylated and expressed regions were identified as novel potential epigenetic targets of metformin. The main functions of the majority of top ranked differentially methylated loci and their representative cell signaling pathways were linked to the well-known metformin therapy targets: regulatory processes of energy homeostasis, inflammatory responses, tumorigenesis, and neurodegenerative diseases.

Relevance of tumour-associated autoantibodies in early detection and prognosis of cancer

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Serum autoantibodies against tumour-associated antigens (TAA) have been extensively studied as cancer biomarkers in diverse types of malignancies. Altogether, the existing data demonstrate the capacity of the humoral immune system to report on malignant processes at certain accuracy; however, to date the existing information on the time point during cancerogenesis when they appear in circulation and their prognostic value is very limited.
In collaboration with clinical partners, we have performed several studies to address these issues in a systematic way in gastric and prostate cancer. From >1300 TAA antigen clone collection identified in our initial studies by using T7 phage display-based SEREX approach [1], we have selected for the most promising TAA clones and used them to develop focussed tumour-associated antigen microarrays comprising 152 and 102 prostate and gastric cancer associated antigens, respectively. The microarrays were used to systematically analyse the prevalence of the cancer-associated autoantibodies in 829 gastric cancer patients and 929 healthy controls from Caucasian and Asian populations, as well as 100 patients with chronic atrophic gastritis and 775 individuals staged according to different grades of intestinal metaplasia [2]. In prostate cancer study, serum samples from 250 prostate cancer patients, 113 patients with biopsy-confirmed benign prostatic hyperplasia (BPH) and 159 age-matched healthy males were analysed; the patients were followed-up for up to seven years, and the information on the diagnosis change from BPH to prostate cancer and the overall survival was obtained.
In gastric cancer study we found a signature of six TAAs predominantly reacting with sera from gastric cancer patients when compared to healthy controls, and the seroreactivity was associated with intestinal type gastric cancer. We detected gastric cancer-associated seroreactivity in 13% of patients with advanced/severe intestinal metaplasia, which was increased in comparison to mild/moderate intestinal metaplasia (5.3%) and was comparable to that seen in early stage gastric cancer patients (12%). Results of this study suggest that humoral immune response against TAAs is generated already during premalignant stages. By using multivariate Cox regression analyses, five TAAs were identified as independent prognostic factors (HR range 11.18-43.59; $P < 0.05$) in gastric cancer patients.In prostate cancer, a diagnostic signature of 25 autoantibodies was identified, capable of discriminating prostate cancer from healthy controls with AUC of 0.69 ($P < 0.001$), and prostate cancer from BPH with AUC of 0.59 ($P < 0.01$). BPH patients that were diagnosed with prostate cancer during the follow-up showed cancer-specific autoantibody responses at the time of first diagnosis. By comparing autoantibody responses in prostate cancer patient groups with high (>7) and low/medium (≤7) Gleason score, we identified a 15-autoantibody signature that can predict prostate cancer patients having high Gleason score tumours with 81% accuracy ($P < 0.001$).
The results obtained within these studies suggest that cancer-associated autoantibodies have limited diagnostic value but might make a valuable contribution through enhancing the

positive predictive power of existing models currently used for the diagnosis and risk assessment in cancer patients.

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Preferential solvation and hydration of proteins in water-organic mixtures

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Preferential solvation is an effective way for revealing the mechanism of protein stabilization or denaturation [1-3]. When a protein interacts with a binary water-organic solvent mixture, the three components do not equally mix. Water or organic solvent molecules exist preferentially in the protein's solvation shell. This difference between the solvation shell and bulk solvent in the solvent components has been termed preferential solvation. Preferential solvation is a thermodynamic quantity that describes the protein surface occupancy by the water and cosolvent molecules. The main focus of our study is to monitor the preferential hydration and preferential solvation of protein macromolecules (lysozyme, α-chymotrypsin) at high, intermediate, and low water content in organic solvents (ethanol, DMSO, acetonitrile). Our approach is based on an analysis of residual enzyme activity and water/organic solvent sorption. Advantages of our approach: (i) The preferential interaction parameters can be determined in the entire range of water content in organic liquids. (ii) Our approach facilitates the individual evaluation of the Gibbs energies of water, protein, and organic solvent.

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Adenosine and cyclic adenosine monophosphate (cAMP) as diagnostic indicators for drug-induced liver disease (DILI) in HIV-infected patients

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Drug-induced liver injury is a major cause of safety-related drug-marketing withdrawals. There are an estimated 40 million HIV infected individuals worldwide, with chronic liver disease being the 2nd leading cause of mortality in this population. Elevated liver functions are commonly noted in HIV patients and the etiologies are varied. Adenosine in the intracellular pool is at the center of energy and nucleic acid metabolism in the liver tissue. Increase of extracellular adenosine levels has been observed in hepatic injury. The increase of adenosine is presumably subsequent to inflammatory tissue damage.

The study included 180 HIV-infected patients. Among the observed 39.3% of patients not receiving HAART, 60.7% - were on ART, according to clinical treatment protocols. In 14.4% of patients had a change of ART regimen due to ineffectiveness or intolerance. Co-infection of HCV / HBV virus was diagnosed in 7% of patients, about which antiviral causal treatment was not carried out. By high performance liquid chromatography (HPLC) in the blood plasma of HIV-infected patients were determined adenosine (Ado) and cyclic adenosine monophosphate (cAMP).

A significant correlation between the activity of transaminases (ALT and AST) and level of Ado has been established. The growth rate of Ado depended on the group of antiretroviral drugs taken and the duration of their admission. There is an increase in cAMP in HIV-infected

patients with laboratory signs of drug-induced liver damage when taking antiretroviral therapy was also identified.

Evolution of dsRNA viruses in protists

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Functional roles of genes and their pseudogenes

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Pseudogenes are the class of long ncRNAs, which are present in large numbers and regulate the expression level of their parental transcripts. Despite the recent progress in pseudogene bioinformatic search and identification, their functional roles are only starting to emerge. For this reason, we have designed a library of siRNAs to specifically target genes and their pseudogenes and performed the screen to analyze their impact on integrin mediated cellular events. The selected hit, namely PTEN gene and PTENP1 pseudogene, were further analyzed in details. Our data suggests the complementary roles of PTEN and PTENP1 in integrin localization, endocytic trafficking and autophagy.

Markers for prediction of early complications after unrelated haematopoietic stem cell transplantation

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Yeast viruses - foes or friends?

Saulius Serva

Life Sciences Center, Vilnius University

Molecular and cellular mechanisms of CDNF action

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MANF and CDNF are novel survival-promoting proteins that potently reduce the neurological symptoms in the animal models of Parkinson's disease. Both are intracellular resident proteins of the endoplasmic reticulum (ER), but can also work extracellularly, at least in the in vivo experiments. The molecular details of either mode of action are poorly known. We followed by immunohistochemistry the fate of recombinant CDNF protein infused to the striata of rats, the locus where it leads to recovery from Parkinsonian symptoms. Intracellularly, CDNF was localized mostly vesicularly in the cytoplasm of neurons and glial cells that, as revealed by electron microscopy, were mostly the endosomes. Importantly, the

CDNF-positive vesicles were also found in the somae of dopamine neurons in the substantia nigra, that innervate the striatum and that are protected by CDNF in the Parkinsonian model. Thus, CDNF is axonally transported from striatum to substantia nigra where it protects the dopaminergic neurons. CDNF immunoreactivity had disappeared from the brain parenchyma after 24 h.

In vitro fibrillization of Alzheimer's amyloid-β peptide

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Alzheimer's disease (AD) is the most prevalent cause of dementia in the elderly population. Molecular events that trigger AD are not yet clear, however, there is increasing evidence showing that this cascade of events is initiated by the amyloid- β (Aβ) peptides that make up the amyloid deposits characteristic to the disease. The peptide fibrillization in vitro is an autocatalytic process involving secondary nucleation events, however, the mechanism of the whole process is unknown. In addition to the peptides the amyloid deposits contain high levels of biometals copper, zinc, and iron. At least Zn(II) and Cu(II) interact with the Aβ peptides with considerable affinity and affect their fibrillization and toxicity on neurons.
Fibrils of Aβ42 grow easily in a test tube where the process can be initiated by adding preformed fibrillar seeds or intensive agitation of the solution. Both Zn(II) and Cu(II) inhibited the fast fibrillization of the Aβ peptide and various metal chelators reversed this effect. Thus, the metal ions inhibit the fibril growth or elongation by lowering the concentration of free peptide and decrease the fibrillization rate. The inhibitory effect of Zn(II) was observed at lower concentrations than that of Cu(II), which has higher affinity towards Aβ. However, the metal ions induced formation of amorphous aggregates and these aggregates transformed into fibrils during incubation. Thus, in the absence of fibrillar seeds metal ions can enhance the fibrillization rate by inducing the assembly of the peptide into initial non-fibrillar aggregates, which further transform into amyloid fibrils. The toxicity of Aβ appear only when the fibrils are growing in the cellular medium. The peptide aggregates cover the cells and cause the apoptosis, the preformed fibrils added to the cellular media has no toxic effect. On the other hand, it was demonstrated that Cu(II)-induced Aβ aggregates are toxic to the rat primary neurons in the presence of the reducing agent ascorbate. Putative mechanisms of fibrils propagation and toxicity are discussed. It should be noted, that the fibrillization behaviour of Aβ42 and its recombinant counterpart with an additional methionine in the N-terminus used often as a model in in vitro studies, differ significantly.

Cellular copper metabolism in health and disease

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Cellular copper proteome is constituted from copper enzymes and copper chaperones. Copper chaperones compose a specific class of proteins assuring safe handling and specific delivery of potentially harmful copper ions to variety of essential copper proteins like Cu-ATP-ases, Cu,Zn-superoxide dismutase and cytochrome c oxidase. Analysis of the metal-binding properties of copper chaperones and their partner proteins allowed us to establish affinity gradients determining copper transport in the cell under normal conditions [1]. Cellular copper metabolism is substantially disturbed in case of Wilson disease, caused by loss-of-function mutation in P-type copper efflux ATPase - ATP7B, which leads to toxic accumulation of copper mainly in the liver and brain. Wilson disease is treatable, primarily by copper-chelation therapy, which promotes copper excretion. Although several de-coppering drugs are currently

available, their Cu(I)-binding affinities have not been quantitatively characterized. Here we determined the Cu(I)-binding affinities of five major de-coppering drugs - D-penicillamine, trientine, 2,3-dimercapto-1-propanol, meso-2,3-dimercaptosuccinate and tetrathiomolybdate - by exploring their ability to extract Cu(I) ions from two Cu(I)-binding proteins, the copper chaperone for cytochrome c oxidase, Cox17, and metallothionein [2]. We report that the Cu(I)-binding affinity of these drugs varies by four orders of magnitude and correlates positively with the number of sulfur atoms in the drug molecule and negatively with the number of atoms separating two SH groups. Based on the analysis of structure-activity relationship and determined Cu(I)-binding affinity, we hypothesize that the endogenous biologically active substance, α-lipoic acid, may be suitable for the treatment of Wilson disease. Our hypothesis is supported by cell culture experiments where α-lipoic acid protected hepatic cells from copper toxicity. These results provide a basis for elaboration of new generation drugs that may provide better therapeutic outcomes [2].

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Multiscale spectroscopies for understanding GPCR mediated cell signalling

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Poster presentations (session I)

1. Comprehensive proteomic analysis of pancreatic ductal adenocarcinoma

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Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest forms of cancer due to the lack of diagnostic tools at the early stage and low efficiency of current chemotherapeutic approaches. Here we present high-throughput differential proteomic analysis of tissue samples from the operations of patients with PDAC, chronic pancreatitis and those without these diseases. We have identified the set of proteins differentially expressed in PDAC patients. These differentially expressed proteins enable us to identify biological processes specific to pancreatic cancer but not pancreatitis patients. We have also extracted from the data a number of already known as well as new potential PDAC-specific markers. Moreover, the analysis of our data using the database of gene expression perturbation with drugs from the Library of Integrative Network-based Cellular Signatures enabled us to extrapolate potential chemotherapeutic agents for PDAC treatment. The results show the promising potential of this bioinformatic approach for anticancer drug discovery. This study also provides new candidate PDAC biomarkers for further validation.

This work was supported by DiaKASA project (Nr. SEN-01/2016) of the Research Council of Lithuania.

2. β-Carotene loaded three-component particles: preparation and stability

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β-carotene is an orange colored compound belonging to the carotenoid family that can be found in many fruits and vegetables. The consumption of this carotenoid is important for maintaining human health and wellbeing because it is enzymatically converted to vitamin A in the human intestine by the β-carotene 15,15'-monoxygenase. Besides its pro-vitamin A function, β- carotene has many other activities that may contribute to human health, such as the ability to exhibit antioxidant activity, reduce the risk of type 2 diabetes, lower metabolic syndrome in middle-aged adults, enhance immune system performance, and reduce the risk of cardiovascular diseases. However, the application of β-carotene is currently limited due to its poor water-dispersibility, chemical stability and bioavailability.
The new water-soluble three-component system for β-carotene delivery was developed. Inclusion complexes of β-carotene with 2-hydroxypropyl-β-cyclodextrin were prepared by co-precipitation method using a β-carotene solution in acetone and a cyclodextrin in water at the concentration of 25 %. Further, the prepared water-soluble inclusion compounds were complexed with pectin. For β-carotene-2-hydroxypropyl-β-cyclodextrin-pectin particles formation, pectic acid and pectin with 26 % and 60 % degree of esterification were used. Optimal encapsulation conditions, i.e. inclusion complex-pectin ratio and pH are examined. The sizes of the obtained nanoparticles were measured next day after preparation and after 30 days of storage in 4 ˚C temperature using DLS method. To evaluate the stability of encapsulated β-carotene against UV raddiation, particles were irradiated by Philips UV-C 30 Watt lamp for certain period of time and changes in absorbance at 450 nm were observed.

3. Identification of small non-coding RNAs responsible for cell wall formation in lactic acid bacteria

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Non-pathogenic lactic acid bacteria (LAB) such as *Lactococcus lactis* and *Lactobacillus casei* have a long history of use for food production such as cheese and yogurt fermentation. In recent years much interest has been shown in their potential in medicine as live drug delivery tools and probiotics. Since bacterial cell wall is a major barrier between the bacterial cell and its environment, it is of utmost importance to determine how the bacterial cell wall forms and changes under stress conditions. Many studies have already been performed in order to understand protein function in bacterial cell wall formation. However, until recently only little was known about the role of bacterial small non-coding RNAs (sRNAs) in this process. These 50-500 nt molecules are now considered as major post-transcriptional regulators of gene expression in bacteria. The aim of this study is to reveal *L. lactis* MG1363 and *L. casei* BL23 sRNA transcriptomes and identify sRNAs responsible for stress-mediated cell wall formation.
In order to determine the network of sRNAs, *L. casei* and *L. lactis* RNA sequencing analyses were performed: ~300 and ~240 putative small non-coding RNAs, respectively, were identified. 27 *L. casei* and 16 *L. lactis* regulatory RNAs belonging to intergenic, antisense, 3′-UTR, or 5′-UTR sRNA classes were validated by Northern hybridization.
To identify *L. lactis* sRNAs differentially expressed in response to lysozyme or penicillin G, sRNA libraries were prepared after the bacteria exposure to cell wall targeting antimicrobials (lysozyme or penicillin G) or growth in optimal medium. Three sRNAs were confirmed to affect bacteria resistance to antimicrobials and potentially regulate cell wall biosynthesis.

In parallel to sequencing, functional analysis for the identification of sRNAs of *L. casei* was performed. After inserting 50-500 bp sheared genomic fragments of these microorganisms to a shuttle vector, *L. casei* genomic library was constructed. Following the selection of bacteria that are resistant to antimicrobials, sRNAs coding *L. casei* genome sequences were found to be responsible for increased ampicillin and penicillin G resistance.
In conclusion, sRNA transcriptome analysis uncovered hundreds of potential sRNA genes in the genome of *L. casei* and *L. lactis*. These results provide an excellent basis for further investigations in the molecular mechanisms of LAB. The work was supported by a grant from the Research Council of Lithuania MIP-059/2015.

4. Factors influencing the reliability and preciseness of trace elements ICP-MS analysis in biological material

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Analysis of trace elements in biological material is a challenging task due to very small concentrations present in the matrix. Inductively coupled plasma mass spectrometry (ICP-MS) is a most suitable technique for the purpose because it exhibits a good precision, excellent sensitivity, and multielemental capability, so that majority of trace elements can be reliably analysed simultaneously. Therefore, the technique of material preparation and effect of storage duration before the analysis also have crucial impact on the reliability and preciseness of trace elements analysis.
The aim of the study was to explore different biological material pretreatment techniques, storage duration effect before the analysis and to find the most reliable ICP-MS analysis mode that exhibits the most accurate reading of lead, cadmium, selenium and manganese in blood and urine.
The research was conducted using the NexION 300D ICP-MS (PerkinElmer, USA) equipped with nickel cones and quartz cyclonic spray chamber as a sample introduction system and applying three different modes: Standard mode, Kinetic energy discrimination (KED) mode and Dynamic reaction cell (DRC) mode, and following manufacturer recommendations. Blood and urine samples were pretreated by direct dilution or mineralization (Microwave 3000, Anton Paar, Austria).
The analyses of trace elements were performed in certified reference materials: Seronorm™ Trace Elements Urine Blank, Seronorm™ Trace Elements Whole Blood L-II (SERO AS, Norway), Clin Check® Trace Elements Whole Blood L-II (RECIPE, Germany). Each sample was analysed three times investigating the reliability and preciseness, analyses were repeated after 1 and 5 months.
The combined results of blood and urine pretreatment technique, ICP-MS analysis using different analysis mode of selected trace elements – lead, cadmium, selenium, manganese, and storage duration effect will be presented in details in the poster.
We conclude that all – the pretreatment technique (direct dilution vs. mineralization), storage duration effect (immediate analysis vs. analysis in 1 month and analysis 5 months of samples stored in the refrigerator (+4°C) and ICP-MS analysis mode (standard vs. KED vs. DRC) have impact on the reliability and preciseness of trace elements analysis in biological material.

5. Antimicrobial peptide from *Pediococcus acidilactici* JEM-1

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Nowadays, the demand for new antimicrobial agents is increasing in food industry as well as in medicine. Pediococcus spp. are lactic acid bacteria classified as probiotics. Pediococcus strains produce pediocin, i.e. bacteriocin having a wide spectrum of antimicrobial activity against Gram-positive bacteria such as *Listeria monocytogenes*, *Enterococcus faecalis* and *Clostridium perfringens*.
Here, the purification and characterization of antimicrobial peptide from *Pediococcus acidilactici* JEM-1 is presented. Bacteria were cultivated in MRS media at 35 ^{o}C for 24 h. For the purification of pediocin, the method of pediocin adsorption to producer cells at pH 6.0 and desorption at pH 2.0 was applied with the following ion-exchange chromatography and gel-filtration. The purity of peptide was confirmed by Tricine-SDS-polyacrylamide gel-electrophoresis and capillary zone electrophoresis. The antimicrobial activity was estimated by agar-diffusion assay using *Bacillus subtilis* as Gram-positive indicator strain. The molecular mass of pediocin was determined by LC-UHR-TOF-MS and found equal to 8114 Da. The analysis of peptide secondary structure by circular dichroism method showed that the composition of secondary structure elements is sensitive to pH value of solution.

6. Vitamin C & Vitamin K_3 system: cell media adaptation and cytotoxicity in normoxia and hypoxia *in vitro.*

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Vytautas Magnus University
Introduction: Vitamin C (VC, ascorbate) or vitamin K_3 (VK, menadione) can actively participate in extracellular and intracellular oxidation-reduction reaction, resulting in dehydroascorbic acid and semi/hydro-chinone formation respectfully. This transformation can generate big amount of free radical, ROS, H_2O_2, especially when intracellular systems can regenerate mentioned reaction products to initial substrates. This characteristic of VC and VK is even more expressed when VC+VK combination are used (synergism). VC+VK is a very potent anticancer system, that have good selectivity and efficiency towards cancer cells *in vitro* and *in vivo.* Nevertheless, mechanism of action has cracks/interpretation and are not well established.
Idea: We believe, that VC+VK system's cytotoxicity hides under multi-process mechanism, but one of the first component toward cells death – oxygen availability for fast extracellular H_2O_2 formation, possibly, followed by localized hypoxia.
Methods: Amplex Red® equipped with fluorimeter was used to specifically identify and evaluate formation of H_2O_2 in cell free media after supplementation with VC, VK, VC+VK substances. Incubator with oxygen level controller was used to modulate atmospheric oxygen level (creation of hypoxia condition - 0.2% O_2), for evaluation of VC, VK, VC+VK cytotoxicity, media adaptation to hypoxia. Clark type electrode with oxygen selective teflon membrane combined with home-made Petri dish measurements chamber was used for evaluating oxygen kinetic in media in experimental condition. Colony forming assay (using mouse hepatoma MH-22A cell line) was used as a model to evaluate cytotoxicity.
Results and discussion: Amplex Red® revealed that VC, VK and VC+VK generates H_2O_2 in cell free media. Generated amounts are similar between VC and VC+VK, but VK formed lower H_2O_2 level. This indicates that VC is main factor for generating main amount of H_2O_2 and no synergism can be seen if VC+VK supplements are used.
Measurements of kinetics of dissolved oxygen in cell free media in hypoxia, showed - around 60 min was needed for media to adapt to new atmosphere. But if media was supplemented with VC, two-phase process could be distinguished. First phase – media was drained out of oxygen in a similar speed as seen in not-supplemented adaptation. Second phase – much slower media adaptation/reaction. This led to believe that VC modulated amount of dissolved oxygen. Similar phases could be seen in media with VC+VK, but in a matter of phase speed

and total adaptation time, VC+VK was much effective/faster, indicating that VC+VK worked synergistically in process speed. VK had no significant effect on media adaptation speed. Furthermore, VC or VC+VK could induce short and local hypoxia in cell media that did not match with pre-set condition and oxygen difusion in incubator, possible because of oxygen consumption for H_2O_2 formation.
VC, VK and VC+VK were cytotoxic to MH-22A cell line in normoxia (atmospheric oxygen level) and in hypoxia (0.2% oxygen). Synergistic cytotoxicity detected in both oxygen level condition when VC+VK are used. Further, cells were more sensitive to cytotoxicity of VC, VK or VC+VK in normoxia condition, possibly because of more available oxygen could participated in generating more H_2O_2. We could not rule out, that VC or VC+VK cytotoxicity caused by reoxygenation after local hypoxia (possible even in normoxia condition).

7. The association between miR-328 expression of whole blood and myopia

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Purpose The aim of this study is to find associations between miR-328 expression of whole blood and myopia.
Methods 112 individuals (42 individuals with moderate or high myopia and 70 healthy individuals) were evaluated. RNA was extracted from peripheral blood samples and expression of miR-328 was assessed by using the Applied Biosystems 7900HT Real-Time Polymerase Chain Reaction System. Refractive error was measured with Sol. Cyclopentolate 1% using an autorefractor (Accuref-K9001, Shin-Nippon, Japan) and calculated by the mean spherical equivalent for each of the two eyes of every individual.
Results The $\Delta\Delta$CT values of miR-328 were compared between the myopic and the control groups. The results showed that $\Delta\Delta$CT values differ statistically significantly between these two groups ($p<0.05$). In myopia group, $\Delta\Delta$CT of miR-328 values are lower than in control group. We found that the area under the ROC curve (AUC) of miR-328 is 0.631, 95% CI 0.522-0.739. Sensitivity varied from 26.2 % to 73.8% and specificity from 34.3 % to 65.7%.
Conclusions miR-328 expression level of whole blood statistically significantly differ between myopia and control groups.

8. Influence of buckwheat leaves and blossom extracts on some antioxidant enzymes activities in mice brain

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Background. Buckwheat (*Fagopyrum esculentum*) is a herbaceous plant, which is known as a dietary source of protein with favorable amino acid composition, fibers, vitamins (B_1 and B_2), starch, essential minerals, and trace elements. Buckwheat grains and hulls contain components with biological activity, such as flavonoids and flavones, tannins, phytosterols, fagopyrins, etc. Flavonoids act as antioxidants inhibiting lipid peroxidation and attenuating damage inflicted by reactive oxygen species. A number of studies have shown that buckwheat possesses strong antioxidant activity, mainly due to high rutin content. Flavonoids in buckwheat decrease blood cholesterol, helping in the prevention of a high blood pressure. Rutin, composed of flavonol quercetin and disaccharide rutinose, has an anti-inflammatory and hypotensive effect.

The aim of this sudy. The present study was conducted to evaluate the effects of buckwheat leaves and blossom extracts on the enzymatic activities of superoxide dismutase (SOD) and catalase (CAT) in mice brain.
Materials and methods. Experiments were done on 4-6 weeks old outbreed mice. Activities of enzymes were determined after 21-day of Buckwheat extracts intragastrically administration. Control animals received the same volume of saline. Whereas buckwheat extracts were made in ethanol, second control group received the same volume of ethanol. The activity of CAT in homogenates of mice brain was determined by hydrogen peroxide reaction with ammonium molybdate, which forms a complex that absorbs light at 410 nm. The activity of SOD was determined according the inhibition of nitroblue tetrazolium reduction rate in the non-enzymatic phenazine methosulfate-nicotinamide adenine dinucleotide system assessed spectrophotometrically at 540 nm. The concentration of protein in the brain homogenates was measured by using the Warburg-Christian method. Results were expressed as the mean ± SEM.
Results. Our data showed that blossom as well as leaves extracts decreased SOD activity in brain of mice by 28% and 27% respectively as compared to the control group, while in ethanol treated brain of mice SOD activity found to be 17% lower. However, activity of CAT significantly increased after both buckwheat extracts treatment as well as under ethanol effect.
Conclusions. Our studies disclosed that repeated administration by Buckwheat blossom as well as leaves extracts has an impact on enzymatic activities of both antioxidant enzymes in brain of mice. The stimulating impact of ethanol on activities of both enzymes of mice brain has also been detected.

9. Transcriptomic analysis of human glioblastoma U87 cells in monolayer and multi-cellular spheroid cell culture models

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The aim of this study was to evaluate the changes in genome-wide gene and miRNA expression in human glioblastoma U87 cells after the transition from standard monolayer to multi-cellular spheroid cell culture conditions.
Microarray analysis revealed differentially expressed genes between 2D and 3D cultured cells. Next generation short non-coding RNA sequencing identified miRNAs with a significant expression change in cell spheroids. Data of these genome-wide expression analyses were validated using RT-qPCR.
Bioinformatical enrichment analysis of microarray data identified 25 significantly enriched KEGG functional gene groups which were divided into 4 major categories: immune response related; cell adhesion, metabolism, and other functional groups. Metabolic mapping analysis showed that main expression profile differences between 2D and 3D MCS model systems arise from upregulation of genes involved in autoimmune response, angiogenesis and cell adhesion mechanisms. Moreover, investigation of KEGG pathway maps led to the hypothesis that transcription in MCS is hypoxia-induced and regulated through a nuclear hormone receptor RORA, which is activated by cholesterol-derived ligands via activation of steroid biosynthesis pathway.
For the purpose of determining whether differentially expressed miRNA is responsible for altered gene expression, we generated correlation matrices of miRNA and their experimentally validated or *in silico* predicted target genes. Interestingly, we have shown that there is no significant inverse correlation of miRNA and their respective target gene expression profiles between 2D and 3D MCS cultured U87 cells.

10. Brain and liver mitochondria response to hyperthermia: changes in respiration system and enzymes activities

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Hyperthermia (HT) (40–43°C) in combination with chemotherapy or radiotherapy is used clinically for cancer treatment. HT increases effectiveness of chemotherapy in ovarian, colorectal, gastrointestinal and other cancers. It was believed that HT selectively kills cancer cells leaving healthy cells undamaged. However it appeared that different types of healthy tissue can be injured by HT as well, moreover their sensitivity to HT is different. More detailed knowledge about temperature effects in healthy tissues is needed in order to use HT treatment without damage of healthy tissues. We aimed to compare HT (40–47°C) effects on mitochondria (Mt) from healthy rat brain and liver tissues. For this purpose we compared Mt respiration with different substrates, and activity of respiratory system enzymes in rat liver and brain Mt.
Under normothermic (37°C) conditions Mt respiration in states 2 and 3 with either 0.5 mM pyruvate + 0.5 mM malate (P+M) or 0.5 mM glutamate + 0.5 mM malate (G+M) and activities of enzymes (glutamate dehydrogenase (GDH) and complex I, but not pyruvate dehydrogenase (PDH) complex) were greater in liver Mt in comparison to brain Mt.
Liver Mt were more sensitive to HT conditions – respiration in state 2 (V_2 is indicator of inner mitochondria membrane permeability) in liver Mt increased at lower temperatures in comparison to brain Mt, oxidizing P+M and G+M. Changes induced in state 3 respiration (V_3) also showed that liver Mt were more sensitive to HT – V_3 notably decreased at 40–42°C in liver mitochondria oxidizing G+M or P+M, while in brain Mt state 3 respiration was stimulated by HT with P+M and inhibited only at 47°C temperature with G+M as substrate. Uncoupled respiration rate V_{CCCP} with both substrates in brain Mt was not affected by HT, while in liver Mt V_{CCCP} was inhibited in the temperature range 42–47°C. Modular kinetic analysis showed, that proton leak across inner Mt membrane increased at lower temperature in liver Mt in comparison with brain Mt. HT did not have effect on complex I activity in brain Mt, but stimulated Complex I activity in liver Mt. Despite large differences in GDH at 37ºC in Mt from both tissues, the response of GDH activity to HT was similar - GDH activity in liver and brain Mt decreased dramatically even at 40°C temperature. HT did not affect PDH complex activity in mitochondria from both tissues, but activation of PDH complex at 47°C temperature was detected only in Mt from brain tissue. Different response of PDH and GDH activities to HT only partially explain differences in HT effects on respiration in Mt from liver and brain.
The obtained results provide evidence that large brain sensitivity cannot be related to energy metabolism in Mt, since Mt from liver tissue were more sensitive to HT.

11. Isoniazid resistant tuberculosis in Latvia

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Introduction: Despite of decreasing incidence of Multi-drug resistant (MDR) *Mycobacterium tuberculosis* (MT) in Latvia, it is still a serious medical and public health problem. Resistance to any of the first line drugs (isoniazid (INH), rifampin (RIF), and ethambutol (EMB)) makes the tuberculosis (TB) more difficult and expensive to treat. INH is one of the most effective first line anti-TB drug. The level of INH resistance among new TB cases in Latvia remains

stable, and it did not change significantly within ten year period: 28.2 % in 2007, 26.3% in 2012, 23.3% in 2015, and 25.2% in 2017. The application of molecular diagnostics reduces time for the detection of resistance, and allows to initiate adequate treatment earlier; therefore, the time of hospitalization could be reduced. While the average time for drug susceptibility testing (DST) is within 6 weeks range (2 – 8 weeks), the molecular test can be done in 2-3 days.
The aim of this study: was to analyze the susceptibility to INH in clinical TB isolates, and to compare the efficiency of molecular tests used for the detection of mutations associated with INH resistance.
*Material and method*s: MT cultures from TB patients were tested for INH susceptibility using automated mycobacterial detection system BACTEC MGIT 960 (Becton Dickinson) for liquid cultures and the absolute concentration method by growth inhibition on solid (Lowenstein - Jensen) medium. Molecular test - Line Probe assay (LPA) (Hain Lifescience) was performed for revealing of mutations in *kat*G and *inh*A genes associated with the resistance to INH.
*Results***:** In total, DST using phenotyping methods was performed for 1536 MT-positive cultures, which were obtained in the time frame from 2015 till 2017. Among them, 1296 were newly registered and 240 were relapse/reinfection TB cases. In total, INH resistance was found in 412 (26.82%) cases: in 312 (24.07%) of newly diagnosed patients, and in 100 (41.67%) of previously treated patients. Both LPA and DST tests were performed for 141 MT DNA isolates. Mutations in *kat*G and/or *inh*A genes were detected in 104 (73.76%) cases, and concordance between molecular and phenotypic testing methods was 94.33% (133 cases of 141) for INH resistance. The discordance between tests was observed for 8 MT clinical isolates, and it could be mainly attributable to the absence of known INH resistance mutations in these samples.
Conclusions: 1. Level of INH resistance was higher in the relapse/reinfection TB cases.
2. Use of molecular diagnostics methods significantly reduces time for detecting of INH resistance. However, both molecular and phenotypic test results should be considered for the accurate diagnosis of MDR TB due to the existence of unknown INH-resistance mutations.
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12. Generation of Carbonic anhydrase IX knockout cancer cell lines using CRISPR/Cas9 system

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Enzyme CA IX is present only in a limited number of normal human tissues, whereas its ectopic expression is strongly associated with many frequently occurring hypoxic tumors. CA IX contributes to maintaining the cellular pH balance in cancer cells under hypoxic conditions, therefore, CA IX is being investigated as a cancer cell surface marker and therapeutic target. At the Department of Biothermodynamics and Drug Design (VU IBT), novel inhibitors of CA IX are designed and their efficiency is studied using human cancer cell lines. The inhibitors must exhibit high target selectivity and the most convenient strategy is testing them on cell lines, which have lost CA IX protein due to CA IX gene disruption. The aim of this research work was to knockout CA IX gene in A549 and MCF-7 cell lines using CRISPR/Cas9 genome editing system. In order to improve transfection efficiency in A549 cells, three CA IX specific gRNA fragments were cloned into three puromycin resistance gene carrying vectors. After transfection of CRISPR/Cas9 plasmids, singe cell clones were isolated, and the clonal populations were expanded. Screening for CA IX gene editing using PCR, revealed no gene alterations in MCF-7 clones. However, in one of the A549 clones, the desired deletion of the gene was identified. CA IX knockout in this clone was confirmed by sequencing, Western

blotting and immunofluorescence microscopy. A549 CA IX knockout cells did not exhibit any changes in morphology and viability, as compared to wild type A549 cell line, implying on the absence of the essential off-target genome editing effects. Newly generated A549 CA IX knockout cell line will be a useful tool in enzyme functional studies and will serve as a negative control in CA IX inhibition assays.

13. Construction and characterization of self-assembling bacteriophage-like particles in yeast

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Statement of the Problem: Virus-like particles (VLPs) have evolved and became widely accepted tools, especially in the field of vaccinology. In fact, some VLP-based vaccines are currently used as a commercial products, while other VLP-based products are at different stages of clinical studies. Several remarkable advantages have been achieved in the development of VLPs as gene therapy tools and new nanomaterials. While icosahedral VLP platforms have been studied in detail, but rod-shaped VLPs have been mostly forgotten. Nevertheless, many icosahedral VLPs are synthesized in bacteria and because of its prokaryotic nature the misfolding of eukaryotic proteins and contamination of purified VLPs with bacterial endotoxins are encountered. Until now, there is no information regarding the generation of tailed-bacteriophage rod-shaped structures in yeast protein synthesis system, which is well established for biotechnological generation of products for human use.
The aim: Continuously increasing interest in different aspects of VLP-based technologies inspired us to expand the knowledge of yeast-expressed bacteriophage tail tube and tail sheath protein self-assemblage into rod-shaped structures and characterize their morphology.
Methodology: DNA sequences coding the tail tube or tail sheath proteins of *Escherichia coli* bacteriophages NBD2 and FV3 as well as *Klebsiella* sp. phage RaK2 were cloned into yeast protein expression vector. The synthesis of recombinant phage proteins was confirmed by protein electrophoresis. Later, rod-shaped structures were sucrose-purified and analyzed by transmission electron microscopy.
Findings: Our work has focused on synthesis of tail sheath proteins gp041 from RaK2, gp053 from FV3 as well as the tail tube protein gp39 from NBD2 bacteriophage in yeast. It was found that in vivo recombinant bacteriophage structural tail proteins in the absence of other phage proteins, self-assemble into tubular structures with different surface morphology. Yeast-expressed tail sheath proteins from RaK2 and FV3 self-assemble into non-flexible comparatively short rod-like structures. However, yeast-expressed tail tube protein from NBD2 bacteriophage sell-assembles into highly-organized extremely long and flexible structures. We demonstrated that rod-shaped structures formed by gp29 from NBD2 bacteriophage are extremely stable at different environmental conditions. To our knowledge, it is the first attempt to produce bacteriophage-originated rod-shaped structures in yeast and characterize their structural morphology. Our findings offer the new tailed-bacteriophage originated tubular structures for innovative biomaterials or potent vaccines for newly emerging diseases.
Conclusion & Significance: This work intends to show the suitability of yeast protein synthesis system to generate high-yields of stable, long and flexible rod-shaped structures originated from *Escherichia coli* infecting bacteriophages NBD2 and FV3 as well as *Klebsiella* sp. bacteriophage RaK2.

14. Clinical utility of blood-circulating androgen receptor variants in castration-resistant prostate cancer patients

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Introduction: Despite the latest progress in prostate cancer (PCa) treatment, castration-resistant prostate cancer (CRPC) remains incurable and is attributed to the highest mortality rates in PCa. Treatment resistance mechanisms are inseparable from androgen receptor (AR) signaling axis, which remains active in CRPC. One of the reactivation mechanisms might be AR splice variants (AR-Vs) that lack ligand binding domain and are constitutively active. Residing not only in tumors but also in blood-circulating tumor cells, PCa-specific AR-Vs might reflect tumor development, disease progression and response to treatment, and therefore could serve as the non-invasive PCa monitoring tools. The aim of our study was to develop novel AR-Vs-based molecular tool to non-invasively diagnose PCa, predict its progression and response to treatment directly from the blood sample.
Materials and methods: 176 PAXgene RNA blood samples were collected from 96 CRPC patients (66 cases have serial samples) during 2016-2018. The response to treatment was rated as a Response, Progression and Death (N=53, 16 and 28, respectively). For the detection in patients' blood, custom made TaqMan assays for AR-V1, V3, and V7 were used for target-specific reverse transcription, pre-amplification and real-time PCR.
Results: AR-V1, -V3 and -V7 were detected in 27 (15%), 68 (39%), and 70 (40%) samples, respectively. 70 samples were AR-Vs-free, and 10 contained all variants tested. After the cases were stratified according to response to treatment, the levels of AR-Vs had a tendency to be lower in responder's blood and higher in the blood of progressing disease cases. Furthermore, V1 was independent prognostic marker for progression-free (P=0.0350) and overall survival (P=0.0914).
Conclusion: Blood-circulating androgen receptor variants can serve as the non-invasive biomarkers for reliable prediction of PCa recurrence and response to treatment.

15. The impact of C1q on Wnt pathway and PPAR-δ activation related to breast cancer cell viability

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It has been recently shown that plasma concentration of C1q protein increases with age and is capable of triggering the Wnt signaling pathway that in turn is known to play an important role in cancer development. Transcription factor PPAR-δ mediates the increased lipid oxidation and higher capability of cancer cells to avoid the apoptosis. In this work we experimentally examined the hypothesis that C1q induce the Wnt-dependent up-regulation of PPAR-δ. The ability of activated Wnt pathway to evoke resistance to apoptosis induced by loss of attachment was evaluated.
PolyHEMA coated cell culture dishes were used for low attachment conditions. Cell viability was measured using Annexin V/ 7-AAD reagent kit and mitochondrial Δψm was measured using JC1 dye by flow cytometry.
Our results showed that activation of Wnt pathway with C1q increased β-catenin and PPAR-δ protein levels in breast cancer cells. Lower rate of apoptosis was determined in C1q treated cells under low-attachment conditions. C1q treatment as well as low-attachments conditions altered mitochondrial Δψm.

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16. Changes in germination, seedling growth, phytohormone content and proteome induced in *Helianthus annuus* by pre-sowing seed treatment with stressors

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Seed treatment with cold plasma (CP) or electromagnetic field (EMF) is a modern eco-agricultural technology for stimulation of plant germination and performance. Numerous studies demonstrated the effectiveness of such treatments for enhancing germination and growth of a large variety of crops, however the molecular basis of seed response to treatments remains elusive.
In order to gain an insight into the molecular mechanisms underlying effect of stressors on plant seeds, we estimated changes induced in differential protein expression of the model plant – sunflower (*Helianthus annuus*). We studied the effects of pre-sowing seed treatment, using vacuum (7 min), radio-frequency EMF (5-15 min) and CP (2-7 min), on germination and growth of the confectionary cultivar "Nyķrségi fekete". The germination tests were performed both *in vitro* and in substrate and the obtained results indicated that the treatments with CP and EMF had no effect on germination yield while vacuum and EMF (10 and 15 min) treatments increased germination rate. We demonstrate that seed treatments with CP induce decrease in seed phytohormone content – decrease in content of abscisic acid and increase in content of gibberelins. Treatments had effect on morphology of seedlings that developed from the CP (7 min) and EMF (15 min) treated seeds resulting in reduced height/weight of stems and increased weight of leaves, respectively.
Differential protein expression in the leaves of sunflower seedlings was assessed using 2D gel electrophoresis. Among the 104 differentially expressed proteoforms significantly higher abundance of 49 and 38 proteoforms and reduced abundance of 26 and 33 proteoforms was detected for the CP and EMF treated experimental groups, respectively. Hierarchical clustering revealed 3 major protein expression groups: two of the groups included majority of the proteoforms that had similarly increased or reduced abundance after the CP or EMF treatment. Meanwhile, another group represented 8 proteoforms that were differentially expressed between the CP and EMF experimental groups. Mass spectrometry analysis revealed several proteins involved in primary and protein metabolism or stress response, however chloroplast proteins with function involved in photosynthesis were dominant among the 40 identified proteoforms. The results suggests that the effect of CP and EMF treatment has important effect on plant development that is the most prominent in photosynthesis processes at the seedling stage. These findings are confirmed by estimation of photosynthetic efficiency and photosynthetic performance index.
Keywords: Cold plasma, Electromagnetic field, Germination, *Helianthus annuus*, Photosynthesis, Phytohormones, Proteome, Stress response.

17. The role of Rab14 in HeLa cell division and invasiveness

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Even though the cell division mechanism has not been fully elucidated yet, it is well known that small Rab GTPases play an important role in cytokinesis by regulating multiple steps of mitosis (Miserey-Lenkei S., 2016). Specialized functions of various Rabs, including Rab 5, 6,

11, 21, 24, 35 in recirculating endosome trafficking as well as in regulating cellular division, are already well-studied; however, the impact of Rab14 on cell division has not clearly defined yet. The presence of Rab14 protein at the intercellular bridge raised the hypothesis that Rab14 might participate at the last stage of cell division. Therefore, the aim of this study was to evaluate the role of Rab14 in HeLa cell division and invasiveness.
Cell division was evaluated by immunocytochemistry, and cell mobility was assessed by a wound healing assay. HeLa cells with different Rab14 expression levels were used in this study: HeLa-Rab14-KD cells with a reduced expression of Rab14 protein; HeLa-Rab14-KO, with no expression of Rab14 protein; and HeLa-FLAG-Rab14-Q70L, with an overexpressed Rab14 protein in its active, GTP-bound state.
The results showed that the down-regulation of Rab14 disrupted HeLa cell division and as a result, the amount of multi-nucleated cells in HeLa-Rab14-KD and HeLa-Rab14-KO cells was increased. Meanwhile, the invasiveness assay showed that reduced Rab14 expression inhibited cell mobility. On the other hand, the overexpression of Rab14 did not affect cell division, but still increased HeLa cell mobility. Moreover, HeLa-FLAG-Rab14-Q70L cells were more likely to form membranous tunneling nanotubes (TNTs) than control cells, while in HeLa-Rab14-KD and HeLa-Rab14-KO cells, the formation of TNTs was significantly reduced. In addition, upregulated Rab14 expression was determined upon the activation of Wnt signaling pathway, which led to increased multi-nucleation in the dividing HeLa cells.

18. Characteristics and differentiation profiles of human stem cells isolated from amniotic fluid of healthy and pathological pregnancies

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Human amniotic fluid is a relatively new and very attractive source of mesenchymal stem cells (MSC) which have the potential to be used for therapeutic purposes. The aim of this study was to characterize amniotic fluid MSCs (AF-MSC) derived from both healthy and pathological gestations. AF-MSCs were obtained using a two-stage isolation protocol, expanded in a monolayer culture where the typical spindle shaped morphology was observed. AF-MSC were characterized by cell surface marker expression, such as typical mesenchymal markers CD44, CD90 and CD105, and negative for CD34, which represents hematopoietic cell marker, We demonstrated that isolated AF-MSCs exhibited expression of specific stemness markers associated with pluripotency, such as Oct4, Nanog, Sox2 and Rex1. Under appropriate conditions these stem cells proved to be capable to differentiate towards adipogenic, osteogenic, neurogenic and myogenic lineages, as determined by morphological changes and expression of lineage-specific genes. During differentiation AF-MSCs display differential expression of microRNAs, it was observed that miR17 and miR21 were both downregulated, while miR34a and miR146a were upregulated during myogenic and neurogenic, downregulated during adipogenic and differentially expressed during osteogenic differentiation when comparing healthy and pathological samples. The results of this study provide new insights about the nature of amniotic fluid stem cells derived from healthy and fetus affected gestations, their characteristics and differentiation capabilities.

19. Protein dynamics governing the function of the DegP serine protease-chaperone

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Protein quality control is one of the essential functions found in cells from all kingdoms of life and it is achieved by the interplay of dedicated proteases and chaperones. The dysregulation of the protein quality control machinery can lead to cell death and often it is found at the foundation of diseases such as cancer, age-related disorders or neurodegenerative diseases like Parkinson's or Alzheimer's [1]. The HtrA protein family (high temperature requirement A) is a group of heat-shock induced serine proteases, which are widely spread across different species [2]. One of the bacterial HtrA protein family members is the homo-oligomeric DegP-protease, playing a crucial role in the biogenesis and degradation of β-barrel outer-membrane proteins within the periplasmic space of gram-negative bacteria [3]. Although it is known that DegP is an essential protein in E.coli during elevated temperature conditions [4], its functional and structural details are so far only partially understood.
The aim of our study is to track the molecular changes of different DegP oligomeric states at the atomic level underlying its function by using advanced NMR techniques and consequently decipher DegPs role in the assembly and maintenance of bacterial outer membrane proteins. Since the size of DegP can vary from 50 kDa to 1 MDa, sophisticated isotope labeling schemes are used to obtain high-resolution NMR spectra.
Our preliminary results from 2D [15N,1H]-TROSY-HSQC as well as 2D [13C,1H]-HMQC spectra indicate an unexpected degree of dynamics of structural elements of DegP within its hexameric state. Furthermore, we initially observed differences in chemical shift within the obtained NMR-spectra of the different oligomeric states studied, possibly indicating structural changes upon transition between the oligomeric states. The observation of possible structural adaptions seems to be modulated by temperature and might prove to be a crucial aspect for regulating DegP activity.
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20. Emergence of Visible Light Optical Properties of Phenylalanine Aggregates

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The ability of phenylalanine to form fibrillar nanostructures was demonstrated on multiple occasions and such an oligomerization reaction could be the cause of cytotoxicity in patients with phenylketonuria. These findings were quickly followed by claims that these fibrils have amyloid-like properties and can be detected through the use of thioflavin T, an amyloid-specific fluorescent dye. However, these experiments did not take into consideration the possible phenylalanine molecule aromatic ring interactions, resulting in pi-stacking aggregates, which have been shown to exhibit visible light spectrum fluorescence. These structures would also have the properties of J-aggregates, resulting in a fluorescence emission red-shift. Such an occurrence could give false positive results during a thioflavin-T assay and lead to a false identification of amyloid-like fibrils.
In this work we monitored the formation of phenylalanine pi-stacking aggregates under a wide range of concentrations and examined their fluorescence emission spectra, how it changed during the aggregation process and how it was influenced by the aggregate size. We also demonstrate that the visible light fluorescence spectrum partially overlaps with the emissions of ThT bound to amyloid fibrils.

21. Cleavage of N4-acetyl-cytidine-modified DNA by Type II restriction endonucleases

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Nucleic acids containing modified nucleobases are of great interest and find a wide variety of applications in the field of biotechnology. The most suitable positions for base modifications are C5 of pyrimidines and C7 of 7-deazapurines, however, little is known about altering other positions of nucleobases such as C6/C8 or O4/N4. Here, we report on the enzymatic synthesis of N4-acetyl-2'-deoxycytidine-containing functionalized DNA and protection of such DNA against cleavage by restriction endonucleases. We find that N4-acetylcytidine is efficiently incorporated into DNA by a proofreading phi29 DNA polymerase at multiple positions. In fact, N4-acetylcytidine is suitable for the synthesis of both short and long modified-DNA fragments during primer extension (PEX) as well as conventional isothermal amplification procedures, respectively. We also demonstrate the influence of the modification on cleavage by diverse type II restriction endonucleases that recognize and cleave sequences containing one, two or three cytosines. Five out of six restriction endonucleases tested were blocked by N4-acetylcytidine-modification. Moreover, we show that both DNA strands are protected from the cleavage. The control sequence bearing none cytosines, yet with modifications outside of the recognition site, was perfectly cleaved. Interestingly, XbaI cleaved the modified short PEX product, however had no effect on the hydrolysis of long modified DNA produced during isothermal amplification. Therefore, it seems that phi29 DNA polymerase is able to generate DNA containing N4-acetylcytidine-modifications on both strands that is resistant to cleavage by XbaI. These results allowed investigation of the competitive incorporation of N4-acetylcytidine in the presence of its natural counterpart. The assay was based on the fact that specific restriction endonucleases cleave the nonmodified DNA, whereas the DNA containing a modified recognition sequence remains intact. The competitive incorporations of N4-acetylcytidine in a ratio 1:1 with cytidine showed that the modified cytidine was worse substrate than dCTP, giving 37 % of incorporation of the modified nucleotide. In contrast, when using an excess of N4-acetylcytidine (10:1), we observe a major portion of modified DNA (61 %). The results are useful both for protection of specific sequences from cleavage by restriction enzymes and for studying the competitive incorporations of various modified nucleotides.

22. Resistance to antibiotics of culturable soil bacteria and whole soil from ecological and intense farming sites

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Antibiotic resistance has become a major clinical and public health problem. At the moment, there is no antibiotic left that did not have a case of resistant microorganism reported. The resistance of non-pathogenic soil microorganisms to modern day antibiotics has come into notice in the recent years. The ecology, evolution and development of natural resistome and its possible spread to humans and animals is an area of interest and concern.
In this study we aimed to evaluate and compare the resistomes of bacterial populations in several farming environments and distinguish the influence of human interference. This could help indicating the possible routes of antibiotic resistance gene dissemination. Antibiotic resistant bacteria were isolated from three intensive and three of ecological farmingsoils. The

minimum inhibiting concentration (MIC) values of antibiotic resistant bacteria were tested. Clinically important genes responsible for enzyme-mediated resistance to β-lactams (including 3th and 4th generation of cephalosporins, carbapenems), quinolones, aminoglycosides and transferable genetic determinants integrons were investigated in culturable bacteria and total DNA extracted from the same soils.
Antibiotic-resistant culturable bacteria from all tested soils were mostly comprised of Pseudomonas genus. Most of the isolates were highly resistant to antibiotics, on the average to 9.8 antibiotics for the six soils analyzed. Interestingly, only a few aminoglycoside and β-lactam resistance genes of clinical importance were detected by PCR in the culturable bacteria, despite the high phenotypic resistance. Similar results were observed when total soil DNA was screened. The mechanisms responsible for antibiotic resistance of soil bacteria differ from those observed in clinical settings and should be investigated as a potential source of new antibiotic resistance genes.
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23. Oxidative phosphorylation activity changes in treatment resistant acute myeloid leukemia

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Acute myeloid leukemia (AML) is a heterogeneous disease characterized by the rapid growth and division of abnormal myeloid blood cells called myeloblasts. The differentiation of myeloblasts is blocked, thus, they accumulate in the bone marrow and peripheral blood. The prognosis of AML, especially in older patients, is quite poor with approximately two thirds of patients relapsing after treatment. Various studies have shown that certain cancers, including some AML subsets, have upregulated oxidative phosphorylation (OXPHOS). Thus, OXPHOS inhibitors could be used to target such cells. To evaluate the potential of OXPHOS inhibitors for the treatment of resistant AML, we studied the effect of two OXPHOS inhibitors Atovaquone and Metformin on treatment resistant AML patients' blood cells exhibiting different OXPHOS activities. AML blood cells were treated with OXPHOS inhibitors alone and with their combinations with Cytarabine (cytosine analog) and Venetoclax (BCL2 inhibitor). After treatment, cell oxygen consumption rate (OCR), ATP production rate, and glycolysis rate were evaluated by Seahorse XF Cell Mito Stress assay. Cell proliferation and viability was assessed using trypan blue exclusion test. Apoptosis associated gene and protein expression changes (such as p53, p21, BAX, Bcl-2) were tested by RT-qPCR and Western blot respectively. The results demonstrate that cell treatment with Metformin alone and in combinations with Cytarabine and Venetoclax strongly lowered OCR and inhibited ATP production in tested AML patients' blood cells. Meanwhile, Atovaquone, did not have such effect. Even though Metformin successfully inhibited OXPHOS, cell proliferation still was not inhibited in a marked difference. This means that OXPHOS inhibition in treatment resistant AML is not sufficient and combined metabolism targeted therapies (targeting not only OXPHOS but also other metabolic processes) should be tested.

24. Global gene expression change during Totiviridae dsRNA viruses infection in *Saccahromyces cerevisiae*

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In this study, we addressed the impact of dsRNA viruses on transcriptional status of native strain M437 bearing L-A-lus and M-2 viruses [L+M+]. These viruses are obligate endosymbionts, whose reproductive success is intimately tied to that of their hosts. The coinfecting M virus contains a single open reading frame coding for a precursor of the secreted toxin. M virus also confers immunity against the toxin to the host cell but is dependent on the L-A virus for its replication, transcription, and encapsidation. Prolonged infection of yeast with L-A-lus and M-2 viruses resulted in versatile coadaptation of viruses and host, thus the strains representing virus-naive conditions - either M-2 free or L-A-lus and M-2 free—were prepared manually. In such a way, transcriptional alterations in wild type strain M437 were described from the perspective of dsRNA-cured cells. We measured transcript levels using RNA-Seq, a robust and an extremely sensitive standard for analysis of global gene expression. The presence and absence of dsRNA virus in particular yeast strains were confirmed by functional tests and verification of the corresponding dsRNA by RT-PCR. We have demonstrated that curing cells from either M-2 or L-A-lus simultaneously with M-2 resulted in moderate alterations of host gene expression. Using Gene Ontology database differently expressed genes were established to term enrichment analysis. We detected host genes and processes involved in M2 dsRNA and L-A-lus virus maintenance and propagation as well as those important for virus-host interaction.
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25. Mechanism of two(four)-electron reduction of nitroaromatics by oxygen-insensitive nitroreductases: the role of a non-enzymatic reduction step

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Oxygen-insensitive NAD(P)H:nitroreductases (NR) reduce nitroaromatics (Ar-NO2) into hydroxylamines (Ar-NHOH) through nitroso (Ar-NO) intermediates. Ar-NO may be reduced both enzymatically and directly by NAD(P)H, however, it is unclear which process is predominant in catalysis of NRs. We found that E. coli NR-A (NfsA) oxidizes 2 moles of NADPH per mole of 2,4,6-trinitrotoluene (TNT) and 4 moles of NADPH per mole of tetryl. Addition of ascorbate, which reduces Ar-NO into Ar-NHOH, changes the stoichiometry NADPH/Ar-NO2 into 1:1 (TNT) and 2:1 (tetryl), and decreases the rate of NADPH oxidation. Ascorbate does not interfere with the oxidation of NADPH during reduction of quinones by NfsA. Our analysis of ascorbate inhibition patterns and both enzymatic and non-enzymatic reduction of nitrosobenzene suggests that direct reduction of Ar-NO by NADPH rather than enzymatic reduction is the predominant mechanism during nitroaromatic reduction.

26. Electron transfer reactions of Plasmodium falciparum ferredoxin-NADP(+) reductase with nonphysiological oxidants

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Ferredoxin – NADP(+) reductase (EC 1.18.1.2, FNR) is primarily a photosynthesis enzyme responsible for electron transfer between ferredoxin and NADPH. Recently this enzyme was isolated from apicoplast containing protozoans with malaria causing *Plasmodium falciparum* being amongst those. Here we examined the steady state kinetics of PfFNR C99A mutant with various nitroaromatic and quinone compounds with a spectrophotometer and a Clark electrode. The enzyme acts according to a „ping-pong" mechanism. The log k_{cat}/K_M increases linearly for nitroaromatic compounds with increasing single electron redox potential with the apparent second order rate constants (k_{cat}/K_M) ranging from 5.5×10^1 $M^{-1}s^{-1}$ for nitrobenzene to 6.8×10^5 $M^{-1}s^{-1}$ for N-methylpicramide. Quinones exhibit a parabolic dependence with juglone having the highest second order rate constant of 6.8×10^5 $M^{-1}s^{-1}$ and their reactivity in general is markedly higher than that for nitroaromatics. Several aromatic *N*-oxides, namely an antitumor agent tirapazamine and its derivatives, were also tested and found to be comparable with quinones yet one order more reactive than nitroaromatics. These findings are in line with the „outer sphere" electron transfer model. We studied the dependence of the second order rate constants on the pH and ionic strength of the medium. The log k_{cat}/K_M increases slightly with the medium becoming less acidic for nitroaromatics and quinones, although there is a decrease with increasing pH for ferycianide with enzyme exhibiting substrate inhibition in acidic conditions (pH 5.5 – pH 6.5). There is poorly expressed bell-shape dependence on the ionic strength of the solution. Time-course of reduction of nitroaromatics in the presence of NADPH regeneration system under the absence of external oxygen supply shows the reaction rate increasing with depletion of oxygen in the solution. Inhibition studies with $NADP^+$ used an inhibitor shows competetive inhibition versus NADPH (K_M^{app} ca. 40 μM) and uncompetetive inhibition versus quinones (juglone was used in the experiments).

27. Elevated acylcarnitine levels induce insulin insensitivity by decreasing phosphorylation of insulin receptor β and downstream signalling

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The accumulation of long-chain acylcarnitines has been linked to insulin resistance and prediabetes, but the molecular mechanisms involved in the development of insulin insensitivity are still not completely characterized. The aim of present study was to investigate the molecular mechanisms of long-chain acylcarnitine action on insulin signalling pathway.
Single intraperitoneal palmitoylcarnitine (PC) dose (50mg/kg) was injected to 5 fed male CD-1 mice, insulin (0.3 IU/kg) was administrated 30 min later. After 1 h of initial PC administration, hindlimb skeletal muscles were collected for Western blot, qRT-PCR, and acylcarnitine content analysis. CHO INSR1248 cells were incubated for 10 min with PC (10 μM) followed by insulin stimulation (10 nM for 10 min). To study molecular mechanisms, protein phosphorylation was determined by Western blot analysis using antibodies against phosphorylated protein kinase B (Akt), insulin receptor β (IRβ) and glycogen synthase kinase 3 β (GSK3β).
Single in vivo administration of PC increased fed mice muscle acylcarnitine content by 3-fold, corresponding to acylcarnitine levels detected after short term fasting. PC administration increased insulin levels and induced glucose intolerance, as well as decreased Akt phosphorylation in muscles. The administration of insulin overcame the PC-induced effects. In muscle tissue PC did not change the expression of genes involved in glucose transport (GLUT1, GLUT4) and fatty acid metabolism (CPT1A, CPT1B, ACSL, PDK4). Additional in vitro experiments in CHO cells revealed, that phosphorylation of IRβ at Tyr1150/1151, GSK3β at Ser 9 and Akt at Thr 308 and Ser 473 were decreased after PC exposure.

In conclusion, our findings show that accumulation of long-chain acylcarnitines can induce insulin resistance by decreasing phosphorylation of IRβ and all intermediate kinases downstream to Akt required for activation of insulin-stimulated glucose uptake. Long-chain acylcarnitine-induced effects on glucose metabolism are not related to changes in gene expression but rather to downregulation of insulin signalling. These findings confirm the regulatory role of long-chain acylcarnitines during transition from a fed to fasted state in order to limit glucose metabolism during the fasting.

28. Characterization of Pantoea sp. infecting broad-temperature range siphovirus vB_PagS_MED16

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A novel broad-temperature range Pantoea phage vB_PagS_MED16 (MED16) has been isolated in Lithuania from thicket shadbush. Based on TEM results, phage MED16 belongs to the family Siphoviridae and has an isometric head (B1 morphotype) about 64 nm in diameter and a non-contractile flexible tail about 145 nm in length. The host range determination test revealed that out of 20 bacterial strains tested, only Pantoea agglomerans isolate BSL is sensitive to MED16. Plating tests revealed that phage can form plaques in the temperature range of 4 to 37°C. The 46.103 bp genome of MED16 has a G+C content of 55.1% and contains 71 probable protein encoding genes and no genes for tRNA. Comparative sequence analysis revealed that 25 out of 71 MED16 ORFs encode unique proteins that have no reliable identity to database entries. Based on homology to biologically defined proteins, 27 ORFs of MED16 have been given a putative functional annotation, including genes coding for structural proteins as well as those associated with DNA metabolism, morphogenesis and phage-host interactions. Phylogenetic analysis, based on the alignment of the essential structural and functional genes, revealed that phage MED16 is the most closely related to phages infecting bacteria from the genera Esherichia, Edwarsiella, Cronobacter, Klebsiella, Lactococcus, Shigella and Salmonella but no close phylogenetic relationship between MED16 and phages infecting Pantoea sp. has been observed. In addition, based on the results of comparative genome sequence analysis conducted during this study, bacteriophage MED16 cannot be assigned to any genus currently recognized by ICTV and potentially represents a new genus within the family Siphoviridae. Thus, the data presented here will expand our knowledge of the genetic diversity and evolution of Pantoea phages, especially Pantoea-infecting siphoviruses, and their phylogenetic relatedness to other bacteriophages.

29. Identification of hypermodified nucleotides in the genomic DNA of bacterial viruses

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The largest variety of modified nucleobases observed in nature are found within genomes of bacteriophages. The DNA modifications of phages vary from simple methylation in the specific positions to the complete replacement of traditional nucleotides by hypermodified bases. The variety of DNA modifying enzymes encoded in genomes of phages can be adapted for different applications in vitro. In order to expand current knowledge of hypermodified nucleotides in the genomic DNA of bacteriophages, and to explore biotechnological potential of DNA-modifying proteins, we performed the genomic DNA (gDNA) restriction analysis of bacteriophages RB69, VR5 and Vid5, and used bioinformatics

methods to search for homologues of the DNA-modifying proteins of RB51, RB69, VR5 and Vid5 phages.
Restriction analysis of RB69, VR5 and Vid5 gDNAs confirmed the presence of potential modifications as follows: methylation and glycosylation in the case of RB69 and VR5, and deoxyarcheosine presented in the genome of bacteriophage Vid5. Bioinformatic analysis revealed that α-glucosyltransferase is encoded in the genome of bacteriophage RB51, while VR5 and RB69 genomes encode hydroxymethylases and potential manosyltransferases. The cluster of genes encoding proteins that carries out modifications of guanosine was identified in the genome of phage Vid5 including putative queosine tRNA ribosyltransferase, which has been selected for more detailed studies.
The genes of potential DNA-modifying proteins, including α-glucosyltransferases of bacteriophages RB51 and T4, potential manosyltransferase of phages VR5, and putative queosine tRNA ribosyltransferase of phage Vid5, were cloned into an expression vectors, containing a histidine affinity tag. It was determined that the recombinant α-glucosyltransferases are UDP-glucose-specific enzymes active in vitro. Meanwhile, the in vitro activity assays of the other enzymes are yet to be optimised. Either way, this work significantly broadens our current knowledge of hypermodified nucleobases in the genomic DNA of bacteriophages.

30. Effect of blockers on ion channels in the tonoplast of Nitellopsis obtusa investigated using patch clamp technique

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Plant membrane transport systems are responsible for a variety of important physiological functions. Among various electrophysiological methods patch clamp technique is particularly useful for researching the behavior of single ion channels in real time in near-physiological conditions. Much knowledge about the function of ion channels can be gained by applying various ion channel blockers. For plant electrophysiological investigations internodal cells of giant algae Nitellopsis obtusa provide a unique model system, well suited for the patch clamp approach.
The plant cell wall prevents access to the plasma membrane but cytoplasmic droplet technique overcomes this problem by exposing vacuolar membrane – the tonoplast. A Nitellopsis cell is placed vertically in a solution approximately isotonic with the cell sap, and cut. The cell sap then flows out of the cell, in a bath solution spontaneously forming spherical cytoplasmic droplets that consist of cytoplasm covered by the tonoplast.
A microelectrode can be pushed against the tonoplast, forming a high resistance (up to 40 GΩ) seal. Currents passing through ion channels in the sealed area can be easily detected in high resolution. Thus patch clamp method enables analysis of electrical activity of ion channels that represents conformational changes of single molecules in real time.
In this investigation two cation channel blockers were applied in tonoplast-attached configuration: Cs+ ions and tetraethylammonium chloride (TEA). Cs+ ions diminished amplitudes of inward currents flowing through found 70 pS ion channels. The effect is attributed to fast ion channel flickering induced by Cs+ ions constantly associating and dissociating from the channel pore. TEA diminished current amplitudes in the interval of voltages from -20 mV to -100 mV. TEA also diminished the conductance of 70 pS channels to 60 pS.
Cs+ ions and TEA block K+ ion channels therefore 70 pS ion channels are presumed to be K+ channels.
The research was partly supported by Research Council of Lithuania (Project No. 09.3.3-LMT-K-712-03-0008).

31. Enzymatic synthesis of AdoMet analogues

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S-Adenosylmethionine (AdoMet) is the second most commonly used (after ATP) cofactor in cells. It mostly acts as a methyl group donor, which is of utmost importance in epigenetic regulation, where both nucleic acids and proteins are methylated by specific methyltransferases using AdoMet. However, methylation is hard to detect. Biotechnological approach termed methyltransferase-directed Transfer of Activated Groups (mTAG) addresses this issue. It utilises methyltransferases capable of transferring chemically active groups from unnatural AdoMet analogues onto their usual substrates. These modifications can then be readily probed and observed. This method has been widely used for in vitro research. However, in vivo application is still challenging. AdoMet and its analogues are not readily absorbed by cells. Thus AdoMet synthetases – the enzymes that naturally synthetize AdoMet from methionine and ATP in cells - were engineered to synthetize the aforementioned AdoMet analogues from corresponding methionine analogues in live cells. In contrast to final cofactors, methionine analogues are easily absorbed by cells from media. However, the engineered AdoMet synthetases still preferentially synthetize AdoMet in the presence of methionine and its analogues, which may result in artefacts when the method is applied for epigenetic research.
The goal of our ongoing study is to investigate recombinant Mus musculus MAT2A AdoMet synthetase mutant variants for their ability to synthesize AdoMet cofactor analogues in the presence of competing natural substrate, which will pave the way for mTAG application for epigenetic research in vivo.

32. DNA N-glycosylase and TET activity with 5-alkylcytosines

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Methylation of cytosine at the 5th position, resulting in 5-methylcytosine, is a well-characterized natural DNA modification. In Eukarya it is one of several epigenetic mechanisms used by the cells to control gene expression. Methylation is introduced by specific DNA methyltransferases and can be actively removed by demethylation pathway comprising TET dioxygenases and base-excision repair mechanism. Biotechnological approach has employed engineered DNA methyltransferase reactions to site-specifically attach longer carbon chains to DNA, thus producing 5-alkylcytosines. The developed method is called methyltransferase-directed Transfer of Activated Groups, or mTAG. It serves as a molecular tool for selective covalent DNA labeling for studies ranging from optical genotyping to whole genome epigenetic research. We examine whether the 5-alkylcytosines can be recognized and eliminated by base excision repair enzymes or the active DNA demethylation pathway, similarly to the 5-methylcytosine.

33. Complex relationships between yeast totiviruses

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The Totiviridae family viruses L-A and M are commonly found in both laboratory and wild-type yeast strains. The dsRNA genome of L-A virus encodes capsid protein Gag and fusion protein Gag-Pol, which functions as RNA dependent RNA polymerase. The dsRNA genome of satellite M virus encodes a secreted protein toxin. A Gag-Pol protein of L-A virus is vital for the maintenance of itself and M virus in yeast. The presence of both of these viruses in yeast cell gives the host so-called killer phenotype. Killer yeasts are able to kill other sensitive yeast cells in their surroundings while being immune to the secreted toxin. L-A spreads by direct cytoplasm contacts occurring during cell division and cell-cell mating. Extracellular stage of L-A virus remains unidentified. Presence of L-A virus alone in yeast does not cause slower cell growth or other detectable phenotypic changes.
There is a variety of different types of L-A viruses. Most of those types are highly similar in their genome and encoded protein sequences. Nevertheless, they differ in their ability to maintain specific satellite viruses. Research performed with overexpression of various types of L-A virus Gag and Gag-Pol proteins revealed complex interactions between different L-A and M viruses. The collected data provides important insight into the evolutionary relations between various types of L-A viruses and suggests possible mechanisms behind phenotype development.
This research was funded by Research Council of Lithuania, grant #SIT-07/2015.

34. Liquid biopsy-based DNA methylation biomarkers of prostate cancer

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Prostate cancer (PCa) is one of the most commonly diagnosed male malignancies worldwide, with castration-resistant PCa (CRPC) being the major cause of death. PCa has various forms and, thus, the well-timed risk stratification is crucial for clinical decision making. The treatment of CRPC is based on the administration of next-generation targeted therapy (abiraterone acetate, AA) or chemotherapeutic agents, but only a part of CRPC cases responds to this treatment positively. In order to facilitate the selection of the most effective treatment schedule, the development of molecular biomarker system, which is able to distinguish aggressive forms of the disease at its early stages and to predict responsiveness to particular treatment, is highly important.
Utilizing epigenome-wide technologies for the analysis of tumor samples, our group has identified several novel DNA methylation-based PCa biomarkers (PRKCB, ADAMTS12, etc.), which were further validated in the Lithuanian and The Cancer Genome Atlas's PCa cohorts. We developed several liquid biopsy-based epigenetic biomarker panels for PCa diagnosis and identification of the most aggressive CRPC cases, aiming to improve PCa risk assessment and to guide treatment decision making.
Targeted DNA methylation analysis in urine samples of PCa patients (~500 in total) was performed by means of quantitative PCR. Analysis of the genes RASSF1, RARB, and GSTP1 showed sufficient diagnostic power and the potential to predict biochemical progression after radical prostatectomy, as well as to improve patients' stratification into PCa risk groups at the time of diagnosis. The methylation of the newly identified PCa biomarkers, i.e. PRKCB, CCDC181, ADAMTS12, and NAALAD2, was detectable in urine from PCa cases with both localized and advanced disease. In urine samples, collected before the first-line therapy with AA, higher promoter methylation levels of ADAMTS12 and GSTP1 were observed in cases that experienced short-term disease progression. Pairwise analysis revealed decreased methylation levels of the two genes when positive response to AA was observed for >6 mo.

In conclusion, DNA methylation-based biomarkers may be utilized for noninvasive PCa diagnostics and risk determination, as well as to assist in treatment selection and monitoring of the cases with aggressive disease.

35. Geobacillus spp. Induced Biosynthesis of Silver Nanoparticles and Their Antimicrobial Activities

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The biosynthesis of silver nanoparticles (AgNPs) by microorganisms is attracting great attention. The biological synthesis of nanoparticles is profitable, effective and environmentally friendly method compared to chemical or physical synthesis of this nanomaterial. AgNPs have unique properties that can be used in many fields. Nowadays, antimicrobial properties of AgNPs are receiving more interest, as growing microbial resistance to classical antibiotics or antifungal compounds increases which is a serious problem. The present study reports the extracellular biosynthesis of AgNPs using secretomes of Geobacillus spp. strains 18, 25, 95 and 612 and 2 mM AgNO3. Ag+ reduction and formation of AgNPs in all Geobacillus spp. secretomes were confirmed by UV-Visible (UV-vis) Spectroscopy and Scanning Electron Microscopy (SEM). Obtained AgNPs were also tested for their antimicrobial activities against pathogenic Gram-positive (Staphylococcus aureus; Streptococcus pyogenes), Gram-negative (Pseudomonas aeruginosa) bacteria and yeast (Candida lusitaniae, Candida guilliermondii). The antimicrobial activity of the AgNPs was evaluated by time-dependency of growth inhibition and the disc-diffusion method. Time-dependency of growth inhibition of Pseudomonas sp. bacteria evaluated with luminescence showed that the best inhibition was received with 0.1 % concentration of two (Geobacillus spp. strains 25 and 612) tested AgNPs. AgNPs produced using secretome of Geobacillus sp. 95 inhibited growth of S. pyogenes, S. aureus and tested Candida species. The results obtained in this study suggest Geobacillus spp. strains 18, 25, 95 and 612 as a considerable tool for production of AgNPs. In addition, antimicrobial activities of AgNPs give an opportunity of their use as biocontrol agents against pathogenic human bacteria and yeast.

36. The role of protein corona and ion dissociation in cytotoxicity mechanism of zinc oxide nanoparticles

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The past decade has witnessed an exponential growth in nanotechnology of engineered nanomaterials (ENs) on the large scale for both industrial and household purposes. ZnO nanoparticles (NPs) are amongst the most common ENs. ZnO NPs also have a wide range of biomedical applications like drug delivery, anti- cancer, anti-diabetic, antibacterial, antifungal and agricultural properties. Although ZnO NPs are generally recognized as safe (GRAS) by the US Food and Drug Administration it still has the limitation of cytotoxicity which is yet to be resolved. Cytotoxicity of ZnO NPs can be caused by the combined action of plain NPs, protein-coated NPs formed in biological environment and dissociated Zn2+. In this study non-covalent protein coating of ZnO NPs, known as protein corona, was formed from model protein bovine serum albumin (BSA) resulting in ZnO-BSA NPs. The aim of this study was to evaluate the role of protein corona and ion dissociation in cytotoxicity mechanism of ZnO NPs in CHO cells. Different parameters and targets of NPs in the cells were evaluated: NPs uptake, NPs-induced changes in cell viability, proliferation, membrane permeability, mitochondrial amount and potential, ROS and ATP generation.
We have demonstrated that low concentrations (0.1-10 μg/ml) of ZnO-BSA and Zn+2 ions had stimulating effect on cell viability. Protein corona enhances ZnO NPs-induced plasma

membrane permeabilization for small and large molecules (propidium iodide and lactate dehydrogenase, respectively) but decreases short-(3 h) and long-term (24 h) ZnO NPs cytotoxicity. Interestingly, NPs slightly decrease mitochondrial amount and mitochondrial potential however NPs increase ATP generation by 2-fold. These effects were more pronounced for NPs with protein corona as compared to plain NPs.

37. Overcoming antimicrobial resistance in bacteria using bioactive magnetic nanoparticles, high pulsed electric and electromagnetic fields

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Various emerging technologies for food processing are introduced every year with a hope to present an economical, effective, and simple methodology for successful biopreservation of food without reduction of food quality. In this work, we present a proof of concept of novel antimicrobial methodology using targeted magnetic nisin-loaded nano-carriers (iron oxide nanoparticles (NPS) (11-13 nm) capped with citric, ascorbic and gallic acids), which are activated by high pulsed electric and electromagnetic fields allowing to overcome the nisin-resistance of bacteria. As a cell model the Gram-positive bacteria Bacillus subtilis and Gram-negative Escherichia coli were used. We have applied 10 and 30 kVcm-1 electric field pulses (100 µs x 8) separately and in combination with two pulsed magnetic field protocols: 1) high dB/dt 3.3 T x 50 and 2) 10 mT, 100 kHz, 2 min protocol to induce additional permeabilization and local magnetic hyperthermia. We have shown that the high dB/dt pulsed magnetic fields increase the antimicrobial efficiency of nisin NPs similar to electroporation or magnetic hyperthermia methods and a synergistic treatment is also possible. The results of our work are promising for the development of new methods for treatment of the drug-resistant foodborne pathogens to minimize the risks of invasive infections.

38. Optimization of media components for production of bacteriocin-like peptide by *Staphylococcus xylosus*

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[1] Vilnius University, Institute of Biotechnology; [2] JSC "Biocentras"

The extensive use of antibiotics leads to an increasing number of antibiotic-resistant pathogenic microorganisms. The development of new antimicrobials is needed for clinical, veterinary, and food applications. Bacteriocins are antimicrobial peptides that are synthesized by almost all bacteria and certain archaea. These peptides are synthesized in ribosomes, some of which are replaced by post-translational modifications. The Staphyloccocus xylosus bacterial strain is commonly used in the meat production due to their antimicrobial and nitrate reductase activity. It is known that the growth of microorganisms and bacteriocin production is affected by medium formulation.

In this study, the bacteriocin-like (BLIS) peptide, produced by Staphylococcus xylosus was isolated and studied against the spectrum of antibacterial activity. It was detected that the BLIS inhibits a growth of many gram-positive bacteria. The significant fermentation variables were selected in accordance with the Plackett–Burman design and the concentration of components was further optimized via response surface methodology.

Four significant variables (meat extract, glucose, tryptone and K2HPO4) were selected for the optimization studies. The maximum biomass and BLIS production was achieved in medium composting of meat extract (13 g/L), tryptone (18 g/L), glucose (4 g/L), potassium phosphate (5 g/L) and NaCl (2 g/L). The 34 % increase of biomass and bacteriocin

production was achieved in the optimized medium as compared with the unoptimized basal medium.

39. Genome-wide DNA methylation profiling in clear cell renal carcinoma

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Renal cell carcinoma (RCC) is the most lethal neoplastic disease of the urinary system with clear cell RCC (ccRCC) as predominant subtype. The main reason of such mortality is lack of widely accepted tumor markers for early clinical diagnosis. Consequently, identification of novel RCC-specific markers has become an urgent need. Alterations of DNA methylation can modulate gene expression and are observed early during cancer development. Moreover, due to the ability to detect these epigenetic changes in the body fluids (such as urine or blood), it may be the valuable tool for early and non-invasive cancer detection. This study aims to explore DNA methylation profile of the Lithuanian ccRCC cases.
Materials and methods. Genome-wide DNA methylation profiling was performed on 11 ccRCC (grouped into indolent, progressive and metastatic cases) and paired non-cancerous renal tissue (NRT) samples, using methylated DNA immunoprecipitation technology and two-color Human DNA Methylation 1 × 244K microarrays (Agilent Technologies). GeneSpring GX v14.9 software was used for the statistical analysis.
Results. Almost 2700 aberrantly methylated DNA regions were identified in all ccRCC samples, comparing with NRTs. In the indolent ccRCC cases, various genomic regions were more frequently hypomethylated, although in the metastatic cases higher methylation level was observed more often. Biological pathway analysis showed significant enrichment of differentially methylated genes associated with cell cycle, apoptosis, epithelial mesenchymal transition and others.
Conclusions. Changes in DNA methylation are the common events in RCC development. Further studies are needed to explore particular genes suitable for early and non-invasive diagnosis of RCC and prognosis of disease aggressiveness.

40. Antimicrobial activity of red pulcherrimin-producing Metschnikowia spp. against Saccharomyces cerevisiae

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Nature Research Centre; Center for Physical Sciences and Technology

There is ongoing search for new wild type yeasts with killer or biocidal properties. Red pigment pulcherrimin-producing yeast Metschnikowia pulcherrima (Candida pulcherrima) has been found to be an effective antagonist against Botrytis cinerea, Candida tropicalis, Candida albicans, Penicillium, Alternaria and Monilia sp. [1,2]. The efficacy was related to elimination of iron from the growth media due to precipitation reaction between iron (III) ions and secreted pulcherriminic acid, a precursor of pulcherrimin. However, attempts to observe antimicrobial activity of M. pulcherrima against S. cerevisiae were unsuccessful.
Red pigment-producing strains comprised 5 to 10 % of yeasts isolated from spontaneous fermentations of various berries harvested in Lithuania over a last 10-year period. Most of them were identified as Metschnikowia spp. As in the case of M. pulcherrima, red pigment pulcherrimin was produced when these Metschnikowia spp. were cultivated on solid iron-containing media. The growth of wine yeasts S. cerevisiae could be inhibited under appropriated conditions such as fast inoculation of relatively large amount of Metschnikowia

spp. on lawns of S. cerevisiae at concentrations of elemental iron in media not exceeding 12 mg/kg. Strain-dependent antimicrobial activity was related to fast elimination of iron from cultivation media due to accumulation in growing cells and subsequent precipitation by secreted precursor.

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2. Oro L., Ciani M., Comitini F., Antimicrobial activity of Metschnikowia pulcherrima on wine yeasts. J. Appl. Microbiol. 116 (2014) 1209-1217.

41. Synthesis of pyrimidines bearing hydroxamic acid and 1,2,3-triazole moieties as potential inhibitors of histone deacetylase

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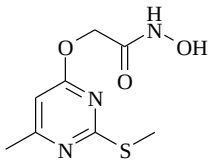
Pyrimidine compounds bearing 1,2,3-triazole and hydroxamic acid groups are known as strong chelating agents which find many uses in chemistry and medicine, especially cancer treatment. The main goal of this work was to synthesize new pyrimidine derivatives **1, 2** containing hydroxamic acid and 1,2,3-triazole moieties as potential inhibitors of histone deacetylase 8 (HDAC8).



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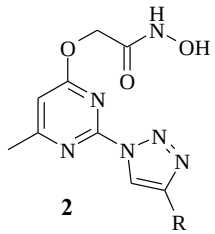

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The hydroxamic acids were synthesized from corresponding esters *via* nucleophilic substitution reaction with hydroxylamine. The 1,4-disubstituted 1,2,3-triazole ring was formed through Cu(I)-catalyzed azide–alkyne cycloaddition reaction.
The binding affinity of tested compounds towards HDAC8 was measured by fluorescent thermal shift assay.

June 28, LBS Conference
Oral presentations

Phospholipid scrambling as a signal of synapti pruning in developing brain

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The development of complex nervous system is accompanied by a generation of superfluous neuronal connections that are removed when neural circuits mature. Why are so many synapses lost, what determines which synapses are eliminated, what are the molecular mechanisms involved, and what are the consequences of not getting it right? Synaptic pruning appears to be highly selective process that ensures selective elimination of some synapses and the maintenance of others. Structural and functional refinement of synaptic network is tightly related to the presence of brain immune cells microglia that actively contact

and engulf unnecessary synapses. Aberrant or impaired microglial function leads to abnormal synaptic densities and dysfunctional connectivity that causes morphological, functional and behavioral deficits. For example, brain imaging and post-mortem studies suggest the role of deficient synaptic pruning in neurodevelopmental disorders, such as autism and schizophrenia. The reduction of brain volume and reduced density of dendritic spines in schizophrenia is suggestive of over-pruning, whereas increased brain volume and dendritic spine densities may indicate under-pruning in autism. Microglial phagocytic function has been implicated to have a central role in synaptic pruning; however, neuronal “eat-me” signal that discriminates weak and strong synapses remains to be identified. Using organotypic hippocampal slice cultures and in vivo mouse models we investigate the role of phosphatidylserine as a neuronal surface signal that labels synapses for elimination thus ensuring proper brain development and circuit maturation.

Mechanisms of neuronal loss in Alzheimer’s disease models

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PNS regeneration in 2D and 3D models

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Regeneration of the nervous system is a complex and often complicated process. It is currently agreed that regeneration of the neurites is possible as long as the neuron body remains intact in the event of injury. During regeneration of the peripheral nervous system, not only growth factors, such as NGF or BDNF play a key role in survival of the neuron and initiation of regeneration, but also other factors such as semaphorin group of proteins. One of the best described proteins with increased expression after injuries is Sema3A. Sema3A acts as a guidance cue during development of NS as well as inhibits neuroregeneration after injuries.
In our studies we were investigating if Sema3A and NGF signalling pathways intersect. By using affymetrix data as well as concentration dependence curves we found, that increased NGF concentration indeed inhibits inhibitory Sema3A action on regeneration of the neurites. Moreover while performing co-culturing experiments of PNS neurons along with Sema3A expressing HEK293 cell line in 3D cultures it was recognized, that the regeneration of the neurites is also guidance cue gradient dependent event, opening further opportunities to investigate intracellular protein gradient recognition pathways from extracellular cues.

Fluorescence anisotropy assay as a novel approach to characterize ligand binding dynamics to G protein coupled receptors

Ago Rinken

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G-protein coupled receptors (GPCRs) transduce signals into cells via guanosine nucleotide binding regulatory proteins (G proteins). The ligand binding to these receptors is the first and often also most crucial step in the signal transduction pathway. During the last decade in addition to conventional radioligand binding, several fluorescence-based methods have been proposed for the characterization of ligand binding to GPCRs. We have shown clear preferences of fluorescence anisotropy-based assay, which allows on-line monitoring of

ligand binding and obtain valuable kinetic data. Ratiometric nature of the assay requires high concentration of receptors, which can be achieved with implementation of budded baculovirus particles, which display GPCRs on their surfaces. Interpretation of the kinetic results of the non-pseudo first order reactions is also more demanding and require special attention, but can be clearly solved for this assay system. We have already working fluorescence anisotropy/baculovirus kinetic assay systems for characterization of ligand binding to melanocortin (MC4R), neuropeptide Y (NPY1R), serotonin (5-HT1AR), dopamine (D1DAR) and muscarinic (M2mAChR) receptors.
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Studies of the extracellular Carbonic anhydrase subfamily: CA VI, IX, XII, and XIV

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Carbonic anhydrases (CA) are ubiquitous, essential enzymes which catalyze the conversion of carbon dioxide and water to bicarbonate and H^+ ions. Vertebrate genomes generally contain gene loci for 15 to 21 different CA isoforms, three of which are enzymatically inactive. Our recent studies have focused on a group of four isozymes which share a common ancestry, namely the transmembrane isozymes CA IX, CA XII, and CA XIV, plus the secreted CA VI.
Our studies have elucidated the evolutionary history of this subfamily, in particular CA VI, by genomic and a phylogenetic studies of sequences. The case of CA VI involves a history of multiple domain rearrangements, and a novel domain combination which is present in non-mammalian vertebrates (fishes, birds, amphibians, reptiles). The novel domain belongs to the pentraxin family, which includes components of innate immunity, such as the C-reactive protein (CRP) and serum amyloid protein (SAP).
We have produced zebrafish (zf) CA VI recombinantly and shown that it has a high CA enzymatic activity, with K_{cat} and K_m similar to human CA I and CA II. Gel filtration and dynamic light scattering show that the protein is a pentameric complex in solution, presumably joined by the pentraxin domains (which form pentamers in the case of CRP and SAP). Further studies of zf CA VI include mass spectrometry, molecular modeling, immunohistochemistry and knock-down of the gene in zf embryos. Most recently, we have obtained results, still unpublished, of the likely presence of the calcium-binding site in the CA-associated pentraxin domains, as seen in human CRP and SAP.
Surprisingly, CA IX has been observed with a nuclear localization and interacting with the nuclear import and export machineries in hypoxic cells in studies by an Italian-Slovakian team. We have studied the presence of nuclear localization signal (NLS) and nuclear export signal (NES) in sequences of CA IX, CA XII, and CA XIV of multiple species, and conclude that all of these transmembrane CA isoforms could be found occasionally residing in the nucleus. Additionally, we have discovered dimerization signal motifs in the transmembrane regions of CA XII and CA XIV.

Synthetic biology: cell factories for future biotechnology

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Synthetic biology is a rapidly emerging field that combines molecular biology and engineering. It represents a paradigm shift – biology is becoming an engineering discipline that aims to use engineering principles to design and construct cells and organisms with novel functions. A great potential of synthetic biology has been rapidly unleashed with several simultaneous recent developments including i) the exponential growth of DNA sequence information in all the branches of life, ii) large datasets and accelerated research on biomolecular systems, and iii) the rapid drop in the price of DNA synthesis during the last decade. In coming years, it is widely believed that synthetic biology will transform entire sectors of human life and the world economy, including the chemical industry, medicine, material science, food production and agriculture. One of the most promising development in synthetic biology is the concept of cell factories. By redesigning genomes and cellular metabolism, one can create microbial cells that produce a wide range of chemicals and pharmaceuticals. The Estonian Center for Synthetic Biology (ECSB) is in the process of creating a research and development platform for the sustainable bio-manufacturing of high value chemicals and pharmaceuticals using cell factories and a synthetic biology approach. The platform will accelerate the transition from proof-of-concept science to commercial products via a system of iterative cycles that carry the technology from the lab to pilot- scale production in bioreactors. The resulting capability enables one to construct cell factories that are considerably faster and cheaper compared with the current state-of-the-art.

Biochemical approaches for the study of organization and biological functions of lipid rafts

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Glycosphingolipids, due to their tendency to form laterally separated liquid-ordered phases, possess a high potential for the creation of order in biological membranes. The formation of glycosphingolipid-rich domains within the membrane has profound consequences on the membrane organization at different levels, and on the conformational and biological properties of membrane-associated proteins and multimolecular protein complexes. Glycosphingolipids at the cell surface interact with plasma membrane receptors (e.g., integrins and growth factor receptors) and adapter molecules forming signaling complexes able to influence the activity of signal transduction molecules oriented at the cytosolic surface of the plasma membrane. The function of these signaling complexes appears to be strictly dependent on their glycosphingolipid composition, and likely on specific sphingolipid-protein interactions.
We have developed a set of different biochemical approaches to study the structure and functions of lipid rafts:
1) Preparation and analysis of detergent-resistant membrane fractions from cultured cells and animal tissues;
2) Immunoisolation of sphingolipid-enriched membrane microenvironments
3) Photolabeling experiments using radioactive ganglioside derivatives
4) Artificial manipulation of the cellular lipid composition.
Along the years, the use of these approaches allowed us to define the importance of lipid rafts in different biological contests, including neoplastic transformation and development of the nervous system.

Branched DNA technology: Great tool to measure RNA in different platforms

Natascha Brinskelle-Schmal

Technical Service | Antibodies & Immunoassays Business Unit, Life Science Solutions, Thermo Fisher Scientific, Bender MedSystems GmbH, Campus Vienna Biocenter 2, A-1030 Vienna, Austria

Cellular heterogeneity is present in all biological samples, including gene expression differences between cells, low- to high-protein levels in cell populations, or unique morphological attributes of individual cells within tissues. Most of our understanding of gene expression is based upon bulk population averages. This has advanced our understanding of general biological function and the identification of informative signatures. On the other hand bulk analysis often leads to conclusions that assume averages reflect the dominant biological mechanism operating within an entire population. Furthermore, it ignores essential cell-to-cell and spatial differences. To fully understand how gene expression heterogeneity contributes to biological function, a single-cell analysis approach must be applied. With the branched DNA technique you get tools that helps to answer both mentioned approaches.

Disposable bioprocesses for production of high quality recombinant proteins

Juozas Šiurkus

Thermo Fisher Scientific Baltics

Approach of Single-use bioproduction process (SUBP) becoming widely accepted by biomanufacturers due to lower cost of development, implementation, operation and required capital investments. It enables to drastically reduce the risks of cross-contamination, ensure continuity from early development through manufacturing, flexibility of scaling up/down and sustainability. During our research we have addressed the major aspects of development of disposable bioprocess, especially scale up/down and transferability of microbial fermentation from conventional steel-tank conditions to Single Use.
Furthermore, we have utilized major advantages of SUBP for the unique niche of manufacturing of DNA-free PCR enzymes. We have developed new closed bioproduction system based on integrated single-use technology including UPS and DST to drastically minimize the risk of DNA contamination inherent to the conventional manufacturing process. To help ensure conformance to strict purity requirements, we subject our DNA-free PCR reagents to stringent quality tests to verify that products are free of contaminating bacterial, human, and plasmid DNA.

When protein quality matters

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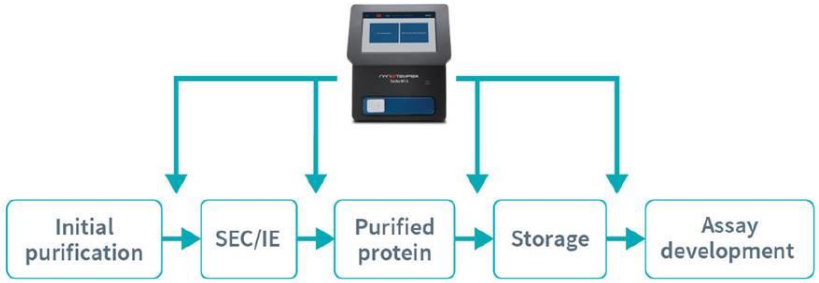
Keywords: protein quality, unfolding, intrinsic fluorescence
For the first time ever, find out the quality of any protein sample in minutes and make your assay development and purification workflows more efficient. Decide which batches, buffers, or conditions are best by comparing the relative stability of each sample along every step of an experiment. Identify unfolding events, important indicators of protein stability, by monitoring temperature inflections (T_i) — that way, you can be confident you're choosing the right sample moving forward.
During this talk we present the newest member of NanoTemper's instrument family - Tycho NT.6 system. It allows rapid protein quality checks at all steps of a protein purification and characterization workflow, enabling researchers to make better informed decisions and ultimately to perform better science.
Tycho NT.6 swiftly monitors protein quality by measuring the Batch-to-batch comparison Storage and stability Folding and functionality Assay development intrinsic fluorescence of a protein at 350 nm and 330 nm upon application of a thermal ramp to generate a representative unfolding profile of the sample protein.

Results are obtained in 3 minutes using small volumes of material and can be used in every workflow when protein quality matters.



BLI systems for regulated environments - providing data integrity of label-free biomolecular binding

Renata Gronczewska

Pall ForteBio

Biolayer Interferometry (BLI) technology based Octet® systems have been widely adopted in early research, and development of drug candidates and biotherapeutics. Now, with a comprehensive set of tools for compliance, this label-free technology is gaining traction in process development and quality control (QC) labs for concentration analysis in cell culture and purification, for kinetic and potency analysis of drug-target and drug-Fc receptor interactions, and for stability analysis by assessing changes in activity in stressed and forced degradation samples. Discover the go-to solution providing versatility and flexibility necessary in development combined with rigor and simplicity needed in a QC environment.

Poster presentations (session II)

1. The role of calcium in the infection of *Lactococcus lactis* with C2 and Sk1 phages

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Lactococcus lactis are one of the most commonly used bacteria in the dairy industry. They are important because of the ability to produce lactic acid from lactose, which is used in the manufacture and preservation of dairy products. These bacteria are often infected by Siphoviridae family bacteriophages, which are naturally found in milk. Due to the phage infections the dairy industry is experiencing great losses in production. The aim of this work was to assay the influence of divalent cations on the infection of c2 and sk1 fages. Divalent cations, such as calcium or strontium, are important for an effective of c2 and sk1 phage infection. Ca^{2+} ions are mostly found in milk. Therefore the further research was carried out by using various concentrations of Ca^{2+} ions. Under the aerobic conditions lysis of *L. lactis* bacteria by both c2 and sk1 phages was the fastest in the medium with 10 mM calcium ion. In the medium with 0,5 mM or 1 mM Ca^{2+} the lysis is slower but more effective. In the microaerobic conditions with 10 mM Ca^{2+} both c2 and sk1 lyse the bacteria in a fast and effective way. In the presence of 0,5 mM Ca^{2+} the infection is slow and ineffective. Without Ca^{2+} supplement the phage caused infection does not occur. After determining that 1 mM is

the optimal Ca^{2+} concentration at aerobic conditions, the process of infection was analysed using TPP^+ and K^+ selective electrodes. C2 and sk1 phages caused the release of TPP^+ and K^+ ions from *L. lactis* in the buffer medium with or without the 1 mM Ca^{2+}. We can conclude that calcium ions are needed in the later stages of infection.

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2. Evaluation of the composition of phenolic compounds in *Elsholtzia ciliata*

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Elsholtzia ciliata (E.ciliata), commonly known as crested late-summer mint, is an annual aromatic plant belonging to the Lamiaceae family. The main chemical compounds found in the Elsholtzia genus are steroids, flavonoids, triterpenes, polyphenols and others. Polyphenols are predominant compounds in Lamiaceae family plants and have gained significant importance as bioactive compounds with substantial health benefits. The main properties of polyphenols are known as antioxidative, immunomodulatory, vasodilatory, inflammatory and neuroprotective. The aim of this study was to evaluate main polyphenols in E. ciliata and increase the efficiency of their extraction from herbal materials as well. Ultrasound assisted extraction (UAE) method was used for producing extracts from E. ciliata. Polyphenolic compounds were separated using an Acquity H-class UPLC system equipped with a Xevo triple quadrupole tandem mass spectrometer. HPLC analyses have been carried out using Waters 2695 chromatography system.

17 phenolic compounds were determined in the ethanolic extract obtained from dried E. ciliata herbal material by UAE method. Phenolic compounds were determined using HPLC-PDA and UPLC-ESI–MS/MS methods. Their mass, spectra and retention times were compared to external standards. In this study 13 new phenolic compounds (quinic acid, neochlorogenic acid, chlorogenic acid, p-coumaric acid, vitexin, ferulic acid, luteolin-7-rutinoside, luteolin-7-glucoside, procyanidin B, apigenin-7-glucoside, naringenin, diosmetin and chrysin) were detected in extracts produced from E. ciliata herbal materials for the first time. Considering the obtained results, the most rational way of extraction of dried E. ciliata herbal material is to select an optimized UAE method. The optimal extraction conditions for UAE were found to be as follows: the solvent 70% ethanol, the extraction time 30 min, the extraction temperature 25 °C. The obtained results are relevant for the modeling, developing and producing of new, effective and natural products that are active in the prevention and treatment of various illnesses. Besides, these results could be used for the further research and development of healthy food products, supplements and drugs.

3. The eficiency of *Listeria monocytogenes* bacteria efflux pumps

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Vytautas Magnus University

Introduction. Antimicrobial resistance is a constantly growing worldwide problem. A key part of antimicrobial resistance is multidrug resistance efflux pumps. Because of these pumps Listeria monocytogenes is a multidrug resistant pathogen, not sensitive to many antimicrobial compounds. It is very important to understand the mechanisms how could we regulate the activity of efflux pumps, because L. monocytogenes is an opportunistic foodborne Gram-positive pathogen causing serious human infections. It is not easy to develop new antimicrobial compounds which would not be a substrate of efflux pumps in addition the sides effects of new compounds are unknown so the knowledge about the inhibition of antibiotics efflux out of cells could increase the effectiveness of treatment.
Materials and methods. We used potentiometric and fluorescence methods to assay the inhibition of L. monocytogenes efflux pumps. We used tetraphenylphosphonium, which is a substrate of these pumps, selective electrode to register the inhibition of efflux. In parallel, the intensity of ethidium fluorescence was determined. We used inhibitors of different families of efflux pumps, such as chlorpromazine, verapamil, reserpine. Also we explored the effect of Phe-Arg-β-naphthylamide (PAβN) and 1-(1-Naphthylmethyl)piperazine which have not yet been used against gram-positive bacteria.
The aim. The aim of our studies was to determine the specificities of tetraphenylphosphonium and ethidium interaction with L. monocytogenes cells and to evaluate the efficiency of efflux pumps inhibition.
Conclusions. TPP+ strongly inhibits the intensity of respiration of L. monocytogenes cells while ethidium – not. We determined that all of used inhibitors increase the accumulation of efflux pumps substrate. In addition, we observed that PAβN and NMP inhibit the efflux of tetraphenylphosphonium but not ethidium. The results obtained from electrochemical analysis whith PAβN selective electrode showed that L. monocytogenes bacteria accumulate a large amount of this compound.

4. Monitoring of energy-dependent efflux in *Candida albicans*

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Patients with AIDS and those who are undergoing chemotherapeutic modalities are always at a risk of developing C. albicans infections. Morbidity and mortality from invasive candidiasis and candidemia have increased significantly over the last four decades. The scientific and clinical findings have shown that the incidence of severe Candida infections is constantly increasing. With the development of azoles and other antifungal medicines, the potential for treatment of these infections has improved significantly. However, the possibility of timely diagnosis of fungal infections is limited, and therefore the outcome of the disease is not favorable. The pivotal membrane transporters that C. albicans is exploiting as one of the strategies to develop multidrug resistance (MDR), belong to either the ATP binding cassette (ABC) or the major facilitator superfamily (MFS) classes of efflux pumps. Overexpression of the efflux pumps Cdr1p, Cdr2p and Mdr1p is the main mechanism of fluconazole resistance in C. albicans.
For studies of yeast efflux activity we applied electrochemical measurements of phenyldicarbaundeborane (PCB-) ions in incubation medium. To activate the MDR pumps C. albicans wild-type cells were cultivated with fluconazole. During experiments in arsenate buffer, where the cells rapidly lose ATP, an additional binding of PCB- is observed. This indicates the suspended activity of cellular MDR pumps due to the lack of energy. No additional accumulation of PCB- was observed in experiments with control cells grown

without fluconazole. Using this assay method different C. albicans isolates were investigated and the results of this study will be presented at the conference.

5. Relationship between quality of functioning and miR34a expression level in glioblastoma

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Glioblastoma is the most common brain tumor, that expresses high aggressiveness, and poor prognosis for the patients. These tumors are very heterogenic, and that burdens the grading of malignancy, prognosis and treatment. Therefore, scientists began to search for biomarkers, that would help specify the characteristics of this kind of tumors, thus improving the treatment. The aim of this pilot study was to investigate the expression level of miR34a in glioblastoma patients and its relationship between quality of functioning. 31 patients with histologically confirmed glioblastoma participated in the study. The level of miR34a expression level in glioblastoma tumor tissue were identified by qPCR using TaqMan Advanced miRNA assay (ThermoFisher Scientific) following the manufacturer's instructions. The European Organization for Research and Treatment of Cancer Quality of Life Questionnaire QLQ-30 and QLQ-BN20 questionnaire were used to evaluate preoperative health related quality of life (HRQoL) and brain tumor related symptoms. Karnofsky performance index, was used for assessment of functional status. Preliminary findings indicate gender specific relationship between relative miR34a expression and functional status of glioblastoma patients. Statistical tendency was observed for higher relative miR34a expression to be related to better subjectively evaluated total level of functioning in males with glioblastoma. In sample of females, higher relative miR34a expression was significantly related to more reported brain tumor related symptoms.

6. Identification of serum based diagnostic and prognostic protein sets in astrocytoma patients

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Astrocytoma is one of the most common brain tumors, histologically and clinically classified into low-grade and high-grade (malignant) astrocytoma. The most disastrous type of malignant astrocytoma is glioblastoma (GBM). The treatment of malignant astrocytoma, in particular glioblastoma is complicated by the absence of reliable tools, which would allow to monitor essential aspects of tumorigenesis such as cell invasiveness and angiogenesis. Serum – based biomarker sets may support diagnosis and prognosis of astrocytoma. The aim of the study was to determine astrocytoma specific set of cancer-associated proteins in patient blood serum. ELISA-based multiplex protein array technology was designed and ten serum proteins related to angiogenesis and invasiveness were analyzed: AREG, ANGPT1, CXCL10, IGF1, MMP2, NCAM1, OPN, PAI1, TGFβ1 and TIMP1. Relative protein serum level was analyzed in 103 blood samples: 59 astrocytoma patient and 44 healthy controls. To find clinically informative protein profile Decision tree classification method was applied at the initial evaluation step involving all 10 proteins. Kaplan – Meier survival analysis was applied to evaluate patient survival in distinct groups.
The relative expression level of individual ten serum proteins showed no differences between patients and controls (Mann-Whitney test, $p>0.05$). Decision tree algorithm identified a panel

of four serum proteins formed by ANGPT1, TIMP1, IP10 and TGFb1, which enabled correct assignment to astrocytoma group with accuracy, specificity and sensitivity of 73.5 % (75/102), 65.1 % (28/43) and 79.9 % (47/59), respectively. Higher probability to survive more than 12 months after tumor resection for glioblastoma patients was associated with the serum based protein set formed of OPN and IP10, enabling correct assignment to survivorship group with the sensitivity of 92.3 %. Kaplan – Meier survival curves constructed using OPN and IP10 protein relative expression cut – off values generated under the decision tree showed statistically significant differences in postoperative survival time for glioblastoma patients (Log – rank test, $\chi 2 = 4$, $df = 1$, $p = 0.044$). In conclusion, profiles of more than one biomarker enable more specific assignment to the astrocytoma and survival group than those based on single protein. Our data provide only first indication of the presence of astrocytoma specific serum protein sets. Prospective and retrospective validation data on larger sample sizes and including other glioma – specific proteins will be necessary to evaluate presented profiles further.

7. Effect of preparation Humate Gel AG ethylene on the production of winter wheat

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Plant growth regulating substances are naturally occurring organic compounds that influence various physiological processes in plant such as the growth and differentiation of plant cells, tissues and organs. They perform these functions at concentrations far below the levels at which fertilizers normally affect plant processes. It is now well established that most soil microorganisms can produce plant growth regulating substances, including phytohormones (auxin, gibberellins, cytokinins and ethylene) and form one of the major mechanisms of plant growth promotion by production of plant growth regulating substances. One of phytohormone group is ethylene; it is a simple gaseous hydrocarbon. Ethylene is apparently not required for normal vegetative growth. However, it can have a significant impact on the development of roots and shoots.

Our goal – to find out whether the tested preparation Humate Gel AG 700 ml/ha affects ethylene production in plants under controlled laboratory conditions.

Commercial preparation Humate Gel AG “HydroThermoDynamic Technology”(Ukraine) was used. The microbiological content of this composition is as follows: Azotobacter rhizobium, Bradyrhizobium, Bacillus subtilis, B. cereus, B. megaterium, Lactobacillus, Trichoderma. Preparation Humate Gel AG was applied as aqueous solution at the optimal concentration rate 700 ml/ha. The studied variety was winter wheat ′Zentos′.

Ethylene content was measured using a FOCUS gas chromatographer (Thermo Fischer Scientific) equipped with a flame ionization detector and a stainless steel column (matrix 80/100 PROPAC R). Ethylene content in winter wheat seedlings was determined by the adapted previously used method.

Phytohormones in the plant comprise single system, but their ratio is not constant and can change ethylene accumulation. We performed tests for the impact of Humate Gel AG 700 ml/ha on the ethylene accumulation in seedlings of winter wheat plants. The obtained results showed that the seedlings of winter wheat ′Zentos′ treated with Humate Gel AG 700 ml/ha accumulated ethylene by 8 % more after the 16-day exposure, compared to the control.

Preparation Humate Gel AG 700 ml/ha concentration produced the strongest influence on the production of phytohormone ethylene in the tissues of winter wheat ′Zentos′ seedlings.

Key words: Ethylene, preparation Humate Gel AG, winter wheat

8. Pressure stability of Hsp90 N-terminal domain: insights from molecular dynamics simulations

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Protein structural characterization is one of the key elements in understanding its mode of action, function, stability, physical and chemical composition and role in the organism. Researchers use variety of tools to investigate the properties of the proteins, however only molecular simulations provide information about protein structure dynamics at the atomic scale. It is well known that high pressure unfolds the proteins and volumetric properties could be analyzed by applying high pressure on the molecule. Protein response to pressure is slow, requiring simulations on the elongated time scale thus increased computational resources are necessary for pressure effects to arise. Several dynamic simulations each 100 ns in length were run at different pressures to investigate the volumetric and hydration parameters of the 90 kDa Heat Shock Protein N-terminal domain. We tested internal structure stability, compressibility, residues, atoms and hydration shell fluctuations as well as conformational dynamics of the protein to discuss hydration and internal voids volume change contributions to protein. Our study provides insights how molecular dynamics simulations could be used as a tool to investigate volumetric properties of the proteins and could be broaden to explore protein stabilization against high pressure upon ligand binding.

9. Formulation and characterisation of effervescent tablets with ginger root extract and naturally-derived excipients

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In the pharmaceutical industry, effervescent tablets serve as a widely used dosage form for a broad range of applications. It is a broadly established type of pharmaceutical dietary supplements and commonly prescribed drug form for young children, for individuals suffering from swallowing disorders, hospitalised patients, etc.
Nowadays, green production and consumption has become an important step towards safeguard of the environment and a global trend. For this reason, the development of effervescent tablets formulations with naturally derived excipients has recently been studied as a promising alternative to synthetic binders, sweeteners, lubricants, and colourants.
In this study, several formulations of effervescent tablets featuring ginger (*Zingiber officinale*) root extract and naturally derived binders, such as lyophilisate of *Plantago ovata* seeds mucilage, guar gum, citrus fruit peels pectin, natural sweeteners – glucose and lyophilised honey – and sodium stearyl fumarate PRUV® (used for improved lubrication efficiency) have been developed and analysed (Fig. 1).

Fig. 1. Preparation stages and testing of effervescent tablets with ginger root extract and naturally derived excipients

The results showed that the physical-chemical characteristics of the prepared tablets satisfy the requirements for effervescent tablets (according to *European Pharmacopeia*). Moreover, the high total antioxidant capacity of the tablets proved the synergetic effect of polyphenolic

compounds in ginger extract and lyophilized honey. *Acknowledgements.* The financial support from MB "Euro Core" is greatly acknowledged. The authors wish to render their thanks to JRS Pharma (Germany) for providing a free sample of PRUV®.

10. Production of recombinant house dust mite allergen fused to maltose-binding protein

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Allergic diseases are among the most prevalent chronic diseases – they affect up to 25% of population in developed countries. This problem is relevant in many countries, including Lithuania, where the tendency of increasing number of allergic patients is observed. Well-characterized allergens with proper antigenic structure are needed not only for immunotherapy, but also for precise diagnosis, since only allergens that cause the disease should be used for this treatment.
The house dust mite (Dermatophagoides pteronyssinus) is one of most important indoor allergen sources and a major cause of allergic asthma, eczema and allergic rhinitis in humans. Sufficient quantities of purified Der p23 are needed for experimental, diagnostic and therapeutic purposes. Bacterial expression of recombinant Der p23 protein fused to maltose-binding protein (MBP) as an allergen carrier has been achieved to obtain soluble, properly folded allergen, having structural and antigenic features consistent with the properties of the allergens from natural sources. Recombinant fusion protein has been purified by affinity chromatography on the amylose-containing sorbent. MBP is an effective fusion partner that not only increases the level of expression, but also helps to fold proteins properly, resulting in their soluble protein form. Antigenic properties of the purified recombinant Der p23 allergen have been tested by Western blot and ELISA using Der p23-specific human IgE. This study demonstrated the efficiency of MBP-based expression and purification platform for the production of recombinant house dust mite allergen Der p23 in E.coli.
This research was funded by the European Regional Development Fund according to the supported activity ‘Research Projects Implemented by World-class Researcher Groups’ under Measure No. 01.2.2-LMT-K-718, project No. 01.2.2-LMT-K-718-01-0008.

11. Synthesis of carbazole and diphenylamine-based derivatives as potential efflux pump inhibitors

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Antimicrobial resistance is a worldwide problem in human and veterinary medicine. The appearance of multiple resistant bacteria of human and veterinary origin is accelerated due to the inappropriate or excessive use of antimicrobials. There are different solutions proposed to solve this problem. The environment contains a lot of man-made products. Such contamination is increasing and spreading into the environment. The major bacterial solution to toxic challenges takes the form of multiple pumping systems that prevent intracellular accumulation of bactericidal and bacteriostatic substances. The ability to pump antimicrobials out of cells is a common feature of most environmental microorganisms. That’s why is very important to discover molecules inhibiting the efflux pumps also to reveal the inhibition mechanisms of it. There is a possibility to discover substances competing with antibiotics for the interaction with efflux pumps. Antibiotic treatment in combinations with such inhibitory compounds is being widely discussed.

Derivatives of carbazole and diphenylamine could be considered as potential efflux pump inhibitors or substrates. Carbazole and diphenylamine are heterocyclic compounds with two benzene rings linked through nitrogen atom and also could be employed as biologically active compounds.

Carbazole derivatives could be employed as antibacterial, antiviral, anti-inflammatory, anticancer, sedatives or tranquilizers agents. Diphenylamines are used as antioxidants, fungicides, analgesic, antiinflammatory, antibacterial, antihistaminic or antihelmintic agents. Any change in the structure of these compounds causes distinguishable difference in their biological activities.

In this work we have synthesized carbazole and diphenylamine-based molecules by one step synthesis. Synthesis paths, purification and structure identification methods will be presented. To determine the susceptibility of the synthesized compounds to the bacteria, to study their interaction with efflux pumps in Salmonella enterica bacteria it is planned in the future research step.

12. DNA polymerase inhibition with derivatives of pyrophosphoric acid analogues *in vitro*

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Retroviruses represent a unique viral reproduction strategy by using a reverse transcriptase for DNA biosynthesis from viral RNA. HIV-1 reverse transcriptase is prominent target for antiviral treatment. Reverse transcriptase of Moloney murine leukemia virus represents group of monomeric retroviral reverse transcriptases, making it valuable tool for molecular biotechnology. The ability to exploit distinct characteristics of reverse transcriptases would allow developing inhibitors against viral polymerases, without targeting of the host polymerases. At the same time the polymerase ability to use modified nucleotides would allow synthesizing DNA with augmented properties.

Small molecule compounds phosphonoformic (PFA) and phosphonoacetic (PAA) acids comprise one of the three types of principal inhibitors of viral polymerases. PFA is the most effective small molecule inhibitor of HIV reverse transcriptase identified so far. Sensitivity of various viral polymerases towards PFA is 100 to 1000 fold higher compared to that of mammalian replicative polymerases. However, it is cytotoxic and used for the treatment of HIV and HIV-associated viral infections in critical cases only. Despite the similar structure, PAA is far less effective against viral polymerases but does not exhibit observable cytotoxicity.

To achieve a better affinity, PAA was linked with different positions of natural nucleotide. Here, we focused on novel analogues of nucleoside triphosphates designed to be catalytically acceptable by the target polymerases for incorporation into DNA. Several different PAA conjugated compounds were prepared, and their incorporation to DNA varied significantly towards different DNA polymerases, including reverse transcriptases. One compound was found to be capable to alter the processivity of DNA polymerases. Similar PFA conjugated compounds are being tested in follow-up studies.

13. Biodegradation of 7-hydroxycoumarin in Pseudomonas mandelii 7HK4

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A gene cluster, denoted as hcdABC and required for degradation of 3-(2,4-dihydroxyphenyl)-propionic acid has been cloned from 7-hydroxycoumarin-degrading Pseudomonas mandelii 7HK4 and sequenced. The bioinformatic analysis shows that the hcdABC genes encode a flavin-binding hydroxylase (HcdA), an extradiol dioxygenase (HcdB), and a putative hydroxymuconic semialdehyde hydrolase (HcdC). The analysis of the activity of the recombinant HcdA in vitro confirms that the enzyme belongs to the group of ipso-hydroxylases. The activities of the HcdB and HcdC proteins have been analysed by using the recombinant E. coli cells. The intermediate metabolites have been identified, that allowed confirmation of the enzyme functions and reconstruction of the catabolic pathway of 3-(2,4-dihydroxyphenyl)-propionic acid. HcdA catalyses the conversion of 3-(2,4-dihydroxyphenyl)-propionic acid to 3-(2,3,5-trihydroxyphenyl)-propionic acid through an ipso-hydroxylation followed by internal (1,2-C,C)-shift of the alkyl moiety. Then, in the presence of HcdB, a subsequent oxidative meta-cleavage of the aromatic ring occurs resulting in the corresponding linear product (2E,4E)-2,4-dihydroxy-6-oxonona-2,4-dienedioic acid. The collected data show that Pseudomonas mandelii 7HK4 is the first known microorganism which degrading 7-hydroxycoumarin via 3-(2,4-dihydroxyphenyl)-propionic acid pathway.

14. Next-generation *Escherichia coli* strains

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E. coli has a long history of use in biotechnology industry and is still a microorganism of choice for most cloning and protein expression experiments. Nevertheless, lack of natural competence, slow growth and frequently encountered expression of proteins as insoluble aggregates force researchers to spend time on optimizations or look for alternatives. To address these challenges and support E. coli as an attractive laboratory tool, we aim to redesign E. coli genome to significantly improve growth rate, protein solubility
and, intriguingly, find ways of natural uptake of DNA. We generated genome-wide libraries of transposon insertion mutants of DH10B-derived universal cloning and expression strain. This study is designed to not only disrupt the genes at insertion sites but also (i) create random fusions of genome encoded proteins with polyhistidine tags to explore surface proteome by enriching tag exposing individuals via affinity purification, (ii) enhance the transcription of nearby genomic loci to identify mutations leading to elevated growth rate.
We established methodologies for efficient in vivo transposon mutagenesis and transposon sequencing using Ion Torrent™ platforms, and applied NGS for the analysis of E. coli surface proteome dynamics. Cell surface analysis will shed light on the potential candidates which could be engineered to mediate DNA uptake eliminating the need of special cell treatment prior to transformation.

15. Platinum II Taq Hot-Start DNA Polymerase: PCR simplified with universal annealing

Skaistė Valinskytė, Edita Elijošiūtė, Tomas Radzvilavičius, Miglė Mikutėnaitė, Jonas Belevičius, Aistė Serapinaitė, Rasa Sukackaitė

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Invitrogen Platinum Taq DNA Polymerase has been trusted by researchers for over two decades and has been used in several thousand publications. Invitrogen Platinum II Taq Hot-Start DNA Polymerase is a new generation polymerase designed for fast PCR with minimum optimization. Platinum II Taq polymerase combines antibody-based hot-start with two innovative technologies. Taq DNA polymerase was engineered by in vitro evolution for higher speed and resistance to common PCR inhibitors, while innovative PCR buffer enabled

universal annealing temperature. Isostabilizing molecules in the Platinum II PCR buffer increase primer–template duplex stability during the annealing step and contribute to enhanced specificity without the need to optimize annealing temperature for each primer pair. Moreover, Platinum II PCR buffer allows flexibility in the length of the extension step. Therefore different targets of diverse length can be cycled together, increasing the laboratory throughput in PCR. Here we present how Platinum II Taq Hot-Start DNA Polymerase provides cycling speed, universal cycling protocol and market leading inhibitor tolerance.

16. Different cell death modalities in normal and cancer cells

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In 1970s, first attempts to classify cell death types were made. Historically, three quite distinct cell death types were suggested: type I (apoptotic), type II (autophagic) and type III (necrotic). The distinction between those types was more or less morphologic. However, specific biochemical markers of a certain cell death modality have emerged. During the last 50 years, a dozen of additional cell death types have been identified. Interestingly, they all are programmed in a cellular genome, except the necrotic death which is in most cases accidental. Here, the importance of cell death research in the context of human cancer is reviewed.

17. MAPK activation in response to chemotherapeutic drug cisplatin treatment in lung cancer cell lines

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It is known for decades that mitogen-activated protein kinases (MAPKs) regulate cell functions, such as proliferation, differentiation and programmed cell death. It has been demonstrated in various systems that MAPK signaling mediates cellular response to stress, including both reactive oxygen species-dependent and -independent effects of anticancer drugs. Thus, MAPKs are candidates in improving effectiveness of targeted and conventional chemotherapies. In present study, the dynamic changes in phosphorylation of MAP kinases JNK, ERK and p38 were investigated during cisplatin treatment, which is a known inducer of apoptosis in various cell models. The effect of cisplatin was studied in comparison with other DNA-damaging and ROS-inducing compounds.
Cis-diaminedichloroplatinum (II) (cisplatin) is used as an adjuvant therapy for non-small cell lung cancer after surgery as well as a component of combinational therapy in the late stages of metastasized lung cancer. To investigate cisplatin-induced cell signaling events, commercial lung adenocarcinoma cell line A549 as well as patient-derived primary lung adenocarcinoma cell lines were used for in vitro studies. Cell death induction was caspase- and p53 activation-dependent. We observed sustained and increasing up to 24 h MAPK activation during cisplatin treatment. DNA-damaging and ROS-inducing compounds of different molecular structure affected MAPK phosphorylation differently. Activation kinetics and the role of a certain protein kinase in cell death induction may differ depending on various background components, including drug concentration and its mechanism of action. As other signaling molecules which co-regulate cell survival along with MAPKs are induced simultaneously by these agents, parallel and loop-back signaling may be responsible for these differences in the same cell.

18. Influence of co-culture on cell proliferation, migration and cytokine expression

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Cells grown in a co-culture are more physiologically relevant therefore, the co-culture systems are the future in regenerative medicine and tissue engineering. The aim of this work was to evaluate interactions of endothelial (EPC) and myogenic progenitor cells (MPC) in co-culture. We found that cells in co-culture tend to migrate more intensive. Analysis of cytokine profile revealed an increased amount of CINC-1, LIX, TIMP-1 and VEGF cytokines in the co-culture secretome compared to EPC and MPC mono-cultures. Two cytokines – LIX and TIMP-1 – are directly involved in the regulation of cell migration, thus this might be one of the factors increasing co-cultures migration process. Further, the effect of the mono and co-culture secretomes on proliferation and migration of cultures was investigated. Data showed a positive effect of co-culture secretome on cell proliferation. Meanwhile, tested secretomes had no effect on the migration process. The expression of phosphorylated FAK, Akt, and ERK1 / 2 kinases was higher in cells grown in serum-containing culture media that in secretomes-containing media.

19. The activity of *Gardnerella vaginalis* recombinant sialidases

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Sialidase activity is one of the variables studied in association with the virulence of G. vaginalis and its role in bacterial vaginosis. In a study of 37 clinical G. vaginalis isolates, all isolates lacking the sialidase A gene (sld) were sialidase-activity negative, while sld containing isolates displayed various levels of sialidase activity in the culture medium supernatant when tested with the fluorogenic substrate 4-methylumbelliferyl-α-D-N-acetylneuraminic acid sodium salt hydrate. To evaluate the enzymatic activity of the G. vaginalis sialidases in heterologous bacteria, the genomic DNA of the cultured G. vaginalis strains was used to amplify the sld genes. The template DNA was extracted from the strains 47.3 and 58.4 (high sialidase activity in culture), and the strains 60.1, 79.2, 86.1 and 114.2 (very low to undetectable activity). Full length open reading frame of sld was amplified to obtain the product corresponding to the sequence encoding amino acids 1–907 of the GenBank YP_003985295.1. The nucleotide sequences of sialidase genes were deposited in GenBank (MG737371–MG737376). Multiple alignment of catalytic domain sequences suggested the presence of all conserved sialidase motifs. The truncated proteins corresponding to amino acids 374–907 of the reference sequence were amplified from the strains 86.1 and 114.2. The lysates of the E.coli strain Tuner™(DE3) carrying the sialidase expression plasmids exhibited specific protein bands of ~110 kDa and ~65 kDa after the induction of heterologous gene expression. The full-length proteins remained in solution after centrifugation and 0.22-μm membrane filtration of the bacterial lysates. The truncated proteins were poorly soluble. Soluble fractions of E. coli containing full-length recombinant sialidases were screened for enzymatic activity and all the samples containing recombinant sialidases exhibited specific activity.
Conclusions: 1. The sld gene was demonstrated to encode a protein exhibiting sialidase activity.
2. The presence of sialidase activity-negative G. vaginalis isolates harboring the sld gene raises a question about the gene regulation.

20. Toxicity of cadmium and effect of *Acanthopanax senticosus* extract on amount of immune response cells in mice spleen

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Introduction: Cadmium is a heavy metal of considerable toxicity with destructive impact on most organ systems. It inhibits the humoral and cellular immune response with the lower doses of the metal used, and opposite effects were detected with the higher doses. Studies of the immunomodulatory effect of Cd in experiment animals are inconsistent due to varying doses, route of administration, and differences in the sensitivity of immune system.
Acanthopanax senticosus (Rupr.et Maxim) Harms (Araliaceae), also called Siberian Ginseng, Eleutherococcus senticosus is a widely used in the Traditional Chinese Medicine. Acanthopanax senticosus extract (ASE) increases splenocytes proliferation and improves immune response of B cells and phagocytosis of macrophages rather than T cells.
Aim: The effects of ASE on accumulation of Cd and on levels of splenic immune cells after treatment with CdCl2 were investigated.
Results: Mice were injected for 6 weeks with cadmium chloride and AS extract solutions of two different concentrations: 0.05 LD50 AS; 0.10 LD50 AS; 0.05 LD50 Cd; 0.05 LD50 Cd and 0.05 LD50 AS; 0.05 LD50 Cd and 0.10 LD50 AS. Periodical injection of cadmium chloride and Cadmium chloride together with AS caused increase of Cd concentration in the spleen. Injections of cadmium increased the number of macrophages. AS and/or Cd did not change the number of T lymphocytes. AS significant increases the number of B lymphocytes, furthermore the number of B lymphocytes was higher in Cd +AS 0.1 LD50 group than in mice injected with cadmium chloride only.
Conclusions: Injections of AS extract combined with cadmium chloride leads to the increase of cadmium concentration in spleen of experimental mice. AS decreases the activity of macrophages and T lymphocytes induced by cadmium and increases the activity of B lymphocytes.

21. Investigation of TGF-β1 and Angiotensin II as potential cardiomyogenic differentiation inducers of human amniotic fluid mesenchymal stem cells

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Human amniotic fluid-derived mesenchymal stem cells (AF-MSCs) are of fetal origin and have multilineage differentiation potential, thus they may be applied for cardiovascular tissue engineering and cell therapy for cardiac diseases. The purpose of this study is to verify Transforming growth factor beta 1 (TGF-β1) and Angiotensin II as potential cardiomyogenic differentiation inducers of AF-MSCs in vitro. AF-MSCs were collected from amniocentesis samples (second trimester) from women who needed prenatal diagnostics (protocols approved by the Ethics Committee of Biomedical Research of Vilnius District, No 158200-123-428-122). Isolated AF-MSCs were positive for typical cell surface markers (CD44, CD90 and CD105) and negative for hematopoietic cells marker CD34 and expressed pluripotency

genes, such as OCT4, SOX2, NANOG and REX1. The differentiation towards cardiomyocyte-like cells was induced using TGF-β1 and Angiotensin II, for 12 days. These agents caused phenotypical alterations of AF-MSCs and up-regulated the expression of cardiac genes-markers (NKX2.5, MYH6, TNNT2 and DES) and the main cardiac ion channels genes (sodium, calcium, potassium) as determined by RT-qPCR. Western blot and immunofluorescence analysis revealed the increased expression of Connexin43, the main component of gap junctions, and the early cardiac transcription factor Nkx-2.5. Also, variations in the levels of chromatin-modifying enzymes DNMT1, Polycomb repressive complex 2 (PRC2) proteins EZH2 and SUZ12, histone deacetylases 1 and 2 as well as modified histones H3 and H4 demonstrated global epigenetic changes during the induced differentiation. Moreover, metabolic switch and mitochondrial function were assessed using Agilent Seahorse XF technology. Our results demonstrate that TGF-β1 and Angiotensin II initiate changes in genetic, epigenetic and metabolic profiles of AF-MSCs leading towards cardiomyogenic differentiation and may be considered as potential tools for the cell therapy and regenerative medicine.

22. Ectoine and hydroxyectoine interaction with mink growth hormone as a model protein

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Ectoine ((S)-2-methyl-1,4,5,6-tetra-hydropyrimidine-4-carboxylic acid) and hydroxyectoine ((4S,5S)-5-hydroxy-2-methyl-1,4,5,6-tetra-hydropyrimidine-4-carboxylic acid) are known extremolytes that accumulate in halophilic and halotolerant microorganisms. Previous studies showed that ectoine increased the thermostability of proteins and supressed the aggregation in their refolding process.
Here, we present the data on ectoine and hydroxyectoine interaction with mink growth hormone as a model protein by UV-Vis, circular dichroism and fluorescence spectroscopy. The anti-aggregatory effect of the additives was investigated at 60 ^{o}C and pH values of 7.5 and 6.0, i.e. above and below the isoelectric point of the protein. The first order rate constants for aggregation reaction were calculated, and the onset temperatures of aggregation were determined by circular dichroism spectroscopy in the absence and in the presence of various concentrations of ectoine and hydroxyectoine. Ectoine was found to be more effective aggregation suppressor as compared to hydroxyectoine. However, circular dichroism spectra showed that ectoine and its hydroxy derivative significantly affected the tertiary structure of the protein. It suggests that the inhibition of aggregation could occur by stabilizing partially unfolded intermediates, not the native globular structure. Moreover, fluorescence spectra showed that the additives did not stabilize the native structure of the protein at higher temperature. However, fluorescense quenching of protein intrinsic fluorescence by both additives was observed, and the apparent static quenching constants were calculated.

23. Ankrd1 kinetics in dilated hearts

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The ankyrin repeat domain 1 (Ankrd1) protein is a cardiac-specific stress-response protein. Ankrd1 is functionally pleiotropic, involved in transcriptional regulation, sarkomere assembly

and mechano-sensing in the heart. Cardiac Ankrd1 has been shown to be highly induced in heart failure and in various cardiomyopathies cases. However, it is still unclear what impact this may have on the pathophysiology of heart failure due to the fact that this protein is playing in both the formation of myocardial damage and its reparation systems. There is also lack of consistent in vivo studies that would allow molecular mechanisms of Ankrd1 in vitro to be directly linked to pathological processes undergoing in myocardium.
The aim of this study was to evaluate Ankrd1 protein levels in BALB/c mice dilated cardiomyopathy (DCM) pathogenesis process.
Experimental mice group was imumunized with heart myosin derived peptide. Animals weight and general condition were followed 60 days after immunization. Cardiac dimensions were registered echocardiographically. After 0, 7, 14, 21, 40 and 60 days mice were sacrificed, blood samples were taken, heart and lungs were weighed and prepared for further histological and molecular studies.
Mice immunization with cardiac myosin induced autoimmune myocarditis/DCM and animals physical activity during experiment echoed the typical symptoms of heart failure development. It was confirmed by progressive dilation of left ventricle, increased systolic and diastolic ventricular diameters was showed. Heart function abnormalities – worsening of systolic function and decrease in ejection fraction also appeared. Further molecular assay of myocardium cells revealed changes of Ankrd1 protein levels in heart muscle during heart failure remodeling.

24. Picomolar inhibitors of Carbonic anhydrase: importance of inhibition and binding assays

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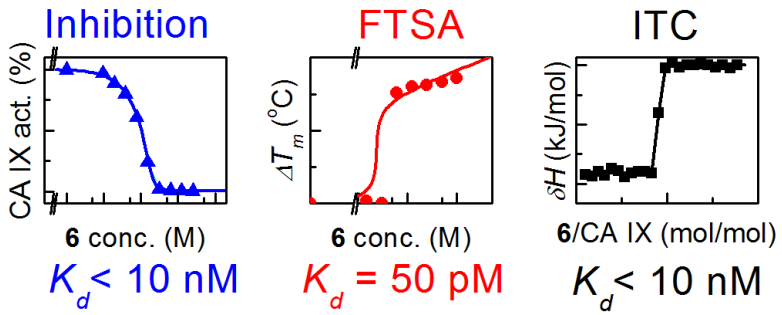
Human carbonic anhydrases (CAs) are targets for drug design due to their role in numerous diseases such as glaucoma, epilepsy, and cancer. Clinically used CA inhibitors–drugs are relatively weak and non-selective for human CA isoforms thus exhibiting toxic side effects. Further drug development should lead to compounds with picomolar affinities and significant selectivities. Currently, the K_i of CA inhibitors is usually determined by the stopped-flow CO_2 hydration assay, the method that directly follows inhibition of CA enzymatic activity. However, the assay has limitations, such as largely unknown concentration of CO_2 and the inability to determine the K_i below several nM. The widely used direct binding assay, isothermal titration calorimetry, also does not determine the K_d below several nM. In contrast, the thermal shift assay can accurately determine picomolar affinities.



The inhibitor dose-response curves were analyzed using Hill and Morrison equations demonstrating that only the Morrison model is applicable for the determination of tight-binding inhibitor K_i. The measurements of interactions between ten inhibitors and seven CA isoforms showed the limitations and advantages of all three techniques. Inhibitor **6** exhibited the K_d of 50 pM and was highly selective towards human CA IX, an isoform which is nearly absent in

healthy human, but highly overexpressed in numerous cancers. Combination of inhibition and binding techniques is necessary for precise determination of CA–high-affinity inhibitor (such as **6**) interactions and future drug design.
Reference: Smirnovienė, J., Smirnovas, V., and Matulis, D. Picomolar Inhibitors of Carbonic Anhydrase: Importance of Inhibition and Binding Assays. *Anal. Biochem*. 522 (2017) 61-72.

25. Fluorescent pressure shift assay – a method to investigate volumetric properties of protein-ligand interaction

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The change in protein volume, induced by binding of a ligand (binding volume), is still a highly unexplored area in molecular biophysics. This is mostly due to the lack of accurate and efficient methods for determining this thermodynamic parameter. Despite the difficulties, investigation of the binding volume is important, because it provides new insights about fundamental protein – small molecule interactions at the molecular scale.
Here we present a technique that allows to determine the binding volume by exploiting ligand-induced stabilization of proteins against pressure denaturation. In this study interactions between carbonic anhydrases and sulfonamide inhibitors were studied using a photon counting spectrofluorimeter equipped with a high pressure system, which sustains a hydrostatic pressure of up to 400 MPa. Different ways of protein fluorescence spectra analysis, including intensity and wavelength shifts, were compared in order to resolve the most appropriate method for tracking changes in protein structure. Various buffer solutions and in some cases denaturing agents (such as guanidine hydrochloride) were used to obtain optimal conditions for the determination of binding volume. Finally, protein-ligand binding constants and binding volumes were determined. Negative volume changes in carbonic anhydrase isoforms I, II and XIII due to acetozolamide and p-carboxybenzene sulfonamide binding were observed. Acetozolamide, the stronger inhibitor, induced more negative volume changes in carbonic anhydrases.

26. Buckwheat seed treatment with electromagnetic field and cold plasma accelerates seedling growth and induces changes in radical scavenging activity of leaf extracts

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Buckwheat, traditional crop cultivated in many countries, is a rich source of vitamins, essential amino acids and phenolic compounds, which are responsible for many of the health benefits and antioxidant properties of this plant. We aimed to investigate the effects of pre-sowing seed treatment with radio-frequency electromagnetic field (EMF, 5-15 min) and cold plasma (CP, 2-7 min), on germination, seedling growth and radical scavenging activity of two buckwheat cultivars – ‘Nojai’ and ‘Vokiai’ (seeds were kindly provided by dr. D. Romanovska from Voke branch of Lithuanian Research centre for Agriculture and Forestry).
Final germination percentage indicating seed viability in control and all treated groups in vitro and in substrate was about 100%. Germination tests in vitro only negligible effects of treatments on germination kinetics of both cultivars. CP and EMF effects on germination in substrate were different from their effects in vitro: both CP and EMF decreased germination rate of ‘Nojai’ (by 14 and 15% CP 2 min and EMF 15 min, respectively) while germination of

'Vokiai' was not affected. Despite suppressing effects on germination rates in substrate, CP and EMF treatments induced positive changes in morphometric parameters of seedlings grown for 6 weeks in the climatic chamber under controlled conditions. For both cultivars height and wet weight of seedlings, the average number of leaves per plant and the number of flowering plants in experimental groups grown from treated seeds was larger (up to 15, 33, 33 and 34%, respectively) in comparison to the corresponding control, while roots were longer only in 'Vokiai' cultivar plants from treated seeds groups (up to 14 %) compared to the control. Radical scavenging activity in the extracts of leaves of young plants grown from seeds treated with EMF (but not CP) was substantially higher – by 43% in EMF 15 min treated 'Vokiai' group and by 50% in EMF 5 min treated 'Nojai' group. Changes, induced by CP and EMF in the content of rutin correlated with changes in free radical scavenging activity.
The obtained results indicate that short time pre-sowing treatment of buckwheat seeds with CP and EMF had only minor effect on seed germination rate, however both CP and EMF treatments increased early seedling growth and accelerated flowering, but only EMF treatments increased radical scavenging activity in leaf extracts.

27. Immunogenicity and functional activity of recombinant pneumolysin

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Pneumolysin (PLY) is a toxic protein produced by Streptococcus pneumoniae. It is a cholesterol-dependent cytolysin forming large pores in cholesterol-containing membranes. Therefore, it is cytotoxic for mammalian cells. PLY mainly causes pathogenic effects contributing to pneumococcal pneumonia and meningitis.
This study investigated functional activity and immunogenicity of full-length hexahistidine-tagged E. coli-expressed PLY (rPLY) in order to generate PLY-specific antibodies that can be employed as diagnostic tools and PLY neutralizing agents. The ability of rPLY to induce cell lysis was performed by an in vitro hemolytic assay using human erythrocytes. In this assay, rPLY lysed erythrocytes in a dose-dependent manner. These data suggest that recombinant PLY expressed in E. coli is functionally active. To investigate the immunogenicity of rPLY, BALB/c mice were immunized with rPLY and the development of antigen-specific IgG antibodies was tested by an indirect ELISA. It was demonstrated that rPLY was immunogenic and induced a strong antibody response in immunized mice. High titers (approximately 1:15000) of rPLY-specific IgG antibodies after the third immunization were determined. In addition, activation of splenic T cells and antigen-presenting cells in response to rPLY was observed. Results gathered here indicate that E. coli-expressed PLY induces a strong immune response in mice.
This study was supported by the Lithuanian Research Council (grant 09.3.3-LMT-K-712-02-0094).

28. Differences in the substrate binding between highly identical catalase-related fatty acid hydroperoxide-metabolizing enzymes

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A catalase-related allene oxide synthase-8R-lipoxygenase (cAOS-8R-LOX) fusion protein is involved in the stress response of corals via arachidonic acid (AA, C20) pathway. It catalyzes

the formation of 8R-hydroperoxyeicosatetraenoic acid (8R-HpETE) and its subsequent conversion into alpha-ketol and cyclopentenone. A highly identical fusion protein, hydroperoxide lyase-8R-lipoxygenase (cHPL-8R-LOX), was identified from soft coral Capnella imbricata. The amino acid identity between cAOS and cHPL domains is 83% and all their catalytically important amino acids are conserved. Despite the high structural similarity with cAOS, cHPL catalyzes the cleavage of 8R-HpETE into the short-chain aldehydes, C8-oxo acid and C12 aldehyde. Based on the X-ray structure of Plexaura homomalla cAOS, positively charged lysines, K60 and K107, in the substrate entry site were proposed to be the anchoring residues of the negatively charged carboxy group of the 8R-HpETE substrate. However, the presence of corresponding residues of C. imbricata cHPL, E60 and K107, indicate that only K107 might be involved in the interaction with the 8R-HpETE substrate.
The first aim of this study was to investigate the involvement of K60 and K107 in the substrate binding by analyzing the substrate entry sites of C. imbricata cHPL and P. homomalla cAOS via in silico and mutational analyses. The second aim was to determine the substrate preference of C. imbricata cHPL and P. homomalla cAOS by measuring the activities with hydroperoxides of naturally occurring polyunsaturated fatty acid (PUFA) substrates and arachidonyl derivatives.
Using homology modelling and docking analysis of the substrate entry site of P. homomalla cAOS with 8R-HpETE, an ionic interaction between K107 and the carboxy head of 8R-HpETE was detected. It was in correlation with the kinetic measurements of cAOS mutants, K60M, K60E, K107M, K107E, where only the K107E mutation caused a significant decrease in the turnover rate of P. homomalla cAOS. In comparison, only the E60 replacements of the cHPL E60M, E60K, K107M and K106E mutants lowered the turnover rate, indicating a different interaction between the carboxy head of fatty acid hydroperoxides and the entry site residues of cHPL.
We also determined that coral cAOS-LOX and cHPL-LOX were capable of metabolizing naturally occurring PUFAs, docoshexaenoic acid (DHA, C22), eicosapentaenoic acid (EPA, C20), alpha-linolenic acid (ALA, C18) and gamma-linolenic acid (GLA, C18). At the same time, the substrate preference of cAOS and cHPL differed remarkably. While cAOS preferred specifically C20 PUFAs, the best substrate of cHPL was the C22 PUFA. Moreover, only cHPL was capable of metabolizing neutral hydroperoxy substrates, anandamide, 1- and 2-arachidonyl glycerols, and methylated fatty acid hydroperoxides, which refers to the unnecessity of the salt bridge formation in the substrate binding and catalysis by cHPL.
In conclusion, K107 of P. homomalla cAOS and E60 of C. imbricata cHPL were determined as the key residues involved in the anchoring of charged fatty acid hydroperoxides. P. homomalla cAOS metabolized only charged fatty acid hydroperoxides whereas C. imbricata cHPL catalyzed both charged and neutral substrates in a similar fashion. Our results indicate that despite the highly identical core structure of P. homomalla cAOS and C. imbricata cHPL, their substrate preference and binding are strongly influenced by their entry sites.

29. The correlation between complete blood count, CRP, platelet activity and NYHA class in chronic heart failure patients

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Introduction: The prognostic impact of CRP in coronary artery disease is well known, however, we do lack proofs of CRP importance in chronic heart failure. Furthermore, heart failure is associated with increased risk of venous thromboembolism, stroke and the formation of platelet-leukocyte aggregates play an important role in the pathogenesis of thrombotic events. In this study we aimed to evaluate the correlation between complete blood count, CRP and platelet activity and compare our results between patients of II-IV NYHA class. It is important to determine early biomarkers for predicting the severity of the illness and the risk of developing thrombotic events.

The aim was to determine the correlation between complete blood count, CRP and platelet aggregation in CHF patients.
Objectives: 1.To determine the significance of differences of complete blood count, CRP and platelets aggregation between patients of different NYHA classes.
2.To determine correlation between complete blood count, CRP and platelets aggregation.
Materials and Methods: Were investigated 99 patients: NYHA II, n= 22; NYHA III, n= 59 and NYHA IV, n= 18. Complete blood count testing was performed on a hematological analyzer COULTER LH 780. The quantification of C-reactive protein concentrations based on turbidimetry was performed using a "Synchron" analyzer. Platelet aggregation was investigated in platelet-rich plasma using an aggregometer (Chrono-Log, USA) by the standard Born method. ADP (3.8 mmol/L, Chrono-log P/N 384) and ADR (4.5 mmol/L), were used to induce aggregation. The spontaneous aggregation (SP) was registered without an inductor. Statistical analysis was performed using SPSS 21.0 statistics pack. Student t test was used to determine the correlation of parametric criteria and Pearson's correlation coefficient. The difference was considered significant if p-value was below 0.05.
Results: Platelet count, mean platelet volume and platelet aggregation was significantly higher among patients with IV NYHA class compared to III NYHA class. There was a significant correlation between platelet aggregation and MPV ($p<0.05$, r=0,223). There was a significant positive correlation between lymphocyte count and platelets aggregation ($p<0.05$, r=0.218), monocyte count and platelets aggregation ($p<0.05$, r=0.205); however, there was a negative correlation between neutrophil count and platelet aggregation ($p<0.05$, r=-0.141). There was no significant correlation between CRP and platelet activity ($p>0.05$).
Conclusions:
1. Platelet count, MPV and platelet aggregation were higher among patients of higher NYHA class. Patients of IV NYHA class had significantly higher platelet count, MPV and platelet activity, compared to III NYHA class patients.
2. Lymphocyte count and monocyte count positively correlated with the platelet activity.

30. The relationship between platelet activity and cortisol and BNP concentrations in the blood of patients with chronic heart failure (CHF).

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Introduction: In response to pressure or volume overload, cardiac ventricular myocytes excrete brain natriuretic peptide (BNP). In patients with chronic heart failure, elevated blood levels of BNP are associated with the severity of CHF and an increased risk of mortality. Also, studies have shown that in the case of long-term fatigue and cardiovascular disease, the hypothalamus-pituitary-adrenal axis function is affected, which is reflected in the insufficiently reduced afternoon cortisol concentration. Processes as inflammation and neuroendocrine dysregulation altered the platelet activity. Therefore, this work sought to identify the relationship between chronic fatigue, patient status and platelet activity.
Materials and Methods: Ninety patients with CHF were studied. They were divided in two ways: 1) by age, from 0 to 49 years old (n = 37) and from 50 to 76 years old (n = 53); 2) according to the severity of status, II NYHA functional class, 17 patients; III NYHA class, 55 patients; class IV, 8 patients. Platelet aggregation was investigated in platelet-rich plasma using the aggregometer (Chrono-Log, USA) by the standard Born method. ADP (3.8 mmol/L, Chrono-log P/N 384) and adrenalin (45 mmol/L), were used to induce aggregation. Spontaneous aggregation (SP) was registered without inductor. BNP concentration in serum was determined by a quantitative immunofluorescence method. Cortisol concentration in serum was measured by an enzyme-linked method.
Results: The study showed a statistically significant correlation ($p <0.05$), between BNP in the blood and the NYHA functional classes III and IV. There was also a significant correlation between adrenalin induced platelet aggregation and severity of the patient's condition ($p <0.043$, r= -0,248). A statistically significant correlation ($p <0.018$) was obtained between

ADP induced platelet aggregation and BNP levels. When assessing the difference between cortisol concentrations in the morning and afternoon between NYHA functional classes, no statistically significant results were obtained, but in terms of numerical values, we can see that the cortisol difference among patients of IV NYHA class is less than of class III, which could mean that the afternoon cortisol is higher than normal in patients of higher functional classes.
Conclusions: 1. In case of an aggravated patient's condition, BNP concentration and platelet aggregation were statistically significantly higher ($p < 0.05$ and $p < 0.043$, respectively). Cortisol concentrations were not statistically significantly related to the severity of patient's condition.
2. The cortisol and BNP concentrations and platelet aggregation did not correlate.

31. Interaction of bacteriophage vB_EcoM_Alf5 (Alf5) tail fiber proteins with bacterial hosts

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Adsorption to the host cell surface receptors is one of the critical stages of viral infection. Tailed bacteriophages (bacterial viruses, phages) use their tail fiber structures for specific recognition of their bacterial hosts. Specificity is determined by the adsorption and binding of the adhesin domain located on the end of the tail fiber to the particular host cell structures. Bacteriophages of the Felixo1virus genus encode two putative tail fiber proteins, but their function has not been explored yet. In the genome of Felixo1virus vB_EcoM_Alf5 (Alf5), putative tail fibers are encoded by the genes Alf5_ORF073 and Alf5_ORF074. We constructed and purified N-terminally His-tagged recombinant proteins Alf5_gp073 and Alf5_gp074. Whole cell binding assays show that the purified recombinant protein Alf5_gp073 binds to the Escherichia coli BW25113 host cells irreversibly, but fails to bind to E. coli BL21, which is not a host for Alf5 phage. Meanwhile, Alf5_gp074 does not bind to either E. coli BW25113 or BL21 strains. However, adsorption inhibition tests showed that Alf5 phage adsorption to the Alf5_gp073-treated E. coli BW25113 cells was not blocked fully, unless Alf5_gp074 was added. These results suggest that both, Alf5_gp073 and Alf5_gp074, act in cooperation during Alf5 phage adsorption to the host cells.

32. Metataxonomic analysis of berries-associated microorganisms

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In recent years, the exploration of various plants and their products for improving human health grew steadily. Among the beneficial berries black chokeberry (Aronia melanocarpa (Michx.) Ell.), sea buckthorn (Hippophae rhamnoides L.), black, red and white currants (Ribes nigrum, Ribes rubrum L. and the cultivar of Ribes rubrum) are particularly popular. The quality of fruits and berries along with the content of active components depend on the cultivation and climatic conditions during vegetation, application of agrochemicals, hydration as well as on microorganisms colonizing the surface of fruits and influencing plant development, adaptation and evolution, in turn affecting plant potential in food production.
The present study is the first work providing comprehensive information on microbial populations of black chokeberry, sea buckthorn, black, red and white currants. Next Generation Sequencing allowed identification of eukaryotic and prokaryotic microorganisms prevalent on specific berries, including uncultivable microorganisms. Our study revealed the broad diversity of berries-associated bacterial and fungal microorganisms. Analysis of

representative microbial OTUs showed a clear separation among inhabitants of sea buckthorn, black chokeberry and currants, indicating plant-defined differences in the composition of the bacterial and fungal microbiota. Among the microorganisms distributed on tested berries, we documented potentially beneficial fungi and bacteria along with potential phytopathogens or those harmful for humans. The high-throughput identification and quantification of microflora composition provided information relevant for plant disease management, increasing the yield of the desired crop, and uncovered the potential role of microbiota in berries-based food production.
This study was supported by a grant from the Lithuanian Research Council (No. SIT-7/2015).

33. Improved photodynamic inactivation of microorganisms by protoporphyrin IX photoproducts

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Photochemical transformations of protoporphyrin IX (PpIX) during potodynamic inactivation result in PpIX photodegradation (photobleaching) and PpIX photoproducts formation. PpIX photoproducts are characterised with enhanced light absorbtion in 640 – 680 nm spectral region. Presumingly, light irradiation at 656 nm wavelenghth would continue microorganism photodynamic inactivation by employing photoexcitation of PpIX photoproducts alone. The aim of this study was to evaluate the phototoxicity of PpIX photoproducts during photodynamic inactivation of S. aureus and C. albicans cells.
C. albicans (or S. aureus) were prepared from overnight cultures on Sabouraud dextrose agar (respectively on Lysogeny broth) medium with shaking at 30 oC (or 37 oC), then collected with centrifugation and resuspended in PBS at pH = 7 to obtain 1.82 × 107 u. ml-1 (or 1.8 × 109 u. ml-1) starting concentration. A 0.1ml of cell suspension was transfered into non-transparent microplate and incubated with 50 μM PpIX for 10 min. Light doses of 10 J/cm2 (405 nm excitation, 45 mW/cm2) and 60 J/cm2 (656 nm excitation, 100 mW/cm2) were applied for photodynamic inactivation. Cell colony counts were performed 18 h and 48 h for S. aureus or C. albicans respectively, and compared to the untreated controls.
1.7 and 0.88 log10 reductions of S. aureus and C. albicans viability were achieved with 10 J/cm2 dose, applying blue light. Aditionally applied red light induced significant ($p<0.001$, Mann-Whitney) decrease of the sensitized microorganisms' viability, resulting 2.44 log10 killing efficacy for S. aureus and 1.16 log10 killing efficacy for C. albicans, respectively.
We conclude that signifficant improvement of photodynamic inactivation efficacy against S. aureus and C. albicans cells was induced by PpIX photoproducts.

34. A methodological approach to investigate an effect of heavy metal speciation on biosorption process using biomass and universal buffer: thermodynamic database creation and modeling

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Biosorption is a mechanism related to ability of biomass cell walls to remove heavy metals from surrounding. Considering physico-chemical interactions with active groups present on the cell wall, biosorption is affected by several chemical and physical factors in the presence of heavy metals as well as kind of biosorbent material. Equilibrium model is the first step in the development of kinetic models to be applied in experimental operations. In this study an importance of database creation and methodological approach to modeling of speciation and sorption of Ni(II) and Cr(IV) in a presence of chitosan in universal buffer media during

biosorption processes has been proposed and discussed. The distribution coefficients were established using common aqueous geochemical modeling program with updated database.

35. Determination of TRPM7 expression in cardiomyocytes of ischemic cardiomyopathy patients

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Introduction: TRPM7 (transient receptor potential melastanin 7) gene appears to be ubiquitously expressed, with highest expression in heart tissues. Our preliminary data have shown that TRPM7 is expressed in both human atria and ventricle cells. However the quantitative analysis of TRPM7 expression has not been performed. It is known that these channels participate in a variety of pathological processes; therefore we aimed to evaluate the levels of TRPM7 expression in cardiomyocytes of ischemic cardiomyopathy patients.
Aim: to determine the levels of TRPM7 protein expression in atrial vs. ventricle cells from patients with ischemic cardiomyopathy. In addition, we looked for possible changes of protein expression levels depending on the patients clinical history on myocardial infarction (MI).
Materials and methods: We used enzymatically dissociated cardiomyocytes. After fixation, permeabilization and prevention of non-specific binding using blocking buffer, cells were incubated with primary mouse monoclonal or rabbit polyclonal anti TRPM7 antibody (1:200) in blocking buffer overnight at 4oC. For negative control, incubation with primary antibody was omitted. Further, the cells were incubated with fluorescently labelled secondary antibody AF488 (1:200), co stained with Phaloidin (1:100) and with Hoechst (25µg/mL), for labelling of the F actin cytoskeleton for contrast staining, and of the nucleus, respectively. Glass-slides were covered with Anti-fade Reagent. Cardiomyocytes were visualized under confocal laser scanning microscope. Using ImageJ, the level of TRPM7 protein expression was evaluated by calculating the amount of protein per pixel, i.e. intensity of fluorescence (in a.u.) recalculated per cell size (in pixels). The statistical significance of data was tested using unpaired Student's t-test.
Results: The quantitative analysis of the TRPM7 in the atria and ventricle cells from patients with ischemic cardiomyopathy revealed that were no significant difference of protein expression levels between both cardiomyocyte types: for atria was 0.15±0.02 (n=31) and for ventricle was 0.11±0.02 (n=15). In order to evaluate possible changes in protein levels due to MI, all the data were grouped according to patients clinical history (with/without MI). As it was expected, in ventricular cells the expression level was significantly higher (0.15±0.02, n=7) with MI than in cell from patients without such pathology (0.07±0.02, n=8). While in atrial cardiomyocytes there was no significant difference in protein expression levels between both groups (with/without MI).
Conclusions: We found that there was no difference of TRPM7 protein expression levels between atrial and ventricular cells from patients with ischemic cardiomyopathy. In addition, our data also suggest that myocardial infarction increases level of TRPM7 in ventricular cardiomyocytes.
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36. Impact of $BaSO_4$ particles on the viability of eukaryotic and prokaryotic cells

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With the constant advances in nanotechnologies and emerging nanoproducts market, the need to investigate not only chemically active but also stable particles such as $BaSO_4$ effects on the environment become evident. Such breakthroughs may result in undesirable

environment developments and negatively affect bacteria, plant life, humans. The inevitable process of car stopping is related with the $BaSO_4$ particles emission because of break pad waning. Approximately 220000 tons of breaking pads per year worldwide are grinded and their small particles spread around the environment. Such enormous quantities may result in biological effect on the environment and human well-being. Risk assessment of barium sulphate nanoparticles require careful evaluation of its mobility, reactivity, environmental toxicity, and stability. Thus far, few studies have been conducted on the toxicity to the environment caused by direct and indirect exposure to barium sulphate nanoparticles. Until now, no clear studies have been carried out to evaluate the effects of barium sulphate nanoparticles on eukaryotic or prokaryotic cells.
For the experiments a stable barium sulphate nanoparticles suspension has been prepared, with particles having a maximum particle size up to 50 nm. Nonionic surfactant Tween 80 added in suspension reduced the polydispersity degree by 2 times and zeta potential 1.3 times, indicating a higher stability of the $BaSO_4$ suspension. When the concentration of barium sulphate nanoparticles was 0.42 mg/ml, it inhibited the growth of *P.aeruginosa* bacteria, this concentration was 2 times higher for *E.coli* and 3 times the *S.enterica*. Due to the nonionic surfactant additive barium sulfate nanoparticles have increased antibacterial activity: *E.coli* and *P. aeruginosa* 2 times, *S.enterica* 16 times. The growth of gram-positive *S.aureus* bacteria was inhibited when the concentration of barium sulfate nanoparticles was 0.1 mg/ml. The acidification of the medium, which may lead to the ionization of low soluble nanoparticles, with HCl to pH 6.2 increased antibacterial activity of barium sulphate nanoparticles on *E.coli* bacteria 4 times, and acidification with H_2SO_4, which may participate not only in the ionization process but in the formation of complex compound with barium sulfate also, increased the effect 8 times. The correlation between the increase of zeta potential of the bacterial suspension and the decrease in the viability of bacteria has been. Toxicity of nanoparticles was evaluated by the determination of viability of Chinese hamster ovary CHO cells.
Keywords: barium sulphate nanoparticles, zeta potential, viability, eukaryotic and prokaryotic cells.

37. The role of Connexin 43 in myogenic lineage-differentiated muscle-derived stem cell apoptosis regulation

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Gap junctions are intercellular channels, made up of proteins called connexins that ensure the metabolic and electrical communication between adjacent cells. However, studies have shown that beside their traditional role in intercellular communication connexins may be involved in various cellular processes, including cell death.
Stem cells are essential for maintenance of tissue and organ homeostasis. Chemotherapy drugs can affect not only cancer cells but also normal cells, including stem cells. In recent years, stem cell-based regenerative therapy has become a strategy to replace the damaged cells with stem cell-derived healthy ones in heart failure, diabetes and other conditions. Evaluation of stem cell death regulation mechanisms can help to improve cell therapy as well as to decrease the side effects of cancer treatment strategies.
The role of connexin 43, most abundant heart connexin, in rabbit muscle-derived stem cell apoptosis has been analysed in this work. Myo cell lines induced to differentiate along the myogenic lineage were used in our studies. Chemotherapeutic agents cisplatin and daunorubicin served as apoptosis inducers.
Cx43-overexpressing Myo cell lines were generated. Imaging of fluorescent protein-tagged connexin 43 as well as Western blotting was used to confirm upregulation of Cx43 expression in transfected cells. Cx43 functionality was confirmed by use of pharmacological

gap-junctional communication inhibitors and enhancer that modulated transfer of Lucifer yellow dye. Cell screening for the Cx43 protein showed the decrease of endogenous but not exogenous Cx43 expression during process of myogenic differentiation. We found that the role of Cx43 in muscle-derived stem cell apoptosis regulation was dependent on cell differentiation state. Unlike in proliferating Myo cells, differentiated cells showed the proapoptotic activity of this protein. Overexpression of Cx43 increased cell death in myogenic lineage-differentiated Myo cells as was evidenced by MTT assay as well as apoptotic cells in culture. In the search for possible intracellular mechanism of action of Cx43 in controlling cell death, increased phosphorylation of MAP kinases ERK and JNK during cisplatin-induced apoptosis in Myo9 Cx43 transfecants was shown. Thus, the results of our study prove the proapoptotic role of Cx43 in differentiated Myo cells.

38. Hyperthermia enhances sensitivity of Ovcar-3 cells to cisplatin

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Ovarian cancer is the most common cancer for women. Hyperthermic chemotherapy is used aiming to overcome the resistance of cancer cells to cisplatin and to reduce cisplatin doses. Due to the shift in energy metabolism of certain types of cancer to aerobic glycolysis (Warburg effect), glutamate dehydrogenase (GDH) was suggested as an attractive anticancer target, because mitochondria in cancer cells became producers of non-essential amino acids from tricarboxylic acid (TCA) intermediates and exports excess of citrate to cytosol to support fatty acid synthesis and NADPH production. Given that GDH is an enzyme converting glutamate to TCA intermediate α-ketoglutarate, it acquires an important role in Ovarian cancer cell viability. However, the interaction of GDH with common anticancer drugs as well as changes in GDH activity upon the hyperthermia treatment of cancer cells remained uninvestigated.

The aim of this study was to investigate the effects of mild hyperthermia (40°C and 43°C for 1h), cisplatin IC50 or combination of both treatments on GDH activity, respiration and cell viability immediately after hyperthermic treatment (0h) and after 24 and 48h recovery at 37°C in Ovcar-3 cell line.

Cisplatin inhibits GDH in Ovcar-3 cells, however not immediately after treatment, but only after 24 or 48h recovery by 40 and 60%, respectively. That possibly indicates to indirect inhibition effect (e.g., effect on GDH expression). Hyperthermia (43°C) in the absence of cisplatin strongly stimulates GDH in Ovcar-3 cells, however this effect is pronounced only immediately after treatment and diminished with time to the levelof control. Combination of cisplatin and temperature had greater (by 10%) effect on GDH activity as compared with cisplatin effect alone, however significant difference only detected at 43°C temperature at 0 and 48h.

Respiration of Ovcar-3 cells was not affected by hyperthermia but inhibited by cisplatin treatment (State 2 by 18%, state 3 by 21%, RCI by 15%). Combination cisplatin with hyperthermia (43°C) increased inhibition effect on respiration by 7%, 22% and 17% of State 2, State 3 and RCI, respectively, compared to inhibition by cisplatin alone.

Ovcar-3 cell viability (estimated by MTT) and colony formation was reduced (10% and 22%, respectively) only by 43°C hyperthermia. Cisplatin alone reduced cell viability by 50% in both tests. Hyperthermia (43°C) in combination with cisplatin increased viability loss by 20% and 30% for MTT and colony formation tests, respectively

We conclude that hyperthermia enhanced sensitivity of Ovcar-3 cells to cisplatin: combinatory treatment inhibited respiration, GDH and reduced cell viability more than cisplatin alone.

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39. Morphological and molecular identification of eight Sarcocystis species from sika deer (*Cervus nippon*) in Lithuania

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Protozoan parasites of the genus Sarcocystis are characterised by an obligatory two-host life cycle. The formation of sarcocysts in muscles or CNS occurs after intermediate host becomes infected by ingesting food or water contaminated with sporocysts. Cervids serve as intermediate hosts of numerous Sarcocystis species; however, the researches on these parasites in the sika deer were scarce. In this study, diaphragm muscles of bred sika deer were examined for sarcocysts and high infection prevalence was established 94 percent (32/34). A total of 37 cysts isolated were morphologically (using light microscopy) and molecularly (using 18S rDNA and mitochondrial cox1 sequences analysis) characterised. Morphological analysis based on shape and size of cyst, and cyst wall structure was not sufficient in some cases to conclusively disclose the species. Whereas, DNA sequence comparison lead to the identification of eight Sarcocystis species, S. entzerothi, S. frondea, S. nipponi, S. ovalis, S. pilosa, S. taeniata, S. truncata and Sarcocystis sp. in the host examined. The 18S rDNA, in contrast to the cox1 was not sufficiently variable to discriminate S. pilosa, S. taeniata and S. truncata from other closely related Sarcocystis species. Identified species differed in their intraspecific genetic variability at both genes analysed. A close phylogenetic relationship was established between above mentioned and other Sarcocystis species, which are known to infect cervids. The phylogenetic results may be useful in suggesting definitive hosts of Sarcocystis species investigated. To conclude, the sika deer is characterised by fairly high Sarcocystis infection prevalence and species diversity.

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40. Promoter methylation analysis of tumor suppressor genes in prostate cancer tissue and urine

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Prostate cancer (PCa) is the second most prevalent malignancy in men worldwide. Despite recent achievements in PCa diagnostics and treatment, new molecular biomarkers that could outperform the currently used ones are highly desirable. Aberrant DNA methylation in promoter region is considered as the earliest somatic genome change in cancer. Thus, promoter methylation of tumor suppressor genes could be used as biomarkers for PCa diagnostics and prognosis.

The aim of this study was to evaluate the promoter methylation status of tumor suppressor genes RARB, RASSF1, and GSTP1 in clinical samples as potential PCa biomarkers.

In the present study, 112 tumor samples and 16 noncancerous prostate tissues (NPT) from 113 PCa patients were analyzed by means of methylation-specific PCR (MSP). Also, DNA from voided urine samples of 44 PCa patients were tested using quantitative MSP.

Significantly different methylation frequencies of RARB, RASSF1, and GSTP1 were observed comparing PCa and NPT samples (all $p < 0.0001$). The sensitivity of these biomarkers for PCa was up to 99%, while specificity reached 87%. Moreover, promoter methylation of

RARB, RASSF1, and GSTP1 was detectable in urine, where mean methylation levels comprised 0.02%, 0.04%, and 0.01%, respectively. In tumors, GSTP1 was more frequently methylated in PCa cases with higher prostate mass ($p = 0.0112$). Methylation of all the three genes was also more commonly observed with increasing Gleason grade group, however, the associations were not statistically significant (all $p > 0.05$). No correlations were detected between gene promoter methylation and tumor stage, prostate-specific antigen level, or biochemical disease progression.
In conclusion, frequent PCa-specific promoter methylation of RARB, RASSF1, and GSTP1 might serve as novel epigenetic biomarkers for PCa diagnostics, potentially applicable for noninvasive diagnostics. Adding RARB, RASSF1, and GSTP1 promoter methylation status to currently used clinical tests has the potential to improve early PCa detection.

41. Optimization of fermentation conditions for recombinant human BiP production in yeast *Pichia pastoris*

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Background. Human BiP, also known as GRP78, is a molecular chaperone mainly located in the endoplasmic reticulum luminal. However growing amount of data also associates BiP with many different functions in subcellular locations outside the ER. Importantly, several diseases have been BiP-related, including rheumatoid arthritis, few types of cancer, autoimmune inflammation and tissue damage; therefore it could potentially be used for therapeutic purposes. The aim of this study was to optimize a high cell density fermentation process for secretion of recombinant human BiP in P. pastoris in defined medium. In our previous work recombinant BiP protein was expressed in Saccharomyces cerevisiae and P. pastoris with the titre reaching up to 10-20 mg/L in shake flasks using complex YEPG/M medium. However secreted BiP titer was 5-10 fold lower when P. pastoris was cultivated in defined mineral media – BSM (basal salt medium). In contrast, the other human ET chaperone – calreticulin was expressed with higher titre in BSM medium compared to complex medium. Thus, lower BiP titer in BSM medium could be related to protein stability, aggregation or degradation.
Results. First of all, in this work we focused on optimization of BSM growth medium composition in shake flasks. It was found that supplementation of BSM with 2mM DTT before induction lead to increased recombinant BiP titer, which was comparable to titer reached using complex YEPM medium. Furthermore, BiP secretion bottleneck in yeast – the translocation of BiP to ER – was relieved by inducing protein expression with defined glucose and methanol mixture instead of sole methanol. Combination of 2:1 ratio of methanol to glucose in feeding solution and addition of 2mM DTT in to the medium before induction increased BiP titer in BSM medium in shake flasks approx. 4-5 times compared to YEPG/M to 70-80 mg/L. Glucose/methanol mixture feeding with addition 2mM DTT before induction was applied in high density Pichia pastoris fermentation in bioreactor. Fermentation using continuous feeding with methanol/glucose mixture was unsuccessful, because BiP titer was considerably lower compared to shake flasks and BiP degradation products were observed. However, using pulse feeding, when methanol/glucose mixture was injected every 8 h, BiP titer reached approx. 130 mg/L after 48h induction and approx. 1.6 times exceeded shake flask titer.
Conclusions. In conclusion, optimization of P. pastoris fermentation conditions for human BiP secretion was achieved. We assume that DTT increases stability of secreted BiP in BSM medium by reducing protein aggregation and/or degradation induced by cysteine oxidation and/or formation of S-S bond. Also, BiP secretion titer was markedly increased by relieving bottleneck of BiP translocation to the ER by decreasing yeast AOX1 promoter activity by adding glucose in to the induction solution.

42. Chemical composition of essential oils of some aromatic and spice plants

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During the last decades the aromatic and spice plants have drawn attention as a subject for research on their biological activities. Essential oils (EOs) which are aromatic volatile liquids, isolated from plants, are known to protect them and often possess antibacterial properties.

The objective of this study was to extract essential oils from some common aromatic herbs and spices, widely used for flavouring of foods, to determine their chemical composition and evaluate antibacterial effects against selected bacteria strains.

The essential oils of dry material were extracted by hydro-distillation method, using a Clevenger-type apparatus. The GC-FID and GC-TOFMS methods were used to analyse and identify 60-70 constituents composing up to 99 % of total EO from the following species: bay laurel leaves (Laurus nobilis L.), common sage (Salvia officinalis L.), coriander seeds (Coriandrum sativum L.), thyme (Thymus vulgaris L.) and oregano (Origanum vulgare L.). Thymol was found to be the main compound in the investigative thyme EO (64 percent), as well as in oregano EO (22 percent), meanwhile eucalyptol was the main component among the aromatic compounds of EO from bay leaves (52 percent), while linalool constituted the major part (71 percent) among the volatiles of EO from coriander seeds.

Taking into account variability in the chemical composition of essential oils depending on the season of harvest, growing conditions and extraction methods, as well as differences in the sensitivity of various bacterial strains, testing of antimicrobial properties was of some interest. The antibacterial activities of selected EOs against six strains of foodborne pathogenic bacteria were tested by using filter disk diffusion method and depended on the bacterial species and EO concentration.

43. Potential stress markers in cat's urine

Raminta Jacevičiūtė, Rūta Budreckienė

Department of Biochemistry, Lithuanian University of Health Sciences, Lithuania

The aim of this work was to perform analysis of biochemical indicators and to evaluate stress markers intensity and dynamic in cats urine.

Biochemical indicators were determined and analysis was performed using urine analyzer URIT-50 in urine of three different stress level cats groups: household cats (stress-free), hospitalized (short-term stressful) and sheltered (long-term stressful) cats. The chromatographic analysis was performed using a gas chromatograph Clarus 680, PerkinElmer. Survey was based on the gas chromatographic analysis and sample preparation method by Sanja Grković with colleagues. The findings of stress markers in cat urine and their comparison was carried out and statistical analysis of acquired test results was evaluated.

The quantity of leukocytes in all three cat groups was increased, traces of ketonic bodies were detected in two samples. Nitrites were indicated in only two urine samples of hospitalized cats. The value of urobilinogen fluctuates from normal to 66 μmol/l, while bilirubin was found in only one of 26 samples. The amount of protein in urine samples varies up to ≥3.0 g/l, while glucose prints were indicated in only two hospitalized cats urine samples. Urine relative density varies from 1.010 to ≥1.030, while pH – from 5.5-8.5. Traces of blood was detected in five samples. While analyzing cat urine chromatograms the main peaks were observed at 28th and 32nd minute, at 7th minute the peak of malon acid was recognized. The most intensive peaks were noticed in the urine of house hold cats, lower intensity – sheltered cats, lowest intensity – hospitalized cats. The results and their fluctuations of urine biochemical indicators do not depend on catecholamines metabolite homovanillic (HVA) acid, vanillylmandelic (VMA) acid and the quantity of their alteration. Since dopamine and its metabolite homovanillic acid was not found, we can make an assumption that this substance is absent in cat organism.

Key words: cat urine, urine tests, catecholamines, homovanillic acid, vanillylmandelic acid.
References: S. Grkovic, R. Nikolic, M. Dordevic ir L. Stojanov, „Urinary catecholamine metabolites: capillary gas chromatography method and experience with 12 cases of neuroblastoma," Indian Journal of Clinical Biochemistry, pp. 178-181, 2005.

June 29, LBS Conference
Oral presentations

The targeting of β-carbonic anhydrase from *Ascaris lumbricoides* for treatment of ascariasis

Reza Zolfaghari Emameh[1]; Marianne Kuuslahti[2]; Daniella Vullo[3]; Harlan R Barker[2]; Claudiu T Supuran[3]; Seppo Parkkila[2,4]

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Carbonic anhydrases (CAs) are metalloenzymes, which play critical roles in many physiological functions such as electrolyte transfer, pH homeostasis, photosynthesis, and calcification [4, 5]. CAs are classified into seven distinct evolutionary families: α, β, γ, δ, ζ, η, and θ. CAs catalyze the hydration of carbon dioxide to bicarbonate and proton. Although, all CA families use zinc ions as metal cofactor, γ-CAs use iron and cobalt and ζ-CAs use cadmium ions, as well as zinc ions in their catalytic active sites. Among these enzyme families, β-CAs are present in prokaryotes and eukaryotes including fungi, plants, protozoans, insects, and nematodes, while this enzyme family is absent in vertebrates. Therefore, inhibition of β-CAs of parasites would be a promising approach to fight against parasitic infections.
Around 760 million people worldwide are infected with *Ascaris lumbricoides* as the causative agent of ascariasis, which is normally caused by feces contamination in water, vegetables, and other food. There are different treatment strategies for the infection, such as application of anthelminthic drugs including albendazole, mebendazole, and pyrantel pamoate. In this presentation, we are going to show the properties of *A. lumbricoides* β-CA (AlBCA) using bioinformatics tools, produced AlBCA as a recombinant protein, and tested its kinetic and inhibition properties.

Type III CRISPR-Cas immunity

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CRISPR-Cas systems provide RNA-mediated adaptive immunity against viruses and plasmids in bacteria and archaea. Easy reprogrammability makes CRISPR-Cas effector complexes (Cas9, Cpf1, etc.) perfect tools for genome editing and molecular biology in general. Type III CRISPR-Cas effector complexes are composed of multiple subunits and simultaneously target both RNA and DNA. We have previously shown that such complex from Streptococcus thermophilus, StCsm, possesses three different activities that are triggered upon target RNA binding: (i) degradation of ssDNA by Cas10 HD domain; (ii) synthesis of cyclic oligoadenylates by Cas10 Palm domain; and (iii) cleavage of the target RNA itself by Csm3 ribonuclease domain [1,2]. The cyclic oligoadenylates, in turn, stimulate a stand-alone non-specific ribonuclease. While the active sites of Csm3 and Cas10 have been identified, roles of other non-catalytic subunits remain unknown. Here we used the

combination of deletion and biochemical analyses to determine the role of non-catalytical Csm subunits in StCsm complex assembly and activity. Although StCsm complex is a tight-knit machinery in which every part plays a role in ensuring optimal nucleic acid cleavage, we show that it is possible to minimize it retaining the functional activity.
1. Tamulaitis, G, Venclovas Č, Siksnys V. (2017) Type III CRISPR-Cas Immunity: Major Differences Brushed Aside. Trends in Microbiology 25(1), 49-61
2. Kazlauskiene M, Kostiuk G, Venclovas Č, Tamulaitis G, Siksnys V. (2017) A cyclic oligonucleotide signaling pathway in type III CRISPR-Cas systems. Science, 357(6351), 605-609

A doubly methylated base N4,5-dimethylcytosine in DNA and its repair

Miglė Tomkuvienė; Marina Alexeeva; Almaz N. Tesfahun; Prashanna Guragain; Rūta Gerasimaitė; Arne Klungland; Saulius Klimašauskas; Svein Bjelland

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Cytosine (C) in DNA is often modified to 5-methylcytosine (5mC) in all domains of life, ranging from viruses to mammals. The modification executes various important functions, one to mention is being a crucial epigenetic mark in the mammalian cells. Despite the significance of this base, damage to 5mC has received little attention. Prokaryotes can also use C methylation at the N4 position. Importantly, N4-methyltransferases are able to convert 5mC into N4,5-dimethylcytosine (4,5mC). We suggest that 4,5mC occurs as a disturbing lesion in DNA, calling for an urgent repair. We screened a series of glycosylases from prokaryotic to human and found significant DNA incision activity of the Escherichia coli Nei and Fpg proteins at 4,5mC residues in vitro. To our knowledge, this is the first description (1) of a repair enzyme activity at a further methylated 5mC in DNA.
1. Alexeeva M et al. (2018) Excision of the doubly methylated base N4,5-dimethylcytosine from DNA by Escherichia coli Nei and Fpg proteins. Phil. Trans. R. Soc. B 373: 20170337.

MicroRNA as biomarkers for non-invasive diagnostics of precancerous and cancerous colon diseases

V. Salteniene[1], S. Juzenas[1], J. Skieceviciene[1], J. Kupcinskas[1], J.Arstikyte[1], Georg Hemmrich-Stanisak [2], Gracie Zhipei Du [2], Andre Franke [2] L. Kupcinkas[1].

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Background and aims: MicroRNAs (miRNAs) are small non-coding RNAs which post-transcriptionally suppress gene expression. These molecules play an important role in oncogenesis and are aberrantly expressed in cells of various human cancers. Colorectal cancer is a leading cause of cancer-related morbidity and mortality in the worldwide. The early detection of colorectal cancer and improved survival, treatment decisions are exclusively based on cancer clinicopathological results. Therefore, the search for new biomarkers to facilitate early diagnosis is particularly warranted. The aim of this study was to investigate miRNA profiles in precancerous and cancerous colon tissues and to assess the relevance of these biomarkers for non-invasive diagnosis of the malignant disease.
Methods: The profiling study consisted of healthy controls (n=32) and patients with CRC (n=20) and AP (n=20). Total RNA was used to prepare small RNA libraries following Illumina TruSeq protocol, which were sequenced on Illumina HiSeq 2500 platform. Small RNA-seq data preprocessing was performed using cutadapt while quantification of miRNAs was carried out using quantifier.pl module from miRDeep2 package (reference miRBase v21). The validation study was performed on the most putative miRNA candidates (22 highly

differently expressed in tissue samples and detected by sequencing in plasma samples of CRC patients), which were quantified using independent cohort of CRC and AP patients as well as healthy individuals (in total, n = 120) and using different miRNA profiling methods. The first validation stage of sequencing results was performed by using Taq-Man low-density array (TLDA). The second validation step of in tissue verified miRNA candidates was performed on plasma samples using RT-qPCR method. The diagnostic performance of circulating miRNAs was evaluated using radial discriminant analysis.
Results: The comparative analysis of small RNA-seq data identified 157 and 104 differentially expressed miRNAs in CRC and AP tissues, respectively. Validation analysis in tissue samples verified 11out of 16 CRC-associated miRNAs (FDR < 0.05 and |log2FC| > 1) and 5 out of 11 AP-associated miRNAs. Validation analysis in plasma samples identified expression alterations of 5 out of 11 CRC-associated miRNAs. However, the expression levels of previously verified miRNAs in AP tissues were not deregulated in plasma samples. The signature of altered miRNAs was able to predict CRC in plasma samples with sensitivity of 0.625 and specificity of 0.875 (AUC = 78,9%).
Conclusions: The expression levels of circulating miRNAs (hsa-miR-223-3p, hsa-miR-1246, hsa-miR-584-5p, hsa-miR- 224-5p and hsa-miR-183-5p) were significantly altered in plasma of colorectal cancer patients; however, due to its only moderate predictive performance (AUC = 78,9%), the miRNA signature is not relevant for clinical decisions. Adenomatous polyp tissue-specific miRNA levels were not altered in plasma, and thus cannot be used in the detection of the pre-cancerous condition.

Standing and active human gut microbiome in pediatric and adult IBD

Jurgita Skieceviciene[1]; Gitana Pranculiene[2]; Philipp Rausch[3]; Gediminas Kiudelis[2]; John Baines[3]; Limas Kupcinskas[1,2]

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Introduction. A global increase of inflammatory bowel disease (IBD) has been reported, especially in countries that previously had low incidence rates. Changed intestinal microbiota leads to the imbalance of anti- and pro-inflammatory response and the development of chronic inflammation. The aim of the study was to investigate the characteristics of the gut microbiota in pediatric and adult (IBD).
Patients and methods. In this study, a set of 105 mucosal biopsies were sampled from individuals presenting with active IBD and healthy controls were analyzed using 16S rRNA gene profiling (V1-V2 region) at both DNA and RNA levels.
Results. Pronounced general disease patterns in the major phyla and patterns of diversity were found, which differ between the standing and active communities. Standing and active community structure differences were determined according to gender, health or age conditions in pediatric IBD cohort. β -diversity with locally restricted disease clusters and more pronounced effects in the active microbial communities were determined. The active members of the *Firmicutes* and *Bacteroidetes* showed significant differences between gender and health conditions. The standing and active microbial community members of *Actinobacteria* showed a consistent increase with age and decrease with disease. Alpha-diversity analysis revealed significant differences between active bacteria community and pathology. Two genera belonging to the Clostridium leptum subgroup, Faecalibacteria and Papillibacter, displayed consistent patterns with respect to disease status and may thus serve as reliable 'microbiomarkers' in the adult cohort.
Conclusion: Structure differences of standing and active intestinal microbiota were associated with age, gender and health status. Diversity differences of closely related active microbial populations were defined by pathology.

Amperometric bioelectrocatalytic systems: new technologies and their applications

Marius Dagys

Life Sciences Center, Vilnius University

Bioelectrochemical systems incorporating enzymes as compound-specific biocatalysts are widely utilized in fast, accurate and robust analytical applications such as glucose test strips. Similar biosensor devices are used for detection of analytes beginning with common metabolites and electrolytes and ending with specific oligonucleotides. Provided the enzymatic electrodes can produce significant current, the application expands to construction of biofuel and biosolar cells. The Department of Bioanalysis of Life Sciences Center specializes in creation of novel amperometric bioelectrode technologies such as: i) carbon-based amperometric urea biosensor, envisaged for use in monitoring the patients with kidney failure undergoing hemodialysis, as well as measurement of urea in fertilizer samples or wastewater; ii) gold nanoparticle-based direct electron transfer bioelectrocatalytic electrodes for efficient conversion of fuel abundant in blood (e.g., glucose and oxygen) for powering microscale implantable devices; iii) bioelectrocatalytic systems applicable in bioreactors for the selective conversion of substrates into useful products; iv) our engineers develop the biosensor devices up to R&D level good enough for licensing the small-scale production to a start-up companies.

The role of liquid biopsy in cancer management

Sonata Jarmalaite; Kristina Daniunaite; Kristina Stuopelyte; Albertas Ulys; Feliksas Jankevicius

National Cancer Institute, Vilnius, Lithuania; Institute of Biosciences, Vilnius University, Vilnius, Lithuania

Liquid biopsy (LB) is a modern alternative to tissue biopsy that allows the detection of cancer-specific changes in circulating tumor cells (CTC) or cell-free nucleic acids in bodily fluids. Currently LB-based analyses can be used for early cancer detection, assessment of response to treatment, and monitoring for disease progression. LB-based approaches are highly valuable in situations when repeated sampling of clinical material is needed, when the main tumor mass is eliminated or undetectable, and provide a more precise representation of genetic heterogeneity of tumor subclones. the application of modern sensitive techniques enables the detection of cancer-specific transcripts, mutations, epigenetic changes in CTC and circulating nucleic acids derived from primary tumor or cancer metastasis. In addition to blood, various other body fluids including urine, saliva, pleural fluid, breast milk, seminal fluid, etc. can contain tumor-specific genetic biomarkers. The genetic information obtained from LB together with novel imaging techniques provide a valuable tool for timely identification of drug resistance and disease progression.
During past ten years, our group has developed several informative and simple LB-based biomarker panels for noninvasive detection of prostate cancer (PCa), identification of aggressive tumors for radical treatment, and assessment of adverse responses to systemic treatment. A panel of DNA methylation markers (RASSF1, RARB, and GSTP1) applied from urine analysis showed sufficient diagnostic power and potential to predict biochemical PCa progression after radical prostatectomy. The test was validated in more than 300 urine samples from patients with localized PCa. In addition, the test was successfully applied for prediction of the disease up-staging when diagnostic biopsy was compared to surgical material. Similarly, the combined analysis of two urinary miRNAs, miR-148a and miR-375, showed high diagnostic potential in two cohorts of PCa cases. Besides, this LB-based test was able to improve the diagnostic power of the PSA test, including the diagnostic “gray zone”.

PCa progression to castration resistant disease (CRPC) requires systemic treatment with chemotherapy or next-generation hormone therapy. LB-based tests are beneficial for monitoring the response to treatment and early identification of the resistance. Resistance to antiandrogen therapy is tightly related to genetic changes affecting the androgen receptor (AR) signaling axis, including the production of AR splice variants (AR-Vs) that lack a ligand binding domain and are constitutively active. LB-based testing was developed for precise monitoring of abundance of AR-Vs in patients' blood. The abundance of AR-V1 and AR-V3 transcripts in the LB specimen was prognostic for disease progression or death. Similarly, the presence of DNA methylation biomarkers in urine collected from CRPC patients before abiraterone acetate therapy was prognostic for poor response to treatment and early disease progression.
In summary, LB-based cancer tests open a new way for non-invasive cancer diagnostics and follow-up of cancer patients during treatment. Cancer-specific biomarker panels analyzed in LB can provide timely information about disease progression or the development of treatment resistance. A wider use of such noninvasive and highly tumor-specific tests in clinical practice has a clear potential to reduce the need for radiologic and other means of monitoring for the disease progression, can enable personalized target-directed selection of treatment regimens and reduce the amount of side effects, thus markedly improving the management of cancer.

Discovery of novel bacterial genes encoding the enzymes acting on modified uracil/uridine derivatives and their use for gene therapy in cancer treatment

Jaunius Urbonavičius; Agota Aučynaitė; Daiva Tauraitė; Rasa Rutkienė; Arūnas Kazlauskas; Adas Darinskas; Rolandas Meškys

Vilnius Gediminas Technical University; Vilnius University; Lithuanian University of Health Sciences; National Cancer Institute

Modified nucleotides are present in several RNA species in all Domains of Life. While the biosynthetic pathways of these nucleotides were well studied in recent years, much less attention was drawn to the degradation of different RNAs and the return of modified nucleotides or their constitutents into the metabolism. Using an Escherichia coli uracil auxotroph strain, we screened the metagenomic libraries for genes, which would allow the conversion of either 2-thiouracil, isocytosine, or 2'-O-methyluridine into uracil and thereby lead to the growth on a defined synthetic medium. We have demonstrated that Domain of Unknown Function 523 (DUF523) containing protein is involved in the conversion of 2-thiouracil into uracil in vivo. We have also purified several recombinant isocytosine deaminases and a nucleotide hydrolase and demonstrated their enzymatic activities in vitro. These enzymes are also capable of converting the potential prodrugs 5-fluoroisocytosine, 5-fluorouridine, 5-fluoro-2'-O-methyluridine, and 5-fluoro-2'-deoxyuridine into a well-known anticancer drug 5-fluorouracil. The human glioblastoma U87MG and colorectal adenocarcinoma Caco-2 cell lines were transfected with the recoded isocytosine deaminase genes, and their citotoxicity together with 5-fluoroisoicytosine was demonstrated. The therapeutic potential of the isocytosine deaminase/5-fluoroisocytosine pair has been demonstrated in vivo, where the co-injection of the isocytosine deaminase-encoding mesenchymal stem cells and 5-fluoroisocytosine have been shown to increase longevity of tumorized mice by 50 %.
References: 1. Aučynaitė, A.; Rutkienė, R.; Gasparavičiūtė, R.; Meškys R.; Urbonavičius, J. (2018). A gene encoding a DUF523 domain protein is involved in the conversion of 2-thiouracil into uracil. Environ Microbiol Rep, 10:49-56.
2. Urbonavičius, J.; Tauraitė, D.; Aučynaitė, A.; Rutkienė, R.; Meškys, R. (2017). A pair of the isocytosine deaminases and the prodrug 5-fluoroisocytosine. Patent Application, LT2017 533.
3. Aučynaitė, A.; Rutkienė, R.; Tauraitė, D.; Meškys, R.; Urbonavičius, J. Novel bacterial deaminases convert the prodrug 5-fluoroisocytosine into the active drug 5-fluorouracil. Frontiers Microbiol. Submitted.

Plant response to seed treatment with cold plasma and electromagnetic field involves changes in seed ROS production, phytohormone amount and protein expression

Vida Mildaziene

Vytautas Magnus University

Fast growth of global population leads to necessity to intensify agricultural technologies while reducing their environmental impact. New emerging interdisciplinary field of research on cold plasma (CP) and electromagnetic field (EF) applications for agriculture is directed towards exploiting of the potential of plant functional plasticity. Seed stress induced by treatment with CP and EF leads to improved seed germination, seedling growth and other beneficial effects. Successful development of reliable agrobiotechnology based on these findings is ultimately dependent on knowledge of the molecular mechanisms involved in CP or EF induced signal initiation, response regulation and development. However, until now more detailed information on the molecular processes involved in plant response to seed treatments was lacking.
We report novel findings important for understanding of CP and EF effects in the context of seed physiology and molecular processes: (1) the extent of the observed effects on seed germination is largerly dependent on seed dormancy status which is characterized by balance between absisic acid, ABA and gibberrelins, GA. Our results showed that seed treatment with CP and EMF induce rapid decrease in ABA/GA ratio in Raphanus sativus seeds, indicating that CP and EMF may be considered as extremely powerful dormancy breaking agents; (2) pre-sowing seed treatments with physical stressors increase EPR signal and modulate H_2O_2 production in seeds of P. abies, so that CP treatments inhibiting germination induce decrease ROS generation and EF treatments stimulating germination result in increased ROS generation; (3) short time (2-15 min) seed treatments with CP and EF induce substantial changes in the amount of secondary metabolites and antioxidative activity in leaves growing seedlings of E. purpurea, T. pratense and F. esculentum. Changes in synthesis of secondary metabolites are important part of plant stress response, increasing seedling establishement and defense potential (they function as antioxidants, antimicrobial compounds as well as means for plant communication); (4) results of differential proteomic analysis performed on control, CP and EF treated H. annuus seeds (4 days after treatment) and leaves of growing seedlings (2 weeks after sowing) have revealed significant changes in expression of more than 30 proteoforms in seeds and more that 100 proteoforms (40% of identified proteoforms involved in photosynthesis) in leaves of seedlings, indicating that plant stress response induced by short pre-sowing seed treatment with CP and EF induced involves multiple changes in plant gene expression that may develop in time.

The study of post-mitotic midbody and factors controlling cell division

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Cytokinesis is a final stage of cell division, during which the mother cell divides, leaving two newly formed daughter cells connected by a thin intercellular bridge (ICB). Residing between the two daughter cells during cytokinesis is a microtubule and protein rich structure, known as midbody (MB). The MB is situated within ICB, with abscission usually occurring only on one side of the ICB. The MB has been well-studied for its role in recruiting abscission-regulating proteins during cytokinesis (Schiel et al., 2012), but its post-mitotic roles remain

poorly understood. Not so long ago, the MB was thought to be discarded after division by either releasing it into an extracellular space or by autophagosomal degradation. However, recent studies have shown that MBs can be retained and accumulate in stem and cancer cells after mitosis (Crowell et al., 2014). Thus, it has been proposed that post-mitotic MBs function as signalling platforms that regulate cell "stemness", as well as aggressiveness of cancer cells (Dionne LK and et al. 2017). For this reason, the function and fate of these MBs remains to be elusive and is the focus of this study.

For that purpose, intact MBs were purified from different cell types and proteomic analysis was completed. Proteomic analysis identified over 600 proteins, including many known MB resident proteins, as well as 18 post-Golgi Rabs. Rab GTPases have been proposed to mediate actin dynamics and endosome targeting at the abscission site. To begin with, dominant-negative mutants were overexpressed in cells to clarify Rabs' function during abscission. Next, shRNA (knock-down (KD)) and CRISPR/Cas9 (knock-out (KO)) were used to down regulate Rab14, a protein that was previously shown to play a key role in cell division. Finally, we tested the involvement of Rab14-interacting proteins in mediating abscission.

The study of the MB identified Rab14 as a novel regulator of cell division, since down regulation of Rab14 disrupted cytokinesis and had a negative effect on cell division. Also, Rab14-KD and -KO prolonged the time that cells require to divide, with the most noticeable difference seen in cells going from anaphase to the final step of cell division - abscission. Another Rab GTPase known as Rab11 was also shown to play a key role in cell division and was even capable of compensating the loss of Rab14 function.

Based on all our data, we propose that Rab14 is a novel regulator of cytokinesis that functions by regulating the targeting of endosomes to the ICB during late telophase, and consequently, affecting the abscission of daughter cells.

*References:*Schiel JA and et. al. FIP3-endosome-dependent formation of the secondary ingression mediates ESCRT-III recruitment during cytokinesis. Nat Cell Biol. 14 (10):1068-78. 2012.

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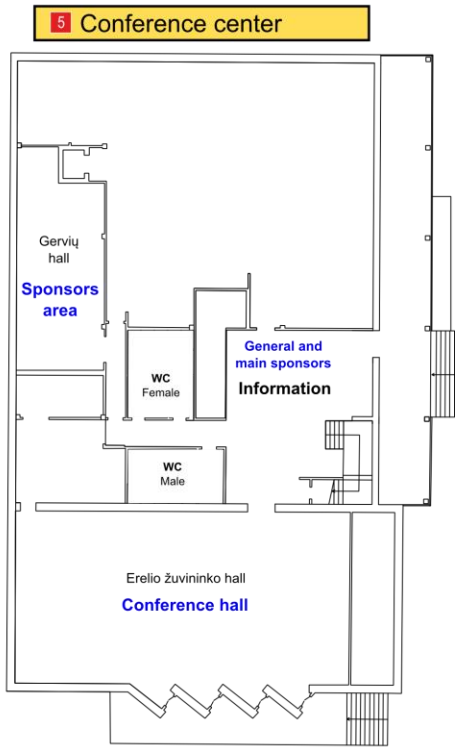
Dionne LK and et. al. FYCO1 regulates accumulation of post-mitotic midbodies by mediating LC3-dependent midbody degradation. J Cell Sci. 130(23):4051-4062. 2017.

Contents

Conference premises



Programme at a glance

Young Researcher Day		LBS Conference					
Time	**Tuesday, June 26**	Time	**Wednesday, June 27**	Time	**Thursday, June 28**	Time	**Friday, June 29**
		08:00	REGISTRATION	08:00	REGISTRATION	08:00	REGISTRATION
			Daumantas Matulis, session moderator		**Ago Rinken**, session moderator		**Saulius Serva**, session moderator
		09:00	OPENING	09:00	**Urtė Neniškytė** (9:00-9:45)	09:00	
		09:15	FEBS National Lecture **Martin Bachmann** (9:15-10:00)				**Reza Zolfaghari** (9:20-9:45)
				09:45	**Vilmantė Borutaitė** (9:45-10:15)	09:45	**Gintautas Tamulaitis** (9:45-10:15)
10:00	REGISTRATION	10:00	**Kaspars Tārs** (10:00-10:25)	10:15	**Andrius Kaselis** (10:15-10:35)	10:15	**Miglė Tomkuvienė** (10:15-10:45)
		10:25	**Jānis Kloviņš** (10:25-10:50)	10:35	**Ago Rinken** (10:35-11:05)	10:45	**Violeta Šaltenienė** (10:45-11:15)
	Vaida Paketurytė, Lina Aitmanaitė, session moderators	10:50	**Zane Kalnina** (10:50-11:15)				
11:00	**Vaida Paketurytė** (11:00-11:15)			11:05	**Martti Tolvanen** (11:05-11:35)		
11:15	**Aivaras Vaškevičius** (11:15-11:30)	11:15	**Vladimir Sirotkin** (11:15-11:40)			11:15	**Jurgita Skiecevičienė** (11:15-11:35)
11:30	**Žilvinas Dapkūnas** (11:30-11:45)			11:35	**Mart Loog** (11:35-12:00)	11:35	**Marius Dagys** (11:35-12:00)
11:45	**Raminta Mineikaitė** (11:45-12:00)	11:40	**Mikhail Kurbat** (11:40-12:00)				
12:00	LUNCH	12:00	LUNCH	12:00	LUNCH	12:00	LUNCH
Mihails Sisovs, Priit Eek, session moderators		**Jānis Kloviņš,** session moderator		**Peep Palumaa,** session moderator		**Jaunius Urbonavičius,** session moderator	
13:00	**Aistė Imbrasaitė** (13:00-13:15)	13:00	**Vyacheslav Yurchenko** (13:00-13:40)	13:00	**Alessandro Prinetti** (13:00-13:40)	13:00	**Sonata Jarmalaitė** (13:00-13:20)
	Martynas Simanavičius (13:15-13:30)					13:20	**Jaunius Urbonavičius** (13:20-13:40)
13:30	**Indrė Valiulytė** (13:30-13:45)	13:40	Vytautė Starkuvienė(13:40-14:10)	13:40	LBS Awards Ceremony (13:40-13:55)	13:40	**Vida Mildažienė** (13:40-14:00)
13:45	**Dukas Jurėnas** (13:45-14:00)			13:55	LBS General Assembly (13:55-15:25)		
14:00	**Priit Eek** (14:00-14:15)	14:10	**Artūras Jakubauskas** (14:10-14:40)			14:00	**Paulius Gibieža** (14:00-14:20)
14:15	**Greta Streleckienė** (14:15-14:30)					14:20	Presentation awards (14:20-14:30)
14:30	**Ugnė Gyvytė** (14:30-14:45)	14:40	**Saulius Serva** (14:40-15:10)			14:30	CONFERENCE CLOSURE (14:30-14:40)
14:45	**Rūta Urbanavičiūtė** (14:45-15:00)					14:40	
15:00	**Viktorija Kurbatska** (15:00-15:15)	15:10	COFFEE BREAK (sponsor area) (15:10-15:45)				
15:15	**Mihails Sisovs** (15:15-15:30)						
15:30	COFFEE BREAK (sponsor area) (15:30-16:00)			15:25	COFFEE BREAK (sponsor area) (15:25-15:45)		
16:00	FEBS Chair of the Working Group on Integration **Jerka Dumic** (16:00-16:30)	15:45	**Poster session I** (15:45-17:00)	15:45	**Poster session II** (15:45-17:00)		
16:30	FREE TIME						
			Kaspars Tārs, session moderator		**Aurelija Žvirblienė,** session moderator		
		17:00	**Urmas Arumäe** (17:00-17:35)	17:00	**Natascha Brinskelle-Schmal** (17:00-17:25)		
				17:25	**Juozas Šiurkus** (17:25-17:45)		
		17:35	**Vello Tougu** (17:35-18:00)	17:45	**Piotr Tarnowski** (17:45-18:05)		
		18:00	**Peep Palumaa** (18:00-18:25)	18:05	**Renata Gronczewska** (18:05-18:25)		
		18:25	**Anthony Watts** (18:25-18:55)	18:25	FREE TIME		
19:00	DINNER	19:00	DINNER	19:00	GALA DINNER		
20:00	OPENING CEREMONY (20:00-21:30)	20:00		20:00 – 00:00	CULTURAL PROGRAM DJ (20:00-00:00)		
21:30							

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