COINS

International conference of Life sciences

Abstract book 2017

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CONTENT

ABOUT CONFERENCE

The Coins'17 - 12th international conference of natural and life sciences which gathers not only students and scholars, but also all people that are working in science fields to discuss, learn and share their scientific experience, find new partners, meet key experts and enjoy exciting programme.

During the conference participants will get acquainted with scientific innovations, perspectives and most relevant topics in the fields of Biotechnology, Genetics, Biophysics, Biochemistry, Nanotechnology, Ecology etc. The Coins also gives an opportunity for BA and MA students and doctorates who are doing their scientific research to present it to a larger audience, get constructive criticism and useful advice.

The Coins'17 is an open scientific environment where everyone interested in natural and life sciences are gathered to build partnerships as well as share and develop new ideas.
Conference is based on curiosity, constructive criticism and a wish to improve.

You can find more information about the conference, lecturers, participants and the whole programme in this publication or online: www.thecoins.eu.

SPREAD THE NEWS AND SCIENCE!

FOREWORDS

Dear Colleagues,

It is my privilege and honour to welcome participants of the 12th International Student's Conference of Life Sciences (COINS) on behalf of Vilnius University and Life Sciences Center. This conference is special because it is entirely organized by students of the oldest university in Lithuania, but on the other hand it is organized in the newest facility of University – Life Sciences Center, which is working just more than a year.

I hope that the conference will benefit from the oldest/newest contrast and I wish you to enrich your scientific knowledge, increase networking and have a good time in the beautiful city named Vilnius!

Prof. Osvaldas Rukšėnas
Head of Institute of Biosciences in
Life Sciences Center
Vilnius University

Dear participants of The Coins 2017,

I feel honored to be the coordinator of The Coins 2017, because I honestly believe in its purpose. In my opinion, during the years of studying young scientists lack encouragement to pursue their path. This is where The Coins conference becomes so important – it informs students about new scientific innovations, gives an opportunity to join the international life-sciences community and familiarize with career perspectives.

That is, I hope, exactly what should help YOU, students, to keep motivated and be passionate about your studies!

Go deeper, seek wider and reach higher during The Coins conference!

Best wishes,
Rugilė Burbulytė
The Coins 2017 coordinator

AMBASSADORS

Prof. Dr. Osvaldas Rukšėnas
Lithuanian neurobiologist, biophysicist;
Head of Institute of Biosciences in Life Sciences Center;
Head of Departament of Neurobiology and Biophysics;
Professor of Biomedical Sciences;
President of Lithuanian Associaton of Neurosciences.

Prof. Dr. Sonata Jarmalaitė
Lithuanian geneticist;
Deputy Director for Research and Education of Lithuanian National Cancer Institute;
Leader of an Epigenetics group of a Division of Human Genome Research Centre, Institute of Biosciences, Vilnius University;
Professor at the Life Sciences Centre of Vilnius University;
Speaker of The Coins 2017.

Egidijus Kinderis
President of Vilnius University Students' Representation,
4th Year Student of the Life Sciences Centre.

PROGRAMME

FEBRUARY 28, LSC (SAULETEKIS AVE. 7)

08:00 - 09:00	Registration and coffee
09:00 - 09:30	Opening Ceremony
	*Session 1 – **Life Sciences Industry***
09:30 - 10:15	**Donata Mauricaitė,** "Enterprise Lithuania" "Lithuania – new destination where trendsetters meet" **Sonata Juciutė, MITA** "MITA – National and International Instruments for Life Sciences Industry"
10:15 - 11:45	**Plenary Discussion** **"From Idea to Product – Success Stories in Lithuania"** **Moderator: Mykolas Katkus** – Experienced stragetic consultant and consultancy business manager Participants: **Algimantas Markauskas** – "Thermo Fisher Scientific Baltics" General Manager and Vice-president for the Baltic region **Monika Kavaliauskė** – Head of Intellectual Property Management and Commercialization Department at Vilnius University **Tomas Andrejauskas** – Vice President of "Lithuanian Biotechnology Association" **Evaldas Pabrėža** – Co-Founder and CEO of "Integrated Optics"

11:45 - 12:45	Lunch break/coffee break
12:45 - 14:15	**Company fair**
	*Session 2 – **Nanotechnology***
14:15 - 15:00	**KEYNOTE: Danny Porath** *"Charge Transport in Single-DNA Based Molecules"* Israel
15:00 - 15:30	Coffee break
15:30 - 15:45	**Shalva Lekiashvili** *"Types Of Pathological Thyroid Gland ATPase and Their Kinetic Parameters"* Georgia
15:45 - 16:00	**Jekaterina Latynis** *"Hydration of Cytochrome c Studied by Vibrational Spectroscopy and Calorimetry"* Lithuania
16:00 - 16:45	**KEYNOTE: Meital Reches** *"Functional Peptide Assemblies on Surfaces: Towards Green Antifouling Materials"* Israel
20:00	Welcome reception

MARCH 1, LSC (SAULETEKIS AVE. 7)

08:00 - 09:00	Registration
	*Session 3 – **Life Sciences in Medicine***
09:00 - 09:45	**KEYNOTE: Aras Mattis** *"Patient iPSC-Derived Hepatocytes Recapitulate NAFLD in vitro"* USA
09:45 - 10:00	**Monika Glemžaitė** *"Osteogenic Differentiation of Human Amniotic Fluid-Derived Mesenchymal Stem Cells"* Lithuania
10:00 - 10:15	**Daina Pamedytytė** *"Expressions of miRNAs and BRAFV600E mutation detection in papillary thyroid carcinoma"* Lithuania
10:15 - 11:00	**KEYNOTE:** Sonata Jarmalaitė *"A molecular approach in cancer diagnostics and treatment"* Lithuania
11:00 - 11:30	Coffee break
11:30 - 12:15	**KEYNOTE: Skirmantas Kriaučionis** *"Roles of DNA modifications in Epigenetic Regulation of Transcription"* United Kingdom

12:15 - 12:30	**Raimonda Kubiliūtė** *"Chemoresistance development in breast MX-1 cancer cell line"* Lithuania
12:30 - 12:45	**Irmantas Mogila** *"Deletion Analysis of Multisubunit CRISPR-Cas Complex"* Lithuania
12:45 - 13:45	Lunch break
13:45 - 14:30	**KEYNOTE: Janis Liepins** *"Purine auxotrophic starvation in budding yeast"* Latvia
14:30 - 14:45	**Giorgi Tchitashvili** *"Polymorphism of CYP2C9 and VKORC1 Genes in Georgian Population"* Georgia
14:45 - 15:00	**Eglė Marija Ramanauskaitė** *"From Stardust to Blood Stalls: How Citizen Science is Accelerating Alzheimer's Treatment Research"* Lithuania

MARCH 2, LSC (SAULETEKIS AVE. 7)

08:00 - 09:00	Registration
	Session 3 – ***Synthetic biology***
09:00 - 09:45	**KEYNOTE: Justas Dapkūnas** *"Interatomic contact areas as means for the analysis and modeling of protein structures and interactions"* Lithuania
09:45 - 10:05	**iGEM Vilnius** Gabrielius Jakutis and Kotryna Čekuolytė *"Synthetic biology: from fundamentals to applications in daily life"* Lithuania
10:05 - 10:50	**KEYNOTE:** Verena Siewers *"Biosensors in metabolic engineering"* Sweden
10:50 - 11:05	**Giulio Preta,** Lithuania "Tethered bilayer lipid membranes as a complementary tool for functional studies on cholesterol-dependent cytolysins."

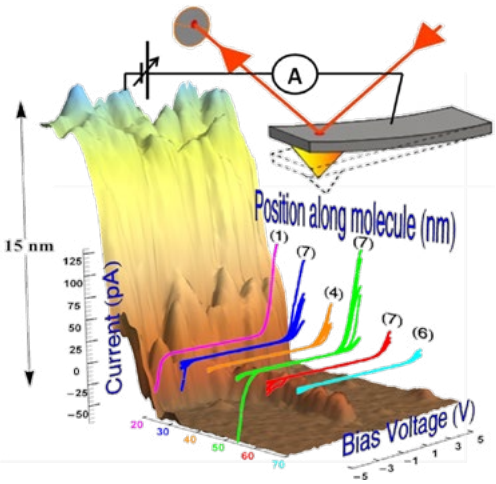
11:05 - 11:30	Coffee break
11:30 - 13:00	**Poster Presentation Session**
13:00 - 14:00	Lunch break
14:00 - 14:45	**KEYNOTE: Irina Borodina** *"How CRISPR-based genetic tools facilitate metabolic engineering of yeast"* Denmark
14:45 - 15:30	**KEYNOTE: Petri–Jaan Lahtvee** *"Quantitative Multi-Layer Stress Regulation Analysis in Yeast"* Estonia
15:30 - 16:00	Awards and Certificates
20:00	Closing Event

KEYNOTE SPEAKERS

Danny Porath (Israel)
Etta and Paul Schankerman Professor in Molecular Biomedicine, Hebrew University of Jerusalem

"Charge Transport in Single DNA-Based Molecules"

danny.porath@mail.huji.ac.il



DNA is primarily and with no doubt the most important biological molecule. But its double-strand recognition, as well as the ability to control its sequence and manipulate its structure open a multitude of ways to make it useful for molecular electronics. Step by step we improve the synthesized constructs and the measurement methods of single DNA-based molecules. In this lecture I will review the field and report on our progress in producing and measuring DNA-based building blocks towards the construction of DNA-based programmable circuits.

Meital Reches (Israel)

Faculty member and group leader in the Institute of Chemistry, Hebrew University of Jerusalem

"Functional Peptide Assemblies on Surfaces: Towards Green Antifouling Materials"

Several natural processes are mediated by the interactions between organic and inorganic materials. The immune response towards an implant inserted into the body is mediated by proteins. Composite materials are formed by the interactions of organic materials (usually proteins) and minerals. Biofouling, the process in which organisms attached to surfaces, is also mediated by organic molecules. Understanding the nature of interactions between organic and inorganic materials will bring to the development of improved implants, new composites and antifouling materials.

This lecture will present single-molecule force spectroscopy measurements of the interactions between individual biomolecules (either amino acid residues or short peptides) and inorganic surfaces in aqueous solution. Using this method, we were able to measure low adhesion forces and could clearly determine the strength of interactions between individual amino acid residues and inorganic substrates. Our results with peptides also shed light on the factors that control the interactions at the organic-inorganic interface.

Based on our knowledge from single molecule experiments, we designed a short peptide (tripeptide) that can spontaneously form a coating that resists biofilm formation. Our results clearly demonstrate the formation of a coating on various surfaces (glass, titanium, silicon oxide, metals and polymers). In addition, we showed that this coating prevents the first step of antifouling, which involves the adsorption of bioorganic molecules to the substrate.

Moreover, the coating significantly reduced the attachment of various organisms such as bacteria and fungi to surfaces.

Aras Mattis (USA)

Head of The Mattis Research Laboratory, Department of Pathology, University of California, San Francisco

"Patient iPSC-Derived Hepatocytes Recapitulate NAFLD in vitro"

With the rising obesity epidemic, non-alcoholic fatty liver disease (NAFLD) is increasing in prevalence affecting nearly 30% of the US population. Ten to twenty percent of NAFLD patients go on to develop non-alcoholic steatohepatitis (NASH), which progresses from hepatic inflammation to fibrosis/cirrhosis and puts patients at risk for hepatocarcinogenesis. In accord with a genetic predisposition for NAFLD/NASH, families exist in which the incidence of the disease is significantly increased. Here we generated induced pluripotent stem cell-derived hepatocytes (iPSC-Heps) from several members of such a family to investigate hepatocyte-specific disease mechanisms. Specifically, we tested the hypothesis that hepatocytes from NAFLD/NASH patients are susceptible to fatty acid-induced toxicity. For this, we obtained fibroblasts by skin punch biopsy and generated 3 iPSC lines per patient using non-integrating episomal vectors expressing the Yamanaka factors. We tested and found that these patients were negative for the patatin-like phospholipase domain containing 3 protein (PNPLA3) I148M variant that is known to predispose to NAFLD. After differentiating the iPSCs into iPSC-Heps, we challenged them with increasing concentrations of unsaturated and saturated fatty acids oleate and palmitate respectively. We found increased steatosis and cell death in palmitate-treated NAFLD/NASH iPSC-Heps as compared to controls matched in gender and

ethnicity. As a potential explanation for the observed phenotype, RNA-Seq of unchallenged iHeps revealed increased expression of sterol regulatory element-binding protein 1c (SREBP-1c) in NAFLD/NASH iPSC-Heps, a master regulator driving de novo lipogenesis. Further analysis revealed elevated levels of the activating sterol regulatory element-binding protein chaperone (SCAP) and decreased levels of the SREBP-1c repressor insulin induced gene 2 (INSIG2) in these cells. As both mouse models and human tissue samples have shown increased endoplasmic reticulum (ER) stress in NAFLD/NASH, we surveyed stress response pathways and found increased levels of phosphorylated c-Jun N-terminal kinase (JNK) and eukaryotic initiation factor 2α (eIF2α). Collectively, our findings suggest that hepatocytes within this NAFLD family are primed for de novo lipogenesis with increased baseline JNK and eIF2 α. Increased steatosis and hyperactive stress response drives increased cell death as a potential mechanism of NASH initiation and progression to inflammation and fibrosis. Moreover, our findings highlight the potential of our patient-specific disease model for elucidating the complex molecular mechanisms that induce and promote NAFLD/NASH.

Sonata Jarmalaitė (Lithuania)
Deputy Director for Research and Education, National Cancer Institute, Vilnius

"A Molecular Approach in Cancer Diagnostics and Treatment"

Recent advances in genomic, epigenomic and proteomic technologies have markedly improved the understanding of the molecular biology of cancer and inspired the development of novel treatment strategies and tools for cancer diagnostics. In recent years, modern approaches have been implemented into cancer diagnostics, including advanced optical methods and genetic or proteomic profiling. Liquid biopsy-based detection of circulating tumor cells or cancer nucleic acids opens a new way for non-invasive diagnostics and follow-up of outcomes after surgery or during postsurgical treatment. Based on the genome- and epigenome-wide analysis of prostate carcinomas, our group has developed several informative and simple urine-based tests for the early detection of prostate cancer and the follow-up of patients during the postsurgical period. Moreover, our biomarker systems enable the prediction of response to treatment and suggest modern means for the selection of the most appropriate treatment in an early-stage or metastatic disease.

Skirmantas Kriaučionis (UK)

Associate Professor in University of Oxford, Ludwig Institute for Cancer Research, Oxford

"Roles of DNA Modifications in Epigenetic Regulation of Transcription"

In complex eukaryotic genomes epigenetic mechanisms provide an extra layer capable of regulating transcription. DNA modifications are one of the key components of epigenetic machinery in vertebrates. Two most abundant DNA modification are 5-methylcytosine (5mC) and 5-hydroxymethylcytosine (5hmC). In humans, defects in enzymes responsible of generating these modifications have been linked to cancer and ICF syndrome. The current view places 5mC as a silencing mark and 5hmC as an intermediate in the removal of 5mC in cycling cells. However, in some post-mitotic cell types, such as terminally differentiated neurons, 5hmC accumulates, suggesting roles outside de-methylation intermediate. Our data demonstrates that 5hmC is capable of affecting transcription of nearly 200 genes in two cell lines by counteracting repressive activity of 5mC.

Janis Liepins (Latvia)
Researcher in the institute of Microbiology and Biotechnology, University of Latvia, Riga

"Purine Auxotrophic Starvation in Budding Yeast"

While virtually all nucleated mammalian cells are able to synthesize purine nucleotides de novo, most of protist and helminth parasites strictly depend on purine scavenging from their hosts. Thes metabolic pecularity, in turn, is an attractive targets for antiparasitic drugs.

Recently auxotrophic yeast strains have been used to model purine auxotrophic, parasitic protists. Adenine auxotrophic *S. cerevisiae* has been used also to screen for protist *Plasmodium falciparum* (malaria) drugs. To effectively screen library of thousands candidates, robust high throughput (HT) yeast based assays are very helpful.

To further exploit *S. cerevisiae* as tool to model parasitic eukaryotes and instrument for drug screening, details on mechanisms governing its phenotype formation during auxotrophic starvation should be clarified.

Yeast strain auxotrophy and lack of particular metabolite in the cultivation broth is example of synthetic starvation. Uracil and leucine starvation leads to lowered stress resistance and shortened half life. Adenine depletion, on the other hand, evokes stress (heat, weak acid and oxidative) resistant phenotype, improves desiccation tolerance and does not significantly affect cell half life. Adenine starved cell's effectively arrest cell cycle in G1 and are insensitive to rapamycin treatment. Interestingly, purine starvation specific,

uniform, phenotype has been observed for two strains (CEN.PK2 and W303), but not for BY4741 the latest is *S. cerevisiae* genetics standard parent strain (EUROSCARF deletion project).

Yeast phenotypes evoked by purine depletion is not only side effects of cultivation in poorly defined medium, but can also serve as a model for several biological phenomena where purine metabolism is distorted.

The results of this research will contribute to understanding of fundamental mechanisms how cell reacts to sudden synthetic starvation and if there are key elements that could be drugable to control eukaryotic parasites with similar metabolic features (auxotrophies).

Justas Dapkūnas (Lithuania)
Researcher in the Department of Bioinformatics, Institute of Biotechnology, Vilnius University

"Interatomic Contact Areas as Means for the Analysis and Modeling of Protein Structures and Interactions"

Structural data on proteins and their interactions is indispensable for understanding the molecular details of biological processes. This data may also guide the design of proteins having desired new features or forming novel interactions. Unfortunately, experimental structures are available only for a small part of known proteins and for an even smaller part of known protein interactions. Nowadays protein structures can be often predicted using computational modeling. However, the analysis of the structures, either experimental or predicted, might be also challenging. As a result, various computational methods are widely used in structural biology.

Here we present a novel approach to protein structure analysis that is based on interatomic contacts. The contacts between atoms are defined using the Voronoi tessellation of macromolecular structures. This method allows straightforward estimation of the contact areas between atoms that reflect the physical interactions in the protein molecule.

There are multiple applications of contact area-based methods for protein structure analysis that clearly demonstrate the usefulness of this concept. For example, contact area differences may be used to compare protein structures and their interaction interfaces using our novel method CAD-score. Clustering of interaction interfaces according to CAD-score was implemented in the PPI3D web server for searching and analyzing of structural data on protein interactions, where it reduces the redundancy of available experimental data and thus simplifies the analysis. This clustering was one of the main reasons why PPI3D was highly effective in finding templates for comparative modeling of protein complexes in recent CAPRI experiment.

Verena Siewers (Sweden)

Docent at Chalmers University of Technology, Gothenburg

"Biosensors in Metabolic Engineering"

Biotechnology has enabled us to design and construct microorganisms that can synthesize almost any natural product of interest. Such microorganisms will play a crucial role when moving towards a more sustainable generation of fuels and chemicals. In our group, we engineer the yeast *Saccharomyces cerevisiae* for the production of fatty acid-derived compounds including fuels such as fatty acid ethyl esters and alkanes, but also higher value fatty alcohols or esters with applications as food ingredients or pharmaceuticals. A second class of molecules produced in our modified yeast strains are isoprenoids, which comprise both low-value fuels and high-value fragrances. However, their initial production levels are often very low. A major challenge is therefore to turn poor producers into efficient ones.

To address this, biosensors that sense the target compound or one of its precursors are a great asset. On the one hand, they make it possible to easily detect and select individual cells with superior production. On the other hand, they allow for the regulation of enzyme production dependent on the metabolic state of the cell. Examples of biosensor applications in metabolic engineering of yeast will be presented.

Irina Borodina (Denmark)
Senior Scientist and Group Leader at the Novo Nordisk Foundation Center for Biosustainability, Technical University of Denmark, Kongens Lyngby

"How CRISPR-Based Genetic Tools Facilitate Metabolic Engineering of Yeast"

The majority of organic chemicals are derived from fossil sources. With the oil and gas resources becoming limiting, biotechnology offers a sustainable alternative for production of chemicals from renewable feedstocks. Yeast is an attractive cell factory for sustainable production of chemicals, due to its safe use status, tolerance of low pH and inhibitors, and amenability to large-scale fermentations. The recent developments in CRISPR-based technology made genetic engineering of yeast cells cheaper and faster.

I will present how CRISPR-based genetic tools facilitate metabolic engineering of yeast *Saccharomyces ceresivisiae*. Using these tools, we engineered yeast cell factories for production of chemicals from renewable feedstocks. Examples include non-native 3-hydroxypropionic acid, a potential platform chemical for acrylics (diapers, acrylic paints, acrylic polymers, etc.) and aromatic secondary metabolites with applications as nutraceuticals and cosmetic ingredients (resveratrol, p-coumaric acid, rosmarinic acid, flavonoids).

Petri-Jaan Lahtvee (Estonia)

Senior scientist and group leader at The Centre for Synthetic Biology, Institute of Technology, University of Tartu

"Quantitative Multi-Layer Stress Regulation Analysis in Yeast"

By with the global market reaching yearly into billions of dollars. However, to make bioprocesses more cost efficient and more generally applied, well performing cell factory with increased production of precursor molecules and robustness towards stress factors must be addressed. In the current study, gradual increase of three different stress conditions (ethanol, osmolarity and temperature) were studied in the yeast *Saccharomyces cerevisiae* at constant dilution rate to understand the regulation of induced stress conditions without the effect caused by decreasing specific growth rate. More than thirty chemostats were performed at constant dilution rate but due to varying stress conditions and levels, significant variations in specific glucose uptake rate were detected. The major common specific glucose uptake rate dependent change took place in mitochondria where mainly oxidative phosphorylation related genes and proteins showed strong specific glucose consumption rate dependence. Latter was also the biggest group of genes that showed significant transcriptional regulation. Rather surprisingly, only relatively small overlap was determined at mRNA and protein levels between different stress conditions. As a common effect, elevated stress caused a decrease in biomass yield which was directly related to increased maintenance energy demand of the cells. However, effects for increased maintenance seemed to vary from one stress condition to another. General underlying energy dependent curve was discovered which also allows one to predict the on-set of overflow metabolism. Various integrative data analysis, including transcription factor analysis, indicated more similar response towards temperature and ethanol

stress conditions, where protein turnover and membrane fluidity were mainly influenced. Osmotic stress showed more distinct patterns as oxidative stress pathways were activated. Gathered quantitative multi-layer datasets together with integrated data analysis sheds light on the regulatory patterns and energy metabolism for each stress conditions separately and defines a general stress response in yeast. Additionally, it gave suggestions for metabolic engineering purposes to produce more robust cell factories.

ORAL PRESENTATIONS

Shalva Lekiashvili

"Types Of Pathological Thyroid Gland ATPase and Their Kinetic Parameters"

SHALVA LEKIASHVILI, Gvantsa Brachveli, Nana Koshoridze

Introduction: Plasma membrane of human thyroid gland, apart from the transport Mg-independent HCO_3^- ATPase, has been found to contain also HCO_3^--ATPase of ecto-ATPase type, whose activity is not conditioned by the Mg-ion and with substrate in the form of free ATP. Activity of such kind is found in both healthy cells and those affected by carcinoma. However, in the latter cells, this characteristic of the enzyme is much higher than the norm.

Aim: We studied certain kinetic properties of the Mg-independent HCO_3^--ATPase, namely relation of its activity to the quantitative content of the HCO_3^--ions and the substrate (free ATP), as well as the pH of the reaction medium.

Materials and methods: The object of our research was the gland tissue extracted by surgery from patients with various thyroid gland pathologies. For determination of HCO_3^--ATPase activity was evaluated using the difference between active and passive ATPase. Protein concentration was measured with a Protein Assay Kit (Sigma, USA), according to the manufacturer's protocol.

Results: The experiments showed that plasma membrane of human thyroid gland shows existence of two types of ATPase, whose activities differ according to the functional state of the

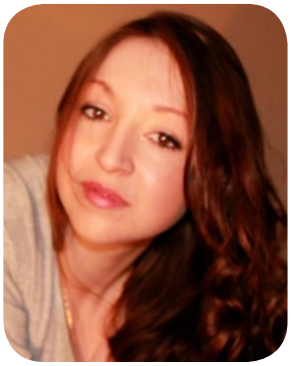
gland. In particular, healthy glands are characterized by high HCO_3-ATPase activity, HCO_3-ATPase being an Mg-dependend enzyme and classified as P-type transport ATPase due to its properties. Alongside it functions the non-Mg-dependent ecto-HCO_3-ATPase, whose activity in the norm is lower if compared to that of the Mg-dependent HCO_3-ATPase. However, as pathological processes develop, its activity significantly rises.

Conclusion: Thus, it can be assumed that in a certain form, it must be involved in formation and development of the pathology. This subject is still under study.

Jekaterina Latynis

"Hydration of Cytochrome c Studied by Vibrational Spectroscopy and Calorimetry"

latynis@gmail.com



JEKATERINA LATYNIS[1], Vitaly Kocherbitov[2], Justas Barauskas[2], Gediminas Niaura[1]

1. Life Science Center, Vilnius University , Vilnius, Lithuania

2. Biomedical Science, Faculty of Health and Society, Malmö University, Malmö, Sweden

Biological function of proteins displays in appropriate conformational structure in presence of particular water quantity. Thus, it is important to know the microscopic dynamics of a protein, i.e., motions of residues and secondary structure, and vision of their bonds' energetics. Recently, we have employed Raman spectroscopy to probe the hydration induced structural changes at various sites of lysozyme (lyz) under isothermal conditions in the range of water contents from 0 to 44 wt %. This study was aimed to investigate structural and thermodynamic

behavior of cytochrome c (cyt c) during the hydration by means of Fourier Transform Infrared Spectroscopy Differential Scanning Calorimetry, Sorption calorimetry studies and compare it to lyz hydration studies.

There was the first reversible structural transition (β-sheets/ unordered structures) in samples containing from 3 to 14 wt % of water with a peak of β-sheets loss in a sample containing 7 wt %, where an enthalpy of cyt c denaturation had a minimum value. We observed an increase of α-helix in a sample containing 14 wt % and above content of water, leading a glass transition onset. An inflexion point of the second structural transition was in a sample containing 30 wt % which coincided with maximum value of cyt c denaturation enthalpy and occurrence of "free water".

Phase transitions for both cyt c and lyz occurred in samples containing relatively close water quantity: the onset of a glass transition was at 13(lyz) /15(cyt c) wt % of water in samples; the end of a glass transition and the start of an elastic non-liquid protein phase was in a range of 20 (lyz) / 22 (cyt c) wt % of water in samples. Finally, a "free water" in protein molecule occurred in the range of 30 (cyt c) / 35 (lyz) wt % of water contents in samples.

Monika Glemžaitė

"Osteogenic Differentiation of Human Amniotic Fluid-Derived Mesenchymal Stem Cells"

MONIKA GLEMŽAITĖ, Rūta Navakauskienė
Department of Molecular Cell Biology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Vilnius, Lithuania

Human amniotic fluid-derived mesenchymal stem cells (AF-MSCs) are a new stem cell source for cell therapy and regenerative medicine. They are foetal mesenchymal stem cells having multilineage differentiation potential and found in amniotic fluid together with other cells. The aim of our research – to investigate genetic and epigenetic changes during osteogenic differentiation of AF-MSCs.

AF-MSCs were obtained from amniocentesis samples from second trimester pregnancy of healthy women who needed prenatal diagnostics but no foetus' genetic abnormalities were detected (protocols approved by the Ethics Committee of Biomedical Research of Vilnius District, No 158200-123-428-122). Isolated AF-MSCs were positive for mesenchymal cell surface markers CD90 and CD105 and negative for CD34 and CD45 and expressed pluripotency genes-markers (*Oct4*, *Sox2*, *Nanog*, *Rex1*). Osteogenic differentiation of AF-MSCs was induced using StemPro Osteogenesis Differentiation Kit and confirmed by staining cells with Alizarin Red and determining increased expression of osteogenic genes, such as *alkaline phosphatase* and *osteopontin*, by RTqPCR. Relative gene expression of pluripotency markers *Sox2* and *Rex1* decreased after differentiation. In addition, expression of specific microRNAs, related to pluripotency maintenance or

osteogenesis, was altered as detected by RT-qPCR. For global epigenetic changes evaluation Western blot analysis was used. The results revealed that levels of Polycomb repressive complex 2 (PRC2) proteins (EZH2, SUZ12) that are involved in silencing lineage-specific genes and maintaining bivalent state in stem cells were reduced after osteogenic differentiation. As a consequence, levels of specific histone markers keeping active state of chromatin (H3K4me3, H3K9Ac and others) increased and markers of repressed state of chromatin (H3K27me3) decreased. Other chromatin silencing enzymes such DNMT1, HDAC1 and HDAC2 were also downregulated during osteogenic differentiation.

In conclusion, our results show that AF-MSCs are able to differentiate into osteogenic progenitors and the differentiation is driven by epigenetic changes mediated by chromatin modifying enzymes, histone modifications and specific microRNAs expression.

Daina Pamedytytė

"Expressions of miRNAs and BRAF^{V600E} Mutation Detection in Papillary Thyroid Carcinoma"

DAINA PAMEDYTYTĖ[1], Vaida Simanavičienė[1], Enrika Leiputė[1], Dalia Daukšienė[2], Valdas Šarauskas[2], Aurelija Žvirblienė[1], Birutė Žilaitienė[2]

1. Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania
2. Lithuanian University of Health Sciences, Kaunas, Lithuania

Papillary thyroid cancer (PTC) is the most common type of thyroid cancer with the highest cure rate. However, tumor recurrence occurs in up to 20% of patients. Currently, recurrence risk stratification is accomplished by using clinicopathologic factors, but they have limited prognostic value in PTC. The development of molecular

determinants of disease recurrence such as protooncogene *BRAF* and miRNAs has the potential to improve the clinical management of patients with PTC by assisting in risk stratification.

The aim of this study was to identify miRNA biomarkers of recurrence in PTC. We selected 3 miRNA (miRNA- 146b, -222 and -21) and measured the expresion levels of these miRNAs in patients with recurrent PTC (Rc-PTC) and without recurrence (NR-PTC). Total RNA was extracted from formalin-fixed, paraffin-embedded (FFPE) samples and the expression levels of individual miRNAs were measured by qRT-PCR. The relative expression was obtained using the ΔΔCt method. RNU48 was used as an endogenous control. One-hundred and six NR-PTC and 60 Rc-PTC samples were analysed. The expression levels of all three miRNAs were increased in PTC when compared to healthy thyroid tissue. miRNA-146b expresion was extremely elevated with 55,6-fold over-expression in PTC. miRNA-222 and -21 were over-expressed 13,8-fold and 3,7-fold, respectively. In Rc-PTC and NR-PTC groups miRNA-146b, -222 and -21 expresion were 1,8- fold, 2,1-fold and 1,4- fold higher in NR-PTC than in Rc-PTC ($p=0,007$, $p< 0,001$ and $p=0,006$, respectively). $BRAF^{V600E}$ mutations were identified by direct DNA sequencing. Genomic DNA was extracted from FFPE samples. Thirty-one DNA sample from Rc-PTC and 83 samples from NR-PTC groups were analyzed for $BRAF^{V600E}$ mutation and it was present in 54,8% of Rc-PTC samples and 59,0% of NR-PTC samples.

In conclusion, these results suggests that all three miRNAs might be a potential biomarkers of recurrence in PTC, while $BRAF^{V600E}$ mutation doesn't seem to be important for relapse.

Raimonda Kubiliūtė

"Chemoresistance Development in Breast MX-1 Cancer Cell Line"

RAIMONDA KUBILIŪTĖ[1,3], Kristina Daniūnaitė[1,3], Rimantas Daugelavičius[2], Sonata Jarmalaitė[1,3]

1. Division of Human Genome Research Centre, Life Sciences Center, Vilnius University, Vilnius, Lithuania
2. Department of Biochemistry, Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania
3. National Cancer Institute, Vilnius, Lithuania

Multidrug resistance of cancer cells is the main cause of the ineffective chemotherapy in most cancer types. An increased active efflux of drugs, mediated by ATP binding casette (ABC transporters) superfamily proteins is the most significant mechanism, by which cancer cells acquire resistance to various chemoterapeutics. Epithelial-to-mesenchymal transition (EMT), when epithelial cells acquire mesenchymal phenotype, metastatic and cancer stem cells features, is related to chemoresistance as well. The aim of this study was to analyze gene expression profile of derived chemoresistant breast MX-1 cancer cell lines.

The study was performed on doxorubicin (DOX) resistant MX-1/D, MX-1/T and MX-1/TD sublines, treated with DOX, tetraphenylphosphonium (TPP), as a model substrate of ABC transporters, or both respectively. Global gene expression profiling was performed using gene expression microarrays. The expression levels of individual genes and the copy number variation of *ABCB1* were assessed by means of quantitative PCR, while the DNA methylation pattern of *ABCB1* was determined by pyrosequencing. DOX resistant cell models, MX-1/D, MX-1/T and MX-1/TD, were established as a valuable tool for chemoresistance studies. Overexpression of *ABCB1* due to gene amplification (> 40 copies) and demethylation of promoter was observed in MX-1/T and MX-1/

TD sublines, however no such alterations in MX-1/D subline were determined. All chemoresistant sublines showed morphological changes consistent with EMT, and the analysis of global gene expression confirmed this process, even in drug unexposed MX-1/T cells. Besides, significant upregulation of key EMT marks *CDH1*, *SNAI1*, *ZEB1*, cell stemness gene *SOX2*, and drug transporter *ABCC1*, were observed in MX-1/D subline. Increased expression of *CDH2* and *ZEB1* was identified after additional exposure of MX-1/T cells to DOX.

In summary, considerable upregulation of *ABCB1* strongly contributes to the development of chemoresistance not only by drug efflux, but eventually by inducing EMT process, whose importance in cancer cells resistance was revealed as well.

Irmantas Mogila

"Deletion Analysis of Multisubunit CRISPR-Cas Complex"

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Introduction: CRISPR-Cas systems provide prokaryotes with adaptive immunity against foreign plasmids and viruses. Immunity is ensured by ribonucleoprotein effector complexes which, guided by crRNA molecules, seek and destroy invading nucleic acids. The best studied effector complexes of Type I and Type II CRISPR-Cas systems target invading DNA. Type II effector complex comprising of a single Cas9 protein is widely established as a genome editing tool. In contrast, Type III effector Csm and Cmr complexes function as RNA-guided DNA nucleases: upon recognition of RNA transcript they start to degrade both RNA and the DNA that is being

transcribed. It is known that Csm3 is the ribonuclease subunit of Csm complex and Cas10 is the deoxyribonuclease. However, the roles of remaining Csm complex subunits are not fully understood.

Aim: We aimed to determine the functions of *Streptococcus thermophilus* CRISPR-Cas subtype III-A effector Csm complex (StCsm) subunits.

Materials and methods: Single-gene deletion variants of StCsm were obtained by standard genetic engineering methods and StCsm complexes lacking individual subunits were expressed in *Escherichia coli* and purified by affinity and size exclusion chromatography steps. The protein and crRNA composition of purified complexes was analyzed by denaturing electrophoresis. RNA binding of deletion variants was evaluated by EMSA and RNase activity was examined by radiolabeled RNA cleavage assays. DNA degradation rate was determined by performing ssDNA cleavage reactions in presence of activating RNA.

Results and conclusions: We show that Csm4 subunit, which forms a subcomplex with Cas10, specifically binds and anchors 5'-end of crRNA and the Csm3 subunit is critical for further formation of StCsm. Our data suggest that proteins Cas10, Csm4 and Csm5 are important for the maturation of crRNA. We also show how effector complex subunits promote the activity of StCsm. These results may pave the way for development of novel molecular tool for programmable RNA silencing.

Giorgi Tchitashvili

"Polymorphism of CYP2C9 and VKORC1 Genes in Georgian Population"

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Introduction: From literature it is known there is a correlation between CYP2C9 and VKORC1 Gene products and warfarin dosage in the treatment of thrombosis. Warfarin is an anticoagulant, causing the inactivation of the VKORC1 gene product, which is one of the clotting factors. The protein product of CYP2C9 gene is involved in the metabolism of warfarin.

Aim: The aim of our research was to study the frequency of different alleles of VKROC1 and CYP2C9 genes for healthy donors and patients with thrombosis, in Georgian population.

Materials and methods: Genotyping of peripheral blood samples for studied genes alleles was carried out using a tube scanner (ESE Quant Tube Scanner - is a small easy-to-use fluorescence measurement system), which gives possibility to identify SNPs.

Results: In the studied group of patients with thrombosis the wild-type homozygous genotype - by the VKORC1 gene was – 60 %; heterozygous – 34 %; mutant homozygous – 6 %. In the healthy donor's group this pattern was a little different: predominated heterozygous genotype (45 %); homozygous wild type was - 40%; mutant homozygous -15 %.

By CYP2C9 gene, in patients with thrombosis, the homozygous wild and heterozygous genotypes were 41% and 44%, respectively;

mutant homozygous were revealed the ratio 11%. On the other hand, in healthy donors, the frequency of wild-type homozygous was 67%, heterozygous and mutant homozygous were 32 % and 1 % - respectively.

Conclusion: VKORC1 and /or CYP2C9 genes polymorphisms are presented in numerous clinical dosing algorithms and clinical trials. It is revealed the significant variation of genotypes in patients with thrombosis, which indicates the importance of genotype testing in treatment process, as well as for the prevention of thrombosis.

Eglė Marija Ramanauskaitė

"From Stardust to Blood Stalls: How Citizen Science is Accelerating Alzheimer's Treatment Research"

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EGLĖ MARIJA RAMANAUSKAITĖ
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Introduction: Citizen Science (CS) – public participation in scientific research, is rapidly gaining weight across disciplines, from ecology to astrophysics and biomedical science. At the Human Computation Institute we have recently adapted an existing CS platform – stardust@home, originally built to look for interstellar dust particles in stacks of aerogel images, to create the very first CS game that accelerates Alzheimer's research.

Aim: The project – EyesOnALZ – was built to speed up promising research at Cornell University, involving the role of reduced blood flow in Alzheimer's disease (AD). Due to a time consuming manual data curation step, each typical study would require at least six months of analysis by several trained scientists, meaning the data could not be analyzed at rates to match the scientific goals.

Method: In a trans-disciplinary effort, we have adapted stardust@ home for a similar task, but a different purpose – annotating microvessels in the brains of mice. The game, "Stall Catchers", allows anyone to look at movies of live mice brain, searching for "stalls" – clogged capillaries. The participants gain points, level up, and help to analyze data several times faster than it would be done in the lab.

Results: In 4 months Stall Catchers reached >3000 participants, with >80 000 real capillary movies were analyzed, reaching ~23 annotations per movie. Our recently conducted validation study confirms that the accuracy of the crowdsourced analysis meets, and perhaps even exceeds, the performance of our in-house lab analysis, with the potential to complete analysis orders of magnitude more quickly.

Conclusion: In such a way we have unconventionally, but very effectively, removed the major bottleneck of AD research at Cornell University, enabling further studies that could help understand the role of reduced flow in the brain of Alzheimer's patients, and lead to the first ever Alzheimer's treatment in a reasonable time frame.

Giulio Preta

"Tethered Bilayer Lipid Membranes as a Complementary Tool for Functional Studies on Cholesterol-dependent Cytolysins"

GIULIO PRETA, Marija Jankunec, Frank Heinrich, Sholeem Griffin, Iain Martin Sheldon, Gintaras Valinčius

Introduction: Cholesterol-dependent cytolysins (CDCs) are a family of ß-barrel pore-forming toxins that are produced by Gram-

positive bacteria. Pore formation is dependent on the presence of membrane cholesterol, which functions as the main receptor for CDCs. Usually, CDCs activity is determined by *in vitro* assays using red blood cells or immortalized cell lines, however recently an alternative bioanalytical technique based on the use of tethered bilayer lipid membranes (tBLMs) was developed.

Aim: The aim of the study was to identify new complementary strategies for characterization of the properties and activity of CDCs, in particular of pyolysin (PLO), a peculiar CDCs since PLO is spontaneously active *in vitro*. The oligomerization process, the membrane insertion mechanism and the dependency from cholesterol were investigated using tBLMs.

Materials and methods: To detect the activity of PLO and other CDCs, biological tests as haemolytic assays and cell proliferation assays were used. Experiments using tBLMs were performed by electrochemical impedance spectroscopy (EIS), atomic force microscopy (AFM) and neutron reflectometry (NR).

Results: Using a combined biological and biophysical approach, we investigated the reconstitution and the activity of PLO. Our experiments with tBLMs clearly showed that cholesterol is acting as a potential binding for PLO, confirming the requirement of cholesterol for the activity of this toxin. Moreover, PLO-induced EIS changes were found to be time and concentration-dependent with significant changes detectable already at 1 nM PLO.

Conclusions: As research group our goal is to demonstrate the effectiveness of tBLMs for *in vitro* studies on CDCs, as well as, for a fast, automated, real time detection of CDCs in cultivation media and biological samples. Further improvements could still be reached related to the sensitivity of tBLMs with more detailed studies on the characteristics and properties of these artificial membranes.

POSTER PRESENTATIONS

Molecular Biology - Biotechnology

1. Deimantė Noreikaitė

"Biofilm Formation of Human Pathogens on 3D Chitin Films from Cockroach (*Blaberus giganteus*)"

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One of the main problem in food industry is products contamination by bacteria, which can cause serious health problems in humans. In addition, some strains can attach and form biofilms on medical devices, which can lead to different types of infections in medical environment. Chitin is one of the most versatile polysaccharide in the world due to its large set of applications in various fields and properties such as non – toxicity, biodegradability and biocompatibility. The aim of this study was to extract a new form of three-dimensional chitin film directly from an insect (*B. giganteus*) body parts (dorsal pronotum and wing) to inhibit biofilm formation of common human diseases causing bacteria. Microbial biofilm formation on chitin films was tested using eight different bacteria strains and control samples (nitrocellulose membranes). In this study, biofilm activity test measurements showed that chitin films completely inhibited biofilm formation by two contagious bacteria - *A. baumannii* and *S. sonnei*. Extracted chitin films can be suggested as coating materials for medical devices and surfaces

in hospitals. Also, obtained chitin films can be used to prevent infectious diseases by coating marine and other food products or covering all usable food storage places with chitin film layer either as an additive in packaging of products with high contamination risk.

2. Darya Volkava

"Optimization of Codon Composition of *acdS*-Gene of Bacteria *Pseudomonas putida* B-37 According to the Codon Usage Frequency in *Nicotiana tabacum* Plants"

D.S. Volkava, S.I. Leanovich, A.A. Melnikava
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Motyvation and aim: 1-aminocyclopropane-1-carboxylate deaminase (ACC-deaminase) is reported to be the key enzyme on the path to decrease the level of stress ethylene which suppresses plants growth, reproduction, and ability to survive under stress conditions. One of the most perspective ways to overcome negative influences of stress ethylene is to create the transgenic plants with bacterial *acdS*-gene coding ACC-deaminase enzyme. High frequency of rare codons for plants usage can be the limit factor for the following translation process in plant cells. Optimization of codon composition of bacterial *acdS*-gene according to the codon usage frequency in plants promotes the expression of the target gene and the accumulation of its product in transgenic plants.

Methods and algorithms: The nucleotide sequence analysis of native *acdS*-gene *Pseudomonas putida* B-37 was conducted by using Graphical Codon Usage Analyser and compared with the codon composition usage in plants genome *Nicotiana tabacum* which

can be found at the Codone Usage Database. The correspondent frequency was observed and adapted.

Results and conclusion: As a result, it was observed that there is eight amino acids from the analyzing sequence, cellular concentration of isoacceptor tRNAs of which in *N. tabacum* stands for 20% or less. By using the property of the degeneracy of the genetic code the modification of the nucleotide sequence of the native *acdS*-gene was made and these codons were changed for synonymous ones with higher rate of usage in *N. tabacum*. Such modification lets optimize the nucleotide sequence of the target gene for the expression in *N. tabacum* plants without any changes in the amino acids composition. So, this manipulation with bacterial *acdS*-gene would provide increase of gene expression in *N. tabacum* and stress stability in transgenic plants.

3. Alesia Melnikava

"Transient Expression of Bacterial *acdS*-gene in *Nicotiana benthamiana* Cells"

A.A. MELNIKAVA, D.S. Volkava
Belarusian State University, Minsk, Republic of Belarus

Introduction: Ethylene is an essential plant hormone also known as a stress hormone because its synthesis is accelerated by induction of a variety of biotic and abiotic stress. The plant growth promoting bacteria containing the enzyme 1-aminocyclopropane-1-carboxylate deaminase (ACC-deaminase) enhances plant growth by decreasing ethylene level under stress conditions. The expression of ACC-deaminase (*acdS*) gene in transgenic plants is an alternative approach to overcome the ethylene-induced stress. Agrobacterium-mediated DNA transfer is currently the most facile and versatile method to deliver gene

constructs into the nucleus for gene function analysis in diverse plant species. Transient gene expression via Agrobacterium-mediated DNA transfer in different plant tissues offers a simple and fast method to analyze transgene functions.

Aim: study was conducted with the aim to determine transient expression of *acdS*-gene of bacteria *Pseudomonas putida* B-37 in plant cells *Nicotiana benthamiana*.

Materials and methods: The *acdS*-gene was amplified by PCR and then cloned into pBI121 vector under the control of the cauliflower mosaic virus (CaMV) 35s promoter. *Agrobacterium tumefaciens* GV3101 strain harboring pBI121-acdS vector jointly with the helper strain 19K were used for Agrobacterium-mediated leaf infiltration in *N. benthamiana* to infect 3-weeks-old plants. Monitoring of transient expression efficiency at 3 days post-infection was conducted by plant RNA extraction and RT-PCR. RNA was extracted from *Nicotiana*'s infiltrated zones and an amount of 1 µg total RNA was used to synthesize first-strand cDNA and then RT-PCR.

Results and conclusion: As a result, *acdS*-gene of bacteria *P. putida* has been observed to be expressed at detectable levels in plant cells *N. benthamiana*. It was shown that bacterial gene can be maintained in plant cells. So, it can be created transgenic plants with overexpressed *acdS*-gene which may have significant effects on plant development under stress conditions.

4. Jolanta Lebedeva

"Genome Mining-Based Identification of Bacteriocins in *Geobacillus*"

LEBEDEVA JOLANTA, Kuisienė Nomeda

Bacteriocins are ribosomally synthesized antimicrobial peptides produced by bacteria which can kill or inhibit bacterial strains closely related or non-related to the producer bacteria. However, little is known about bacteriocins produced by Gram-positive thermophilic bacteria. The interest in them is considerably increasing because they are more stable and resistant to high temperatures therefore may be applied to protect heat-treated food products. The activity of bacteriocins against food-borne and pathogenic bacteria opens wide opportunities for application of these molecules in food technology and healthcare fighting multiple drug resistant bacteria.

The aim of this study is to evaluate the potential of thermophilic bacteria genus *Geobacillus* to produce novel bacteriocins. We employed *in silico*-based screening approach to mine potential bacteriocin clusters in genome-sequenced isolates. These gene clusters were further classified according to their gene organization and the homologies of their structural and biosynthetic genes.

An investigation on to-date available whole genome sequences of *Geobacillus* revealed that most species of the genus have good potential to produce a wide variety of antimicrobials and there is high occurrence of putative biosynthetic gene clusters. We identified 103 putative bacteriocin gene clusters from 59 chromosome and 3 plasmid sequences. The most commonly identified classes of bacteriocin were sactipeptides and head to tail cyclized peptides. Some of the gene clusters show similarity

with those of known bacteriocins, while some are uncharacterized or show limited homology suggesting *Geobacillus* bacteria could be a good source for novel bacteriocin discovery.

5. Ieva Radvilė Žalytė, Laurynas Mockeliūnas

"Genotoxic and Biochemical Effects of Contaminated Soil on Tradescantia Clone #4430"

IEVA RADVILĖ ŽALYTĖ, LAURYNAS MOCKELIŪNAS, Tatjana Čėsnienė, Raimondas Šiukšta

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Introduction: Since the environmental pollution is a worldwide phenomenon, the investigation of the effects of toxic and genotoxic agents on ecosystems and human health is of principal importance. *Tradescantia* micronucleus (*Trad*-MCN) and *Tradescantia* stamen hair mutation (*Trad*-SHM) bioassays are known to be highly efficient in determining genotoxicity of gaseous and liquid environmental agents, whereas lipid peroxidation is a valuable biochemical marker for oxidative stress evaluation.

Aim: To examine the genotoxicity of soil from the territories of closed industrial enterprise "Kuro aparatūra" (KA) in Vilnius and Verkšionių gravel quarry (VER) using a sensitive bioindicator *Tradescantia* clone #4430.

Materials and methods: *Trad*-SHM and *Trad*-MCN assays were applied to investigate the effect of short-term exposure to aqueous and DMSO extracts of contaminated soil. Four types of

biomarkers were scored – pink cells, colourless cells and branched hairs in stamen hairs, and micronuclei in tetrads of pollen mother cells. *Trad*-SHM test as well as lipid peroxidation assessment were also used to investigate the effect of long-term (0.5 year) exposure to contaminated soil.

Results: The results of soil chemical analysis, cytogenetic tests and lipid peroxidation assessment were partially consistent. Sample KA5 was found to be the most contaminated according to the chemical analysis. However, soil sample VER2 was found to induce higher level of lipid peroxidation than sample KA5 and to be more genotoxic than sample KA5 according to the results of *Trad*-MCN test. *Trad*-SHM showed bigger genotoxic potential of soil sample KA5 except for the formation of colourless cells.

Conclusions: *Tradescantia* test-system proves to be a reliable tool for assessing the genotoxicity caused by contaminated soil. Nevertheless, reassessments need to be performed in order to find appreciable correlations between the results obtained from cytogenetic tests and the extent of lipid peroxidation.

This work was supported by a grant from the Lithuanian Research Council MIP-042/2015.

6. Alisa Palavenienė

"*Os Sepia* as a Versatile Marine-derived Material for Dermatological Applications: a Preliminary Study"

ALISA PALAVENIENĖ, Sigita Petraitytė, Monika Budvytienė
Kaunas University of Technology

Introduction: Nowadays the search for natural compounds with multifunctional therapeutic properties is of great interest. *Os Sepia* or cuttlebone (CB), a marine-derived by-product, is known for its unique physicochemical characteristics and versatile composition. CB was shown to be a promising material in soft and hard tissue engineering. Up to now very few investigations on the antimicrobial activity and haemostatic properties of CB were performed.

Aim: The aim of our research was to investigate antimicrobial and haemostatic activity of *Os Sepia* with respect to its possible applications in dermatology.

Materials and methods: Three groups of powdered CB were prepared: non-modified, treated with 1% sodium dodecyl sulphate (SDS) or treated with 40% NaOH for deproteinisation. Samples were dissolved in an acidic solution prior to all experiments. Antimicrobial activity was assessed using an agar well diffusion method. Blood clotting tests were performed using a Cobas t411 coagulation analyser (Roche Diagnostics Limited, Switzerland).

Results: Strains of bacteria found on the surface of a skin, such as *Staphylococcus aureus*, *Pseudomona aeruginosa* and *Lactobacillus plantarum* were chosen for antimicrobial activity evaluation. All samples showed no antibacterial activity against selected bacteria strains. For the assessment of haemostatic properties of

CB the International normalised ratio (INR), the activated partial thromboplastin time (aPTT) and the fibrinogen level (FIB) were analysed. aPTT values of non-modified and SDS-treated CB samples were 65.5 sec and 41.5 sec, respectively, indicating the haemostatic properties of the latter.

Conclusions: The results showed ambivalent therapeutic potential of *Os Sepia*. Haemostatic properties of *Os Sepia* treated with anionic surfactant were demonstrated. Though antimicrobial activity against pathogenic bacteria was not proved, CB could be a promising material for probiotic bacteria *Lactobacillus plantarum* encapsulation and subsequent use for dermatological applications. However, further investigations need to be implemented.

7. Elizabeth Siniauskaya

"Assessing Minimal Residual Disease (MRD) in Childhood JMML Using Single Nucleotide Mutations of NRAS Gene as Molecular Markers"

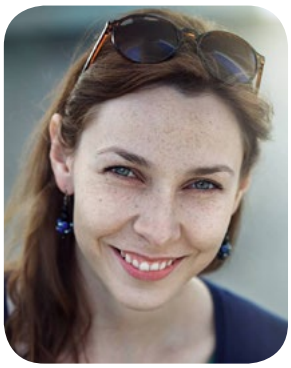
SINIAUSKAYA E., Migas A.

Juvenile myelomonocytic leukemia (JMML) is a rare, aggressive pediatric disorder. Driver mutations are largely mutually exclusive and thus far converge on a single primary pathway (Ras/MAPK). Hematopoietic stem cell transplantation (HSCT) is the only curative approach for most children with JMML (and for most with somatic *NRAS* mutations), but the risk of relapse after HSCT is high. Minimal residual disease (MRD) is the major cause of relapse. Similarly to other types of leukemia MRD monitoring for JMML patients can be used as a prognostic and stratification factor. To date, no standard MRD detection protocols and guidelines for data analysis are available for JMML.

We aimed to create an adequate laboratory protocol for assessing MRD in pediatric cases of JMML. Total PBMCs DNA for analysis was acquired from BM aspirates of two pediatric patients with JMML at different time-points during the pre-/post-HSCT observation. Patients were diagnosed and treated in BRCPOH, and had the same clonal somatic point-mutation in 2 exon of *NRAS* gene (rs121434596, NM_002524.4:c.38G>A). For MRD assessment we used allele-specific oligonucleotide PCR (ASO-PCR) in real time with SYBR®Green. Mutant to wild-type allele ratio was calculated manually using ΔCt value. For data visualization we used SigmaPlot. Having conducted a prior evaluation of sensitivity (10^{-3}–10^{-2}) and efficiency of both (wild-type and mutated) alleles amplification we estimated allele frequencies in patients' samples using real-time ASO-PCR. Using small amount (100 ng) of DNA per reaction we observed a certain dynamic in range of 0.1–1 on log scale for patients' samples obtained in hematological remission. Amplification level of healthy controls was that of -0.53 on log scale. We've developed a working protocol for MRD assessment that allows us to monitor low (nonrelapse) levels of MRD. The current method can be modified further for use with other SNPs and integrated into clinical monitoring routine for JMML.

8. Aušra Stumbrytė

"Combined Effect of HPV and Genetic Polymorphisms in *TP53*, *MDM2*, *MDM4*, *MTHFR*, *CCR5* and *CASP8* in Lung Cancer"



AUŠRA STUMBRYTĖ, Agnė Kunickaitė, Živilė Gudlevičienė, Daiva Dabkevičienė, Regina Liudkevičienė, Genovefa Garmienė, Saulius Cicėnas

Introduction: Laryngeal cancer is the sixth most common

cancer worldwide. The mortality in Lithuania is estimated to be 4.8 per 100 000. Smoking and alcohol are considered to be the main risk factors. Genetic variations and the presence of human papillomavirus (HPV) are also assumed to play an important role in the process of carcinogenesis. Single nucleotide polymorphisms (SNPs) are the most common form of human genetic variations which lead to higher cancer susceptibility.

Aim: The aim of this study was to evaluate the presence of human papillomavirus infection, genetic and harmful factors such as alcohol and smoking in laryngeal cancer.

Materials and methods: A total of 49 patients with laryngeal cancer from National Cancer Institute were examined from 2012 to 2015. HPV screening and SNP detection in biopsy samples were performed using PCR with specific primers.

Results: In order to evaluate the main risk factors which, determine carcinogenesis of larynx cancer, we rated the prevalence of HPV, smoking and alcohol consumption. The HPV infection related genes' SNPs were also included. HPV positive infection was found in 43% (21) of 49 patients. Among these patients, 92% (45) smoked and 55% (27) consumed alcohol. The 95% (20) of HPV infected patients depended to A9 phylogenetic group (types of 16, 16/19) while only one patient belonged to A7 group. 96% of them smoked and 84% used alcohol.

Conclusions: In our study identified frequency differences showed, that not infectected HPV group del/del polymorphic variant of CASP8 gene was found more often (25%, infected - 9%). MTHFR gene T/T variant was found more in HPV-infected group (19%) and not infected only 4%. CCR5 gene polymorphic variant wt/Δ32 frequency in infected samples was 10%, while in non-infected group - 29%.

9. Agnė Kunickaitė

"Combined Effect of HPV and Genetic Polymorphisms in *TP53*, *MDM2*, *MDM4*, *MTHFR*, *CCR5* and *CASP8* in Lung Cancer"

AGNĖ KUNICKAITĖ, Aušra Stumbrytė, Živilė Gudlevičienė, Daiva Dabkevičienė, Regina Liudkevičienė, Genovefa Garmienė, Saulius Cicėnas

Introduction: Non-small cell lung cancer is one of the most common malignant tumours and the leading cause of all cancer related deaths worldwide. Approximately 1.8 million people all over the world were newly diagnosed with lung cancer in 2012. Epidemiological data in Lithuania reflects similar tendencies with 1421 new cases (8.0% of all cancers) and 1355 deaths (16.9%).

Various studies showed that lung cancer is caused by both genetic and environmental factors and especially their interactions. Although exposure to carcinogens is considered to be the main cause, genetic variation and human papillomavirus (HPV) may contribute to lung cancer risk.

Aim: The aim of this study was to evaluate the distribution of HPV and different SNPs involved in the carcinogenesis and its impact to patients survival.

Materials and methods: 92 lung cancer patients from National Cancer Institute were included in the study during 2012-2014 year. HPV screening in tumour samples was performed using PCR with specific primers. Polymorphisms were detected using PCR with specific primers followed by restriction.

Results: HPV infection was stated only in three patients with NSCLC. After genes polymorphisms analysis it was found that patients with *MDM2*c.-5+309G>T gene T/G polymorphic variant had a better survival rate (*P*=0,383). A/A polymorphic variant of the *MDM4*c.1q32A>C gene resulted in a better survival rate (*P*=0,836). C/T and C/C polymorphic variants of the *MTHFR*c.677C>T resulted in similar survival rates as the ones observed in the same test group. Del/del allelic variant of *CASP8*c.-652 6N ins/del gene SNP led to lower survival rates. The analysis of the *CCR5*- Δ32 gene, better survival rate was noted in the patients who were identified with wt/Δ32 polymorphic variant (*P*=0.854).

Conclusions: Patients who were identified with *CASP8* ins/ins and *TP53* Arg/Pro gene polymorphic variants, survival rate was twice longer than those who were identified with *MDM4* A/A, *CCR5* wt/Δ32, *MTHFR* C/T, *MDM2* T/T.

10. Rūta Maleckaitė

"DNA Methylation Analysis of Metallothionein Genes in Breast Tumors"

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2. Nacional Cancer Institute, Vilnius, Lithuania

Introduction: Breast cancer (BC) is the most common malignant disease of women worldwide. BC can be cured completely if it is diagnosed at early stage. Unfortunately, traditional pathologic and immunohistochemical characteristics lack sensitivity in discovering the disease at its initial cancerous lesions in breast tissues. Nowadays, DNA methylation at promoters of particular

genes as could be used as an effective measure to detect early molecular changes in breast tissues.

Aim: To investigate the promoter methylation status of metallothionein coding genes as potential diagnostic biomarkers of BC.

Methods: In total, 78 BC and 29 non-cancerous samples from patients diagnosed with fibroadenoma or fibrocystic breast changes mainly (control group) were included in the present study. The samples were collected during 2007-2009 at National Cancer Institute. Promoter methylation status of genes *MT1E*, *MT1G*, and *MT1F* was analyzed by means of methylation-specific PCR.

Results: Genes *MT1E*, *MT1G*, and *MT1F* were methylated in 40%, 37%, and 0% of BC samples, respectively. Methylation frequency of *MT1E* was significantly lower in the control group (14%, P = 0.0114). Hypermethylation of *MT1G* and *MT1F* genes was less common (29% and 0%, respectively) in tumors and the frequencies did not differ from controls (both P > 0.0500). In BC, promoter methylation of *MT1E* increased with higher tumor grade (P = 0.0196), while *MT1G* was more frequently methylated in BC cases with increased levels of estrogen receptor (P = 0.0399). No significant associations were observed between promoter methylation of metallothionein genes and patients' age, histological tumor subtype, tumor stage, and other pathologic indicators.

Conclusions: In the present study, a significant promoter methylation difference of gene MT1E was identified in BC as compared to controls. After further validation, aberrant methylation of this gene might be proposed as a novel biomarker for diagnostics of BC.

11. Ieva Sadzevičienė

"Epigenetic Biomarkers For Breast Cancer Diagnosis And Prognosis"

IEVA SADZEVIČIENĖ[1], Kristina Daniūnaitė[1,2], Valerijus Ostapenko[2], Sonata Jarmalaitė[1,2]

1. Human Genome Research Group, Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
2. Nacional Cancer Institute, Vilnius, Lithuania

Introduction: Breast cancer (BC) is the most common malignant disease among women in the world. BC can be effectively treated at early stages, while the tumor is still confined to the organ. However, traditionally used pathologic and immunohistochemical characteristics cannot accurately predict disease progression or clinical outcome. Epigenetic (DNA methylation) biomarkers are more accurate and sensitive for the early disease detection and important for molecular BC subtyping.

In the present study, we **aimed** to evaluate the promoter methylation status of selected tumor suppressor genes as potential diagnostic and prognostic biomarkers.

Methods: In total, 124 samples of BC and 29 noncancerous samples from patients diagnosed mainly with fibroadenoma and breast fibrocystic changes were included in the present study. The samples were collected during 2007-2009 at National Cancer Institute. Twelve genes (*p14*, *p16*, *RUNX3*, *DAPK1*, *GSTP1*, *RARB*, *MGMT*, *ESR1*, *ADAMTS12*, *APC*, *RASSF1*, and *PRKCB*) were analysed using methylation-specific PCR method.

Results: In BC samples, promoter hypermethylation was most frequently detected in *PRKCB* (86%), *ADAMTS12* (71%), *RASSF1* (68%), *APC* (60%), *ESR1* (47%) genes and significantly differed from the control group (0%, 4%, 11%, 3% and 14%, respectively; all P < 0.0001). Hypermethylation of *p14*, *p16*, *RUNX3*, *DAPK1*, *GSTP1*, *RARB*, and *MGMT* genes was less common (3-23%) in BC. Differences of promoter methylation frequencies were observed according to BC histological subtype, tumor stage, expression of HER2 receptor, and patients' age. In HER2-positive tumors, hypermethylation of *PRKCB* (100%), *ADAMTS12* (89%), *APC* (69%), and *RUNX3* (57%) was more frequent in comparison to tumors lacking HER2 (84%, 69%, 59%, and 37%, respectively).

Conclusions: Our study identified a set of genes as novel potential biomarkers that might aid in BC diagnostics and better characterization of molecular subtypes. Future investigations would be needed to determine the value of these biomarkers for non-invasive diagnostics of BC.

12. Indrė Pauraitė

"MCF - 7 cells immunophenotype determination after the treatment with ionizing radiation"

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Oncological diseases still cause most health problems in our country and all over the world. Over the past few years the study shown that some treatments are becoming less effective. For example cancer cells after the treatment with ionizing radiation

become resistant. Therefore, scientists become more interested in revealing resistance mechanisms in tumor cells.

The main purpose of our research was to determinate viability of MCF-7 cells and to estimate CD44, CD24 molecular markers expression after the treatment with ionizing radiation.

In our study we used human breast adenocarcinoma MCF-7 cell line. Cancer cells were affected 2 Gy dose per day, four days a week (4x2Gy) using Varian Clinac 600C/D linear accelerator. Viability of cells were determined using Adam-MC automated cell counter. CD44, CD24 molecular markers expression in control and irradiated cells were estimated using BD Accuri™ C6 flow cytometer.

In our experiments we determined that immediately after the treatment with ionizing radiation, viability of irradiated cells decreased, but recovered over the time, while viability of control cells were stable during all experiments. Further we estimated that CD44 molecular marker expression in control and irradiated cells were higher in irradiated cells than control cells. CD24 molecular marker expression was stable in the limits of errors.

Overall it is important to conduct these studies that helps better understand the impact of ionizing radiation in cancer cells and also helps to prepare for further studies.

13. Miglė Kalvaitytė

"The Impact of PLA Scaffold Micro-Structurization on Rat's Dental Pulp Stem Cells Osteogenic Differentiation *in vitro*"

MIGLĖ KALVAITYTĖ, Milda Alksnė, Egidijus Šimoliūnas, Virginija Bukelskienė

Introduction: Tissue engineering is a multidisciplinary science which main purpose is to develop artificial tissue. Artificially engineered bone tissue grafts have many advantages compared to real bone grafts, such as limitless supply and no disease transmission. Because of this, they might be used in regenerative medicine. Artificial bone creation requires appropriate cell source and scaffold selection, as well as determination of optimal cell culture conditions, which have not been elucidated yet.

Aim: This work is focused on the impact of biodegradable polylactic acid (PLA) scaffolds surface micro-structurization on rat's dental pulp stem cells (DPSC) osteogenic differentiation in vitro.

Materials and methods: Three-dimensional porous micro-woodpile geometry and wavy topography PLA scaffolds were created by 3D printing. They were used as extracellular surroundings for cell behavior study in vitro. DPSC were isolated from rat dental pulp by combining two methods: outgrowth from intact tissue and direct isolation with magnetic beads coated with antibodies against cell surface marker CD44. These cells were immunologically characterized by flow cytometry and their pluripotency was examined by differentiation assay. DPSC osteogenic differentiation on PLA scaffolds was evaluated by staining mineralized matrix with Alizarin Red S and measuring

alkaline phosphatase activity. Results were normalized by cell number, calculated from calibration curve.

Results: In vitro study indicated that DPSC, isolated from rat dental pulp, proliferate better on microporous PLA scaffolds compared to wavy ones. Alizarin Red S staining and alkaline phosphatase activity results also showed that microporous surface topography, with pores of 300 µm diameter, were more suitable for osteogenic differentiation than control wavy surfaces.

Conclusion: PLA surface topography influenced DPSC osteogenic differentiation capacity. Ordered scaffolds can be successfully applied as templates for cell proliferation and differentiation.

14. Elona Jankauskaitė

"Effects Of Sex Hormones On Cell Death In Cells With Mutations Responsible For Leber's Hereditary Optic Neuropathy"

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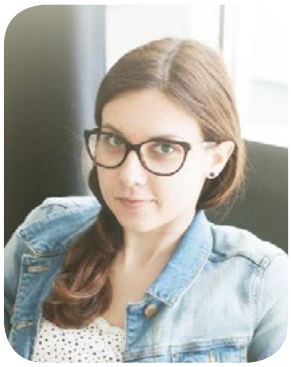

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Leber's hereditary optic neuropathy (LHON) is a maternally inherited form of incurable central vision blindness due to isolated atrophy of the optic nerve caused by point mutations in mitochondrial DNA (mtDNA). In most cases, mtDNA is 100% mutated in every cell, but only retinal ganglion cells (RGC) are affected. LHON occurs mostly in young adults affecting both eyes

simultaneously or sequentially in period of several months or weeks. It is not known why only RGCs are affected and why men are four to five times more likely to develop the disease.

The aim of this study is to determine the possible effect of sex hormones on the cell death mechanism in LHON patient and control cells.

Human optic nerve cells are unavailable for studies, therefore a lymphoblast cell model was used. Lymphoblast cell lines were established from whole blood samples obtained from 3 LHON affected individuals with confirmed the most common m.11778G>A mutation and 3 healthy age-matched controls. The effect of sex hormones (estrogen and testosterone) on initiation of apoptosis was investigated via the canonical apoptotic pathway which involves activation of aspartate-specific proteases (caspases). Apoptosis was confirmed by PARP cleavage. For autophagy detection one of LC3 protein isoforms were used.

Primary results indicate higher a level of apoptosis in LHON patient cells compared to controls. In addition, apoptosis in LHON cells was confirmed to occur via a caspase-independent (CICD) pathway. Interestingly, in some patient cells autophagy and CICD were detected simultaneously.

For unknown reasons apoptosis in patient cells is confirmed to be CICD, which means that apoptosis is promoted by another (possibly defective) mechanism leading to the specific atrophy in the optic nerve. However, it was not confirmed that any sex hormone plays a specific role in these processes.

15. Wiktor Tokarek

"Phaeodactylum Tricornutum as a Potential Bioremediatory Agent in Mercury(II)-Contaminated Waters"

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Introduction: *Phaeodactylum tricornutum* is a unicellular diatom species, widely used in basic research as a model organism. It can be used in the field of wastewater management and heavy metals bioremediation. Mercury is an extremally serious pollutant, able to harm humans and a wide range of other organisms.

Aim: To determine the impact of the exposure to the low and high concentrations of mercury(II) chloride on the pigment composition of diatom cells. We also checked, weather this diatom species is able to change the concentration of soluble heavy metal ions.

Materials and methods: Diatom cells (*Phaeodactylum tricornutum* CCAP 1055/1) were grown in Guillard's f/2 medium with added silicate. The culture media were contaminated with $HgCl_2$ (the final Hg^{2+} concentrations were 0.01, 1 and 10 mg/L). We assessed the optical density of the cultures and performed HPLC analysis of the pigments content. Heavy metal concentrations were measured using the ICP-MS technique.

Results: At the Hg^{2+} concentrations of 1 and 10 mg/L, mercury contamination greatly handicapped the growth of *P. tricornutum*. Except for the last few days of the culture period, no clear logarithmic growth phase was observed. At the lowest Hg^{2+} concentration, the growth characteristics resembled those of the control cultures. We also observed the drop in the concentration of soluble mercury ions at the end of the culture period (to around 70% of the initial value).

Conclusions: We highlight the importance of *P. tricornutum* is the studies of heavy metals impact on the physiology of microorganisms. Furthermore, this diatom species could be used in potential bioremediation applications.

Acknowledgements: Faculty of Biochemistry, Biophysics and Biotechnology of Jagiellonian University is a partner of the Leading National Research Center (KNOW) supported by the Ministry of Science and Higher Education.

Physiology

16. Karolina Valavičiūtė

"Dorsolateral Prefrontal Cortex Coordinates Impact for Transcranial Magnetic Stimulation Effectiveness"

KAROLINA VALAVIČIŪTĖ, Vladas Valiulis, Kastytis Dapšys, Valentinas Mačiulis

Introduction: Transcranial magnetic stimulation (TMS) is a safe and effective way to treat treatment-resistant depression and other various psychiatric dissorders. Nowadays many researchers try to find, which stimulation target is the most effective for

treatment. From the literature we chose 3 of the most discussed targets for our research.

Aim: Our aim is to asses, how TMS target coordination influences the changes in electroencephalography (EEG) frequency power spectre and clinical therapeutic effect and to establish optimal TMS target coordinates, based on our results.

Materials and methods: We investigated the impact of TMS coordinates for inpatients from the Republican Vilnius Psychiatry Hospital. We chose 3 different coordinates for our research: 1) Teneback 1999 study TMS therapy coordinates; 2) Fox 2012 study TMS therapy coordinates; 3) Fitzgerald 2009 study TMS therapy coordinates. TMS therapy was applied for patients with treatment-resistant depression, total 10 – 15 procedures (in 2 – 3 weeks) as a high frequency (10 Hz) 20 electromagnetic waves impulses, aimed to chosen coordinate. Clinical therapeutic effect was estimated by following 3 scales: 1) Montgomery – Asberg depression scale; 2) Hamilton depression rating score; 3) Beck depression iventory. Physiological changes were estimated by EEG frequency power mean. A total of 35 patients were studied (24 from Teneback coordinates target group, 11 – Fox and 10 - Fitzgerald).

Results: Results are currently being processed and analysed to be shown in a poster.

Conclusions: Different patterns of physiological change and variability in clinical response are to be expected from the different TMS target coordinate use.

17. Attila Gáspár

"Effects of Chronic 4-aminopyridine Treatment on Behaviour and Memory Processes in Rats"

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Introduction: Epilepsy is one of the most frequent neuronal disorders. There is a strong connection in the background activity of epileptic and learning processes like long-term potentiation (LTP) of synapses, the cellular mechanism of learning. In our experiments, we used 4-aminopyridine (4-AP), a potassium channel blocker, to evoke epileptic seizures.
Aim: Our aim was to investigate epilepsy-related acute and chronic changes in general behaviour, learning and memory formation using behavioural and electrophysiological methods.

Materials and Methods: Rats were treated with 4-AP for twelve consecutive days. Rats were examined immediately after the end of treatment ("acute group") or 3 month later ("chronic group"). To examine learning and memory processes we used eight-arm radial maze and novel object recognition tests. Furthermore we also investigated locomotor activity and anxiety in open field test. For the electrophysiological measurements we prepared surviving brain slices. After-discharges evoked by brief bursts of high frequency electrical stimulation were detected with parallel recording of intrinsic optical activity in the entorhinal cortex to test the sensitivity of the slices. To evoke LTP, we stimulated Schaffer-collaterals with high frequency tetanic stimulation.

Results: Open field tests showed that acute and chronic 4-AP treated rats were less anxious and were more active than rats from acute and chronic control group. In the novel object recognition test, control rats spent significantly more time with the novel object than 4-AP treated animals. In the radial maze test the treated animals had lower performance. LTP studies showed that in the acute 4-AP treated group the efficacy of LTP induction was moderately higher. We found that it was easier to induce after-discharges in slices from treated rats and burst length was longer than in control slices. The optical signal showed correspondence with the electrophysiological results.

Conclusions: Altogether we can say that long-term 4-AP treatment caused short-term changes on the electrophysiological level, however the memory tests demonstrated long-term alterations in the animals.

18. Rolandas Stonkus

"Emulation of Spiking Neural Network-based Auto-associative Memory on the Spikey Neuromorphic Platform"

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Introduction: Spiking neural networks (SNNs) represent a third generation of artificial neural networks and are inspired by the computational principles of the brain. SNNs consist of biophysically realistic spiking neuron models, and information transmission is encoded in precise timing of spikes or sequences of spikes. Synaptic weights are driven by the biologically inspired learning

algorithms, such as Hebbian or Spike-timing-dependent synaptic plasticity (STDP) learning rules. SNNs are being increasingly used in real-world tasks, however, simulations of large-scale SNNs are computationally expensive. Therefore, neuromorphic computing platforms are being developed to allow fast and energy-efficient simulations. Such systems, e.g. Spikey, SpiNNaker and TrueNorth implement physical models of neurons and synapses and highly accelerate network emulations.

Aim: We aimed to estimate the simulation efficiency of the SNN-based auto-associative memory model, implemented on the Spikey neuromorphic system, with respect to established reference simulator Brian.
Materials and methods: We implemented the SNN-based auto-associative memory model on the Spikey neuromorphic system and in the Brian simulator. The network model consisted of the Leaky integrate and fire neurons connected by the excitatory and inhibitory synapses. Synaptic weight changes were modeled using a classic STDP learning rule.

Results: Our results showed that emulation of the auto-associative neural network on the Spikey system is substantially faster if compared to the Brian simulator. Differences in recall accuracy of the Spikey and Brian network models were not significant.

Conclusions: Neuromorphic computing platform Spikey offers the potential to emulate SNN in the energy-efficient and fast manner. However, Spikey system has its limitations, particularly in network size, synaptic weight control, neuron models.

19. Veronika Bódi

"The Acute Effects of Fumonisin B1 on Brain Slice Preparations"

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Introduction: Fumonisin B1 (FB1) is a mycotoxin produced by microscopic moulds. Its importance is in infecting our major crops – like maize –, moreover due to its high melting point, even heat treated food can contain it.

Aim: The aim of this study was to prove that FB1 has excitatory effects on the neuronal networks with in vitro acute toxin treatment.

Materials and methods: We have treated rat brain slices with artificial cerebrospinal fluid containing the toxin in two different concentrations (50 µM and 100 µM) for 30 minutes. After the treatment we have investigated electrically evoked and spontaneous field potentials in the hippocampus, the secondary somatosensory and lateral entorhinal cortices.

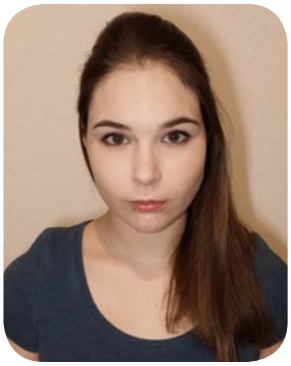
Results: In the hippocampus, during the stimulation tests right after the treatment, the toxin in the lower concentration didn't have significant effects on the neurons, while in case of the higher concentration, we observed an increase in amplitude of evoked potentials. After the induction of LTP (long-term potentiation), we could see that treating with the higher concentration made the initial excitatory effect result in the depletion of neuronal networks, and the setback of the effect of the potentiation.

In the cortex during stimulation tests I've experienced similar results as in the hippocampus since the lower concentration had lesser effect on the neurons, while the higher concentration increased the evoked potentials significantly. In case of spontaneous activity recorded in a convulsant (magnesium-free solution), the impact of the elevation of toxin concentration was clearly excitatory. FB1 treatment causes longer lasting bursts which occur earlier with higher amplitudes.

Conclusion: Altogether we can say that the toxin influences the basic excitability of neuronal networks in brain slices and also elementary processes of learning (LTP). Pathological, epileptic events were also altered, parallel with the increasing sensitivity of neurons.

20. Tímea Májer

"The *ex vivo* Examination of the Entorhinal Cortex: the Kainate Receptors Role in Development and Synchronized Functioning"



MÁJER TÍMEA, Major Katalin, Világi Ildikó

Introduction: Epilepsy is a common disease which often occurs already in childhood. The prominent features of this disease are the strong, unprovoked hypersynchronous neuronal activities of the brain, which appear in recurrent seizures. Kainate receptors may have a modulatory role in this mechanism. Although the activation of these receptors do not initiate seizures, understanding the role of them is fundamental for further research.

Materials and methods: In our experiments we used combined hippocampal-entorhinal rat brain slices from different ages. During the tests, first we used modified ACSF to provoke convulsions, than

we treated the slices with a specific GluK1/2 antagonist (UBP-296). Electrophysiological signals of spontaneous seizure activity were recorded by Multi Electrode Arrays (MEA chip). In all age-groups differences in field potential- and single cell activity were analysed. The frequency, duration, amplitude of spontaneous events and spike activities were compared.

Results: The highest seizure frequencies was detected at the 2 weeks old animals, while the lowest at the 3-4 weeks old ones. The duration was the longest at the 3 weeks old group. The effect of the antagonist was the most powerful in the adult group. Inhibitory effect of the kainate antagonist was significant in all age-groups except in the 3 weeks ones. We examined cellular activity in layer II/III independently from the layer V. We found that burst pattern in layer II/III was very similar to the whole slice's events, while in layer V we do not find any difference between the age-groups.

Conclusions: Based on our results from different age groups we can summarize that kainate receptors participate principally in the propagation of seizures activity, however, they have no significant role in the initiation. Base of the background mechanisms change through development. It is worth to investigate these receptors further as they could be new potential targets of antiepileptic treatments.

21. Emilija Kurlytė

"Intraperitoneal vs. Intravenous Chemotherapy: Treating Ovarian Cancer"

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Introduction: Ovarian cancer is characterized as aggressive and usually asymptomatic disease therefore diagnosed at late-stage, so it is important to look for the best treatment. One promising way is intraperitoneal (IP) chemotherapy. Some studies have demonstrated its survival advantage in comparison to intravenous (IV), although more adverse effects have been noticed and it is unknown if it is long-term beneficial.

Aim: To compare outcomes of IP and IV chemotherapy, progression-free survival (PFS) and side effects of drugs.

Materials and methods: Retrospective study of 36 patients with epithelial ovarian cancer at Vilnius University Hospital Santariskiu Klinikos. 15/36 patients treated with IP and 21/36 – IV. Analyzed data: demography of patients, cytoreduction rate, differentiation grade, existence of metastasis, influence of neoadjuvant chemotherapy, PFS, side effects. Associations between categorical variables were determined using Shapiro-Wilk test, t-test of independent samples.

Results: Follow-up time – 30.5 months. Average age of patients: IP – 47.5 ±7.9 years, IV – 52.8 ±12.4 years. Average PFS when treated with neoadjuvant chemotherapy is longer using IV method (IP - 13.3 ±2.5 months, IV – 21.5 ±10.7 months, p=0.001). Average PFS, PFS: after radical debulking (R0), if G3 differentiation grade, if metastatic disease, with adjuvant chemotherapy did not vary statistically significant. Adverse effects that were noticed more often in IP group: drug induced neuropathy, neutropenia, obstipation, dyspepsia, fatigue, abdominal pain, infection at drain site. Side effects that occurred more often in IV group: anemia, thrombocytopenia, hepatotoxicity, arthralgia, rash, ototoxicity. Similar frequency: cardiotoxicity, nephrotoxicity, postoperative adhesions, insomnia.

Conclusions: Based on results, there were no statistical significance in both patient's groups average PFS, although treatment starting with neoadjuant chemotherapy is better with IV. Both ways are well tolerated, we did not observe extremely severe side effects.

22. Neringa Guobytė

"Analysis of Patient Data Who Tested Positive for a Molecular Assay of 7 STIs"

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Introduction: Sexually transmitted infections (STIs) are the infections which can be caught during all types of sexual intercourse, by sharing infected needles, during pregnancy. Over 1 million people in the world are infected by STIs every day.

Aim: To evaluate the data of medical history, diagnostics, treatment and its effectiveness to the patients who have tested positive for the molecular testing of 7 sexually transmitted pathogens.

Materials and methods: In Vilnius University Hospital Santariskiu klinikos Centre of Dermatovenerology 245 patients were diagnosed with an STI by molecular analysis. The effectiveness of the treatment was evaluated for 103 patients who came for a follow-up. The statistical significance was set at 0.05.

Results: 75% (n=184) studied patients were women, men constituted 25% (n=61). Patients mostly complained of joint pain. One pathogen was identified in 73.9% of patients and a few – in 26.1% of patients. Most commonly identified pathogens: *U.*

parvum (53.5%), *U. urealyticum* (11%), *U. parvum* together with *M. hominis* (8.6%). 75.5% of women and 83.6% men were treated. The efficiency of the treatment was measured in the *U. parvum* group: 46.8% of patients received a total of 2.8 g of doxycycline (77.3% cure rate) and 8.5% of patients received a total of 4.2 g of doxycycline (100% cure rate), (p=1.000). 12.8% of patients received a total of 1.5 g of azithromycin (66.7% cure rate) and 31.9% of patients received >1.5 g of azithromycin (66.7% cure rate), (p=0.065).

Conclusions: The risk of getting infected by STIs for men is 3 times higher than that for women. U. parvum infection was diagnosed for more than half of the patients. 78% of patients were treated and three-quarters of them were cured. No statistically significant correlation was identified between the positive treatment effect, the chosen medicine and the extended duration of its use.

23. László Szente

"Endocannabinoid Interactions in the Regulation of Behavioral Responses to Traumatic Events"

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Introduction: Endocannabinoid (eCB) signaling is an important regulator of the behavioral responses to traumas, however, the specific roles and possible interactions between endocannabinoids anandamide (AEA) and 2-arachidonoylglycerol (2-AG) are not well understood.

Aim: We aimed to assess the specific effects of AEA and 2-AG and their possible interactions on acute responses to a traumatic event and the dynamics of traumatic memory.

Materials and methods: We enhanced AEA and 2-AG signaling in rats via systemic pharmacological treatments and examined its effects on acute fear responses during exposure to a series of electric footshocks and acquisition of traumatic memory during reminders to the shock context. We also investigated local eCB effects by direct treatments in the ventral hippocampus (vHC), the prelimbic cortex (PrL) and the basolateral amygdala (BLA), respectively. Furthermore, we studied the impact of enhanced eCB signaling on the extinction of traumatic memories by systemic treatments before the first contextual reminder.

Results: Systemic enhancement of 2-AG signaling dampened acute fear responses, which effect was abolished by AEA. Interestingly, local enhancement of AEA signaling in the vHC but not in the PrL or BLA decreased acute fear responses. Systemic enhancement of AEA led to strong contextual fear memory which effect was inhibited by 2-AG. The latter effect can be linked to vHC and PrL eCB signaling processes, respectively. In contrast, enhanced AEA signaling in the BLA prevented the formation of traumatic memories which effect abolished by 2-AG. Enhancement of 2-AG or AEA signaling before the first contextual reminder facilitated extinction of traumatic memory.

Conclusions: Our findings show that AEA and 2-AG functionally interact in the regulation of behavioral responses to trauma. Acute fear responses are possibly regulated by a complex interaction between local eCB signaling mechanisms, while acquisition of traumatic memories are predominantly regulated by vHC and PRL AEA under 2-AG control.

24. Vilius Vaitkus

"How COPD Patients Understand Their Disease and What are Their Future Expectations?"

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Introduction: Chronic obstructive pulmonary disease (COPD) affects more than 65 million people around the world. Despite the wide prevalence of the disease, patient awareness is still not sufficient.

Aim: To assess how COPD patients understand their disease - its etiology, risk factors, treatment. To evaluate inhalers techniques mistakes and COPD impact on patient social life, emotional status, future expectations.

Materials and methods: In the year 2013–2017 a prospective study in the Vilnius University Hospital Santariskiu Clinics center of Pulmonology and Allergology was performed. A questionnaire created about patients understanding of the disease, risk factors, treatment, use of inhalers, impact on social life, expectations for the future.

Results: 81 COPD patients (male 92 %, mean age 68 years) were questioned. The average time of illness was 14 years. 47 % of the patient know the exact name of the disease they are suffering

from, 10% could not say what disease they had. 46 % think that the reason of getting COPD was harmful work environment, 40 % named smoking as the main reason. When testing patient inhaler technique, up to 52% made mistakes. The most common observed in the use of metered dose inhalers error was not shaking inhaler before the use. The most common use of dry powder inhaler mistake was too weak inhalation. Most of the patients named COPD as their biggest burden. The patients scored their life quality 5,5±2 points on a scale from 1 to 10. 29,3% believed that their disease will get worse in the future, while 43,21% hoped to feel better.

Conclusions: COPD patients lack knowledge of their disease, treatment, and smoking. The majority of the patients don't quit at all. The quality of life is affected not only by worsening of physical status but also by patients social problems associated with the disease.

25. Olena Pavlyushchik

"Seasonal Variation of DNA Damage in Male Patients with Metabolic Syndrome"

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Introduction: Increased levels of DNA damage have been observed in individuals with metabolic syndrome (MetS); however, the results were often contradictory. Seasons reportedly have an effect on DNA damage in healthy individuals, but the cell

response may differ in patients with MetS, which can contribute to inconsistencies.

Aim: The aim of our study was to determine the season-dependent variations in DNA damage levels and to assess the differences between donors with the diseases associated with MetS and healthy individuals.

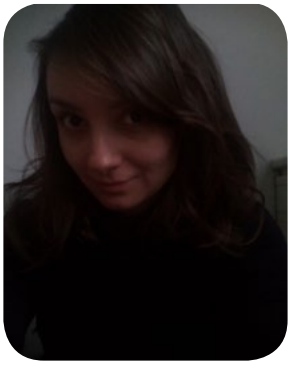
Materials and methods: Biomarkers of DNA damage, cell death and micronuclei of peripheral blood leukocytes, were measured by flow cytometry in the group of men diagnosed with essential hypertension (n=170), men with diabetes (n=126) and the corresponding control group (n=64). All donors were divided into summer and winter groups according to the time of sampling. Differential white blood cell count was carried out.

Results: The patients with the conditions related to MetS had higher levels of cell death and micronuclei compared with the healthy donors ($p<0.05$). However, taking into account the season of sampling, differences in the levels of DNA damage biomarkers were observed only between the winter groups, because healthy donors had significantly higher cell death rate in summer ($p<0.05$), while the cells of patients with MetS did not show any response to seasonal change. In winter, micronuclei of patients with MetS correlated with segmented leukocytes ($r=0.23$, $p<0.05$), which according to the literature, may signify higher DNA damage in hematopoietic stem cells; healthy donors had a negative correlation between the parameters ($r=-0.46$, $p<0.05$).

Conclusions: Differences in cellular response to seasonal changes were observed between healthy and donors with MetS factors. The results may be important for using cell death and micronuclei as biomarkers of MetS and for the studies of nature of DNA damage in patients with MetS.

26. Bibiána Török

"The Effect of Vasopressin Antagonists on Maternal-Separation-Induced Ultrasonic Vocalization and Stress-Hormone Level Increase During the Early Postnatal Period"



BIBIÁNA TÖRÖK, Anna Fodor, Sándor Zsebők, Rita Börzsei, Szilárd Pal, Dóra Zelena

Introduction: In adults vasopressin exerts anxiogenic effect, but less is known about the perinatal period. As a sign of distress rat pups emit ultrasonic vocalizations (USVs), when they are separated from their mothers. Previously we found reduced USV in 7-8-day-old genetically vasopressin-deficient Brattleboro rats and now the contributing vasopressin-receptor subtypes were evaluated in Wistar pups.

Aim: Here we aimed to establish, which vasopressin-receptor subtype can be responsible for the observed behavioral and HPA-axis effects in the early postnatal period.

Materials and methods: USV was recorded for 10 minutes 30 minutes after V1a-, V1b- or V2-receptor antagonist treatment (SSR49059, SSR149415, SSR121463B; 3-10-30 mg/kg, intraperitoneal). Sedation was studied by righting reflex and negative geotaxis and stress-hormones were measured by radioimmunoassay.

Results: The vasopressin-deficient pups showed decreased USV and adrenocorticotropin levels even after a saline injection, with unchanged corticosterone levels. 30 mg/kg V1a-antagonist reduced

USV, but increased stress-hormone levels. 3 mg/kg V1b-antagonist decreased USV and adrenocorticotropin. 3 mg/kg V2-antagonist enhanced USV, while 30 mg/kg increased stress-hormones.

Conclusions: We confirmed that genetic vasopressin-deficiency has anxiolytic effect already during the early postnatal age. Pharmacological analysis showed that antagonizing both the V1a- and V1b-receptors reduced USV, but stress-hormone changes contribute only to the V1b effect. Antagonizing V2 receptors may induce an imbalance through salt-water homeostasis leading to anxiety and increased stress-hormones. Take into consideration the possible side-effects a mixed V1a/V1bR antagonist treatment might be more beneficial as anxiolytic than either antagonist alone.

27. Kotryna Jurevičiūtė
"Vitamin D Status in Lithuania"

KOTRYNA JUREVIČIŪTĖ, Andrius Bleizgys

Introduction: Epidemiological data on vitamin D status in the Lithuanian population are limited.

Objectives: The aim of the study was to evaluate vitamin D levels for different age and sex groups and also their dependence on the seasons of the year, based on the analysis of serum 25-hydroxyvitamin D [25(OH)D] levels.

Materials and methods: This cross-sectional study included totally 19,282 participants (13,580 women; 5,702 men; mean age, 34 ± 23 years; range, 0-95 years), who were examined in the years 2012-2015. Serum concentrations of 25(OH)D, determined using the Cobas e 411 analyzer, were obtained from "Medicina Practica" laboratory.

Results: The mean 25(OH)D concentration in the studied population was 64,8 ± 41,6 nmol/l; 66,42% of the patients had 25(OH)D levels of less than < 75 nmol/l; 17,26% had levels of more than 100 nmol/l; and only 16,32% demonstrated the optimal levels of 75 to 100 nmol/l.

Conclusions: The levels of 25(OH)D observed in this study demonstrated that majority of the sample was vitamin D deficient all year long, especially during winter and spring. Women were tested more than twice as frequently as men and were more often diagnosed with deficiency, whereas men were more likely to show excessive vitamin D levels. The results of our study support the previously reported data on vitamin D levels, yet should be treated with caution due to demographic structure of the analyzed sample. The structure differs from that of the Lithuanian population, in particular in terms of weight of the age group 0-9 years, where 67,34% patients had excessive vitamin D levels.

28. Tomas Makaras

"Rapid Detection of Sublethal Toxicity Using Fish Locomotor Behavior"

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The aquatic environment is commonly polluted with complex effluents containing various chemical mixtures and is almost never exposed to single contaminants. Mixtures are composed of different chemical substances that, as a result of joint action, can produce combined effects even though their components are below their regulatory relevant effect concentration. Bioassays are considered to be an efficient and reliable tool for the impact assessment of multi-component mixtures on organisms, but they

are time- and cost-consuming. Therefore, an increasingly popular approach to the impact assessment of multi-component mixtures involves examining changes in the behavior of test animals over a short period of time using computer-assisted electronics and video tracking systems.This study examined changes in the locomotor activity of rainbow trout (*Oncorhynchus mykiss*) juveniles exposed to sublethal concentrations of hexavalent chromium (Cr^{6+}) (as a single metal ion) and landfill leachate (complex mixtures containing organics and metals) using a newly-designed recording system for fish movement tracking and analysis. Results of the study showed that under the effect of both test substances (at higher concentrations), intensity of fish locomotor activity significantly increased after 5 minutes of exposure. However, the juveniles exposed to leachate were more responsive to all tested sublethal concentrations than those exposed to Cr^{6+} solutions. The video-based movement analysis system was shown to be useful for rapid, quantifiable and high throughout assessment of toxicity in fish. We suggest that behavioral indicators such as swimming capability should be more widely included in fish bioassays for environmental contaminants. Future research should be focused on behavioral patterns of particular fish species so as to gain the comprehensive understanding of how fish respond to different pollutants and their mixtures. That would enable fast and efficient assessment of the toxic effect that ambient concentrations of multicomponent mixtures have on the aquatic environment. Besides, more studies are required to elucidate whether it is possible to identify the origin of pollutants based on the elicited fish locomotor activity patterns.

This study is funded by Research Council of Lithuania, Project No. 108/2015.

Biochemistry

29. Ignas Sabeckis

"Physicochemical Properties of 3D Chitin Extracted from *Blaberus giganteus* Cockroach Wing and Dorsal Pronotum"

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Chitin is very attractive research material of its applications in many different fields. It has interest due to ecofriendly, antioxidant and biocompatible properties. In previous studies, chitin was produced only in powder, granules, sheet form and fully characterized using various analytical tools. However, three-dimensional chitin, naturally present in many living organisms, has not been isolated or characterized. The aim of this study was to extract three-dimensional chitin from Blaberus giganteus cockroach and investigate its physicochemical properties. Herein, original shaped three-dimensional chitin was successfully extracted from the wing and the dorsal pronotum of cockroach. Physicochemical properties of chitin extracted from wing and dorsal pronotum were observed using Fourier transform infrared spectroscopy (FTIR), Scanning Electron Microscopy (SEM) and Thermogravimetric analysis. Similarity was detected in TGA analysis, where three-dimensional chitin extracted from wing and dorsal pronotum were both in alpha form. Despite, slight difference was discovered in TGA analysis were three-dimensional chitin extracted from the dorsal pronotum showed higher thermal stability than from wing.

SEM analysis showed that nanofibers and pores are present in both tested samples. This study clearly showed that three - dimensional chitin extracted from wing and dorsal pronotum of cockroach has high thermal stability and its microstructure is full of nanofibers and pores. It could be a great material for creating filters, coating surfaces or wound dressing due to its high thermal stability and biocompatibility. Furthermore, this study reflects how chitin can be seen from biotechnological perspective.

30. Aistė Kveselytė

"Synthesis of novel indole scaffolds: Towards biosensors"

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Indole carboxylic acid derivatives have a wide variety of applications, considerable number of them being related to the biological activity of compounds mentioned above.

The aim of our research is to find efficient ways to produce indole-based scaffolds that would be useful for further research in fields related to biology and medicine.

In the present work, different pathways of 5,7-diaryl and 5,7-dibromo substituted indolin-2-yl butanoic acid and its derivatives synthesis from spiro[indole-2,2'-piperidin]-6'-one were examined. Target compounds were obtained in good or excellent yields. Efficient synthetic pathway was determined.

The obtained compounds could be suggested as potential agents

in the bacterial quorum sensing research.

32. Justina Gružauskaitė

"Gold-coated Magnetic Nanoparticles for Wiring of Oxidoreductases"

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Magnetic nanoparticles (MNPs) have been widely used in the immobilization of many bioactive substances. MNPs have large surface area to volume ratio and can be attracted by a magnetic field and are easily separable in solution. Similarly, substances to which MNPs have been attached can be separated from a reaction medium, or directed by an external magnetic field to site specific targets. The aim of present study is to synthesize core/shell MNPs and investigate their properties with the special emphasis to the possibility of the obtained nanocomposites to enable the electron transfer between two oxidoreductases.

MNPs were synthesized by co-precipitation method and then coated with gold via methionineinduced reduction method. The synthesized MNPs and gold coated MNPs (AuMNPs) were characterized by X-ray diffraction (XRD), atomic force microscope (AFM) and electrochemical method. The activity of enzymes – laccase and glucose dehydrogenase (GDH) – was verified electrochemically using AuMNPs modified glassy carbon electrode (GCE). The electron transfer in wired system which consists of electron donor part – GDH, electron acceptor part –

laccase and electron transducer part – AuMNPs (GDH/AuMNPs/laccase) was investigated by observing the terminal electron acceptor – oxygen consumption.

The cyclic voltammogram of AuMNPs modified GCE in sulfuric acid have the representative to gold peaks of oxidation and reduction current. The XRD pattern of MNPs indicated that the MNPs were pure magnetite and the pattern of AuMNPs confirmed the presence of gold shell. The AFM micrographs show that the diameter of AuMNPs increases comparing with MNPs. The increase of biocatalytic current of AuMNPs and laccase or GDH modified electrodes in the presence of the respective substrates of the enzymes – oxygen or glucose – suggests the direct electron transfer between the enzymes active center and modified electrode. The oxygen consumption in GDH/AuMNPs/laccase system in the presence of glucose shows that AuMNPs act as electron transducer between the enzymes active center.

33. Eglė Malachovskienė

"Degradative Impact of Fungi on Newly Synthesized Copolymers of Glycerol Diglycidyl Ether and Different Diols"

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Introduction: Depleting oil reserves, accumulation of non-degradable polymers and continuous rise of consumer needs for plastics encouraged the development of polymeric materials derived from renewable resources. Therefore, the degradability at the end of their useful life and the identification of microorganisms

with degradative potential upon these materials is now essential field of research throughout the world.

Aim: to evaluate the degradative impact of fungi on newly synthesized copolymers from renewable resources in order to select fungal strains with degradative potential.

Materials and methods: The new copolymers of glycerol diglycidyl ether and different diols (1,4-cyclohexanedimethanol **(1),** 1,1,1-tris(hydroxymethyl)propane **(2),** hydroquinone **(3),** bisphenol A **(4)**) and control specimen without diol (0) were obtained from the Department of Polymer Chemistry and Technology, Kaunas University of Technology. The resistance of copolymers to consortia of fungal strains and microbially active soil (soil burial test) was evaluated according to the standard LST EN ISO 846: 1999. Antagonistic interactions between selected fungal strains and extracelullar enzyme assays were estimated by agar-block method and by qualitative methods respectively.

Results: According to antagonistic interactions and extracelullar enzyme assay results three fungal strains – Aspergillus ustus 0923, Talaromyces flavus 0891 and Trichocladium asperum 0936 were selected as a consortia of microorganisms for the estimation of the bioresistance of copolymers. The mass loss of copolymers after one month of exposure differed depending on their chemical structure. The highest value of the weight loss of 8.7 % was recorded for the sample of copolymer **(3)** and it was 4.6 times greater than control – **(0).** The lowest mass loss of 2.0 % was observed for the sample of copolymer **(4).** The mass loss of copolymers from the soil burial test showed resembling results to those obtained by bioresistance study.

Conclusions: During this investigation we assessed that mass loss of copolymers depends on their chemical structure and on

microorganisms with degradative potential. A consortium of three fungal strains will be used for further studies in order to improve the biodegradation process of the copolymers.

34. Diana Reznikova

"Lipid Peroxidation and Antioxidant Defense Systems in *Sideritis taurica*'s Extract-treated Diabetic Rats"

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The genus *Sideritis* (Fam. *Lamiaceae*) comprises about 140 species distributed in several countries of the Mediterranean region. Several species of the genus were investigated for their chemical content, also certain biological activities were reported. The present study was designed to investigate the antidiabetic capacity of *Sideritis taurica*'s extract on lipid peroxidation and antioxidative systems in alloxan-induced diabetic rats.

Instead of water *Sideritis taurica*'s extract was given to laboratory animals for a week with a standard diet. 30 male rats were divided into 3 groups: control, alloxan-induced diabetic and diabetic plus *Sideritis* extract treatment groups. Liver samples were taken from all animals and analyzed for superoxide dismutase and catalase activities and malondialdehyde levels. Blood samples were taken to analyze the level of lipids, in particularly, cholesterol and triacylglycerides.

It was found that superoxide dismutase and catalase activities in the diabetic-treatment group were higher compared to the diabetic group, whereas malondialdehyde level was the same as in the intact control group. The level of lipids, cholesterol and

triacylglycerides in diabetic group were significantly higher than those in the intact group. These values were slightly higher than the values in the intact group.

It was proven by these data that the levels of lipid peroxidation in diabetic rats was high, whereas there was a slight increase in the basal antioxidant enzyme activities. However, extract of *Sideritis taurica* may attenuate oxidative stress by enhancing antioxidant enzyme activities and decreasing lipid peroxidation level in experimental rats with diabetes.

35. Moonil Kim

"MOSFET-based Detection of p53 in Spiked Serum"

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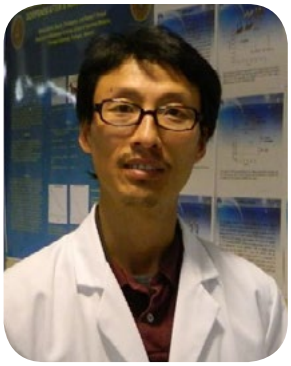

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Introduction: In recent years, much attention has been paid to FET-type biosensors due to their many advantages, which include miniaturizability, high signal-to-noise ratios, and fast response times. The n-type MOSFET with drain and source regions composed of doped phosphorus ions was employed for the monitoring of p53 in spiked serum.

Aim: The aim of this study is to test the abilities of wild p53 and mutant p53 in spiked serum to interact with the cognate DNA binding sequence using a MOSFET biosensor.

Materials and methods: An n-type MOSFET device was fabricated

in order to detect p53 protein. Hundred nM of p53 in spiked serum was added to the cognate DNA-coated gate surface. The evaluation of DNA-protein binding was conducted via the measurement of changes in the drain current.

Results: Upon treatment with the serum sample spiked with wild p53, the resulting current change in the drain was approximately ΔI = +51.2 uA. It is assumed that the results are related to the fact that wild p53 binds to DNA, and mutant p53 lacks DNA binding activity. Meanwhile, we found no significant potential difference in the drain-to-source current in response to the serum sample spiked with mutant p53; if any, it was negligible with a current change of ΔI = -4.9 uA, which is one order of magnitude smaller than that observed with wild p53.

Conclusions: FET-type biosensor might be promising for the monitoring of p53 in spiked serum on the basis of its DNA binding activity, providing us with very valuable insights into the monitoring for diseases, particularly those associated with DNA-protein binding events.

36. Raminta Mazėtytė

"pH Influence on Spectroscopic Properties of Glucose Oxidase"

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The usage of biosensing systems is promising - rapid and accurate method for detection and analysis of various compounds.

Nowadays one of the best-known and the most common biosensors are the glucose biosensors. They can accurately detect concentration of glucose in blood during a short period of time. The active part of the biosensor is a glucose oxidase (GOx) enzyme immobilized on the surface of the electrode. When constructing an enzymatic biosensor, one of the most important aims is to determine properties of an enzyme under different environmental conditions.

The purpose of this research was to evaluate absorption and fluorescence spectra changes in different acidity environments.

Spectroscopic properties of glucose oxidase (GOx) and flavin adenine dinucleotide (FAD) were investigated in different acidity environments. The study of the absorption and fluorescence spectra and the measurements of relaxation times were carried out using a buffer with pH values from 2 to 8.

The experimental data analysis showed that long component of FAD's and GOx's fluorescence decay dominated in the acidic environments. This long component of fluorescence decay is associated with the „planar" spatial configuration of FAD and the short component of fluorescence decay is associated with the „twisted" structure of FAD. In alkaline solutions of FAD, the ratio of a short and long component begins to change from 1:16 to 2:3.

During this study, it was found that at pH 3 solution acidity, the fluorescence intensities of FAD and GOx at 530 nm were the most intense. At the optimum pH (6) fluorescence intensity of GOx was the lowest. The increased intensity of the fluorescence band of GOx is associated with dissociation of FAD from the enzyme. Also, the increase of GOx fluorescence is due to the substantially increased fluorescence of FAD at the acid buffers (pH 3 - 4).

37. Ivan Reznikov
"Preparation and Characterization of Activated Carbon from Hydrolysis Lignin"

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Due to different environmental problems the search for new sorbents, as well as the development of new technologies is quite topical nowadays. The greatest interest is concentrated in large-scale production, such as, for example, wood processing and wood-chemical industry. This can be explained by two reasons. On the one hand, we deal with significant amount of waste. On the other hand — wood and its components are raw materials, that can be used for producing sorbents, wherein charcoal is already a unique sorbent.

The aim of this work is to study the conditions of preparation of activated carbon on the basis of hydrolysis lignin by characterizing it on different organic markers. Hydrolysis lignin produced by the Bobruisk's plant for biotechnologies (Belarus) was used. The moisture content of the sample was 7.0±0.4%. As activators, different inorganic and organic compound were used.

It was found, that initial size of lignin particle influences on the sorption capacity of methylene blue and iodine. The dependence is exponential and the lower the size, the higher the sorption capacity. For organic compound, atmosphere was found to be critical, due to the chemistry of the pore forming processes. As for ratio, certain correlation was found between the optimal proportion and maximum sorption capacity, wherein the ratio close in value to capillary-saturated state.

Different nature of pore forming process was observed for activated carbon, in which as activator NaOH and KOH was used. It was found, that increase in temperature from 6000C to 8000C affects positively on sorption capacity, whereas the increase in activation time affects negatively on chemical activation process.

The maximum sorption capacity of 321.30 and 334.17 were obtained for NaOH and KOH chemical activation respectively.

38. Joanna Stocka

"THF Pseudorotation Under FTIR Matrix Isolaton and CPMD Study"

JOANNA STOCKA

Tetrahydrofuran (THF, oxolane), cyclic ether with formula $(CH_2)_4O$, is widely used the industry applications despite its probable toxic effect to the environment. Nowadays THF is used as a precursor of biologically active molecules, as a monomer in polymerisation reactions, what is more THF might be treated as a simple model of deoxyribose in the DNA chain in electron interactions researches. The high-level ab initio calculations show that the global minimum of the THF molecule in a gas phase has an envelope (C_s) structure. According to the latest benchmark calculations the absolute energy difference between the global minimum (C_s) and the first local minima, the twisted conformation (C_2), is only 0,59 kJ/mol^{-1}. Such low differences between global and local minima suggest almost barrierless adaptation from one conformer to another, called pseudorotation, at finite temperature

The spectra were collected after 1 hour deposition of the THF:N_2

mixture (ratio 0.5:500 mba) in 9 K and after annealing (heating up to 25 K and cooling down to 9 K). Matrix isolation infrared absorption experiments reveled that both conformers are observable in the low temperature nitrogen matrices.

The dynamics of the model THF in N_2 system and the structural rearrangements between the local and global minima have been studied at finite temperature by Car-Parrinello molecular dynamics (CP-MD) simulations. CP-MD calculations proves that the global minimum structure is envelope conformer. We can observe transformation of the conformation from envelope to twisted and backward, what suggest that the nitrogen matrix might induce the pseudorotation even in such low temperatures.

39. Aistė Skeberdytė

"Targeting Colorectal Cancer Stem Cells by Combined Therapy with Ionophores and PDK Inhibitors"

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Cancer Stem Cells (CSC) have been identified as a minor subpopulation of a neoplasm which possesses the ability to self-renew, differentiate and initiate tumorigenesis. Emerging evidence indicates that this small subpopulation is the main reason of unsuccessful cancer treatment and tumor relapse.

The majority of chemotherapeutic agents currently available on the market target the rapidly-dividing cell cycle, which gives a "free-pass" to CSC due to their quiescent slow-cycling phenotype. This knowledge allows to raise a hypothesis that the most promising therapeutic approach to treat cancer is by combining two compounds with different cytotoxic mechanisms: one that targets specifically CSC and another that has a cell cycle non-specific outcomes.

The aim of this study is to investigate the effects of selective CSC inhibitor – salinomycin in combination with dichloroacetate (a pyruvate dehydrogenase kinase inhibitor) on the colorectal cancer cell lines and determine the optimal cytotoxic dose of these agents. Two colorectal cancer cell lines (DLD1 and HCT116) were selected to test the effects of these agents in the broad range of concentrations in monotherapy as well as in combined therapy. Following assays have been performed in order to investigate effects of this therapy:

- Cell viability was estimated by a standard MTT assay in 2D culture;
- Effects on 3D cell culture were assessed by growing spheroids on agarose;
- JC-1 assay was performed to evaluate the changes of transmembrane mitochondrial potential before and after the therapy;
- Protein docking analysis was used to discover potential protein-targets of this therapy.

40. Greta Musteikytė

"Optimization of SOD1 Aggregation Conditions *in vitro* and Impact of Environmental Factors to Aggregation Kinetics"

GRETA MUSTEIKYTĖ, Vytautas Smirnovas

Introduction: Amyotrophic lateral sclerosis (ALS) is a neurodegenerative disease that results in motor neuron death and has no approved treatment yet. Around 20% of ALS cases are caused by Cu, Zn human superoxide dismutase (SOD1) aggregation into amyloid-like fibrils. Wild type SOD1 is found to play the key role in the development of sporadic ALS (90% of ALS cases), as well as co-aggregated with mutant SODs in familial ALS (fALS). However, most of research done in this field is based on fALS-associated mutant SODs, while inherited type of the disease comprises to only 10% of ALS cases.

Aim: The aim of this research was to optimize conditions for SOD1 in vitro aggregation and test the impact of several compounds to aggregation kinetics.

Materials and methods: Recombinant his-tagged SOD1 was purified from *E. Coli* by Ni^{2+} affinity chromatography and dialysed against 50 mM EDTA to obtain apo form of the enzyme. Aggregation experiments were carried out in 10 mM potassium phosphate buffer solution, pH 7.4. Process of aggregation was monitored by measuring fluorescence intensity of ThT (fluorescent dye that specifically binds to amyloid-like structures) in Synergy H4 Hybrid Multi-Mode microplate reader.

Results: Optimal conditions to follow SOD1 aggregation process *in vitro* were found to be 0.2 mM monomeric SOD1, 0.5 M GuHCl, 5 mM DTT and 0.02 mM SOD1 in amyloid-like form (seeds).

Conclusions: Determination of optimal aggregation conditions leads to further investigation of enhancers and prospective inhibitors to SOD1 aggregation.

41. Martynas Simanavičius

"Production and Characterization of Monoclonal Antibodies against Yeast-Expressed Hepatitis E Virus Capsid Proteins "

MARTYNAS SIMANAVIČIUS, Paulius Lukas Tamošiūnas, Rasa Petraitytė-Burneikienė, Aurelija Žvirblienė, Reimar Johne, Rainer G. Ulrich, Indrė Kučinskaitė-Kodzė

Introduction: Hepatitis E virus (HEV) is one of the most common reasons of an acute viral hepatitis in the world. Diagnosis of HEV infection is usually based on a detection of virus specific IgM and IgG antibodies. However the assays currently in use have not been completely validated for a detection of different HEV genotypes. In Europe the most common HEV genotype 3 (HEV-3) is harbored by domestic pigs and wild boars and can be transmitted to humans and cause the disease. Recently discovered rat HEV is related but distinct from human pathogenic HEV genotypes. The role of wild rats as a reservoir in the transmission of HEV to humans and the zoonotic potential of rat HEV are unclear.

Aim: Production of monoclonal antibodies (MAbs) against full-length yeast-expressed HEV-3 and rat HEV capsid proteins (CPs)

in order to later develop sensitive and specific assays for studies of HEV seroprevalence.

Methods: The MAbs were generated by a classic hybridoma technology and characterized by immunochemical techniques.

Results: 19 hybridomas producing MAbs of IgG isotype were generated. The cross-reactivity of the MAbs with recombinant HEV proteins by an indirect ELISA and a Western blot was investigated. 6 MAbs reacted exclusively with rat HEV CP. 3 of them recognized conformational epitopes. 8 MAbs reacted only with HEV-3 CP and 5 recognized conformational epitopes. In contrast, 5 MAbs demonstrated a cross-reactivity with both proteins, with 3 of them being reactive with conformational epitopes. By a competitive ELISA five different MAb-binding sites within CPs of HEV-3 and rat HEV was determined.

Conclusions: The MAbs described in the current study could be useful tools for a development of IgM/IgG capture and competitive ELISAs for detection and differentiation of HEV-3- and rat HEV-specific antibodies.

This research was funded by the Lithuanian Science Council Grant No. MIP-039/2015

42. Rūta Mickienė

"Antifungal Effect of Essential Oil Vapours *Monarda Didyma* L., *Angelica archangelica* L., *Myrrhis odorata* L."

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Essential oils ubiquitous in plants are of considerable interest and have received more and more attention due to their bioactive functions. These components are known as secondary plant metabolites and possess antifungal properties. Essential oils obtained from *Monarda Didyma* L., *Angelica archangelica* L., *Myrrhis odorata* L. were tested for antifungal activity against *Aspergillus niger*, *Penicillium commune*, *Alternaria Alternata*, *Trichoderma viride*. The compounds estragole, thymol, pinene, terpinene-α, sabinene, myrcene, humulene-α, bergamotene-α, bisabolene-β, caryophyllene, anethole, germacrene, nerolidol and other compounds were tested for their ability to show antifungal activity. The antifungal activity of secondary metabolites in *Monarda Didyma* L., *Angelica archangelica* L., *Myrrhis odorata* L. originated from the sector of medicinal plants, botanical garden of Vytautas Magnus University Lithuania, were tested by the method of *Alvarez-Castellanos et al*. against different fungi species. The antifungal activities of essential oils were described by determination of the minimal inhibitory concentration. Antifungal activity against *Alternaria Alternata* was achieved with the essential oil dereived from *Monarda Didyma* L., at 27 × 10-3 mg/ml air concentration. Inhibition rates of *Angelica archangelica* L., *Myrrhis odorata* L.

were 35% at 50% ×10-3 mg/ml air concentration, respectively. The composition of oils was analysed by gas chromatography-mass spectrometry. Our results indicate that essential oils and components for *Monarda Didyma* L., *Angelica archangelica* L., *Myrrhis odorata* L. could be useful as control agent for some fungi. However, for the practical application of those oils and their single components as novel fungicides, further studies are necessary on the safety of these materials to humans and on the development of formulations to improve the efficacy and stability and to reduce cost.

43. Odeta Baniukaitienė

"Sodium hyaluronate modified with silicon dioxide particles"

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Introduction. Nowadays, great attention is focused on 3D scaffolds for bone tissue regeneration. A variety of materials are offered for the fabrication of the scaffolds. Significant attention is focused on natural polymers due to their biocompatibility, non-toxicity, hydrophilicity, and biodegradability. The aim of this work was to prepare 3D sodium hyaluronate-based scaffolds coated with SiO_2 particles for bone tissue regeneration.

Materials and methods. Sodium hyaluronate-based gel was prepared by crosslinking polymer in an alkaline solution by 1,4-butanediol diglycidyl ether and coating it with SiO_2 particles by tetraethoxysilane hydrolysis and condensation reactions. The obtained gel was lyophilized in the Christ ALPHA 2-4 LSC freeze

dryer. The morphology of the scaffolds was analyzed using a high resolution field emission scanning electron microscope (SEM) Quanta 200 FEG (FEI Company, Netherlands). Chemical composition of the scaffolds was determined using Ouantax EDS system (Bruker AXS Microanalysis GmbH, Germany).

Results. Sodium hyaluronate-based gel was lyophilized in selected conditions and the porous matrix was created ensuring space for vascularization and bone tissue formation. Small aggregates appeared on the surface of the scaffolds after tetraethoxysilane hydrolysis and condensation reactions. Following on, the coated scaffolds were analysed for elemental composition. The EDS spectra of the scaffolds revealed the presence C, O, N and Na as the main elements of sodium hyaluronate. Furthermore, in the spectra there was a strong Si atom signal revealing SiO_2 presence on the polymer surface.

Conclusions. Sodium hyaluronate-based scaffolds were successfully coated with silicon dioxide particles by tetraethoxysilane hydrolysis and condensation reactions. The morphology suitable for bone tissue regeneration was successfully created by the lyophilization of the gel.

VILNIUS UNIVERSITY

As the oldest and largest of Lithuania's higher education institutions, Vilnius University is an active participant in international scientific and academic activity and embodies the concept of a classical university – the unity of studies and research. Vilnius University has long been an integral part of European science and culture since its establishment in 1579. As one of the oldest higher education establishments in Central and Eastern Europe, it has had a marked influence on the cultural life of Lithuania as well as her neighbouring states.

One of the main aims of the university is to position and distinguish itself in European research and education with top-level research. Vilnius University has taken upon itself the responsibility for maintaining the highest level of research and studies – fulfilling the needs of the state and society for higher education. It has recently and significantly improved the university's infrastructure through active involvement in European structural funds' projects.

Today, Vilnius University has over 22,000 students and over 1,830 teaching and research staff. The university has 12 faculties, 7 institutes, 3 university hospitals and 4 study and research centres. It has one of the richest libraries in Europe, an astronomical observatory, a botanical garden and the cherished Church of St. Johns'. The university structure also embraces several museums, a dormitory campus, laboratories , workshops, summer resorts and student traineeship bases.

The university enjoys a unique academic atmosphere and academic freedom where priority is always attached to intellect, wisdom and tolerance. Vilnius University remains young, dynamic, progressive and open to the world's cultural and scientific values.

LIFE SCIENCES CENTER

The campus of Vilnius University at Saulėtekio Avenue was recently expanded by a new building of the Life Sciences Centre (LSC) covering the total area of 24 thousand square meters.

LSC operate on the basis of an agreement between the three academic branch units – Institutes of Biochemistry and of Biotechnology and the VU Faculty of Natural Sciences.

Activities of the LSC facilitate scientific research, studies and technological development in the fields of biochemistry, biotechnology, molecular biology, genetics, neurobiology, molecular medicine and other related sciences.

The LSC is a part of the 'Santara Valley' project. Together with the 'Sunrise Valley' project they both seek to stimulate a breakthrough in research development and the commercialization of research. Both projects have been initiated by VU in cooperation with other national institutions.

The 'Sunrise Valley' project concentrates on the research potential in the field of laser and light technologies, materials science, nanotechnologies, semiconductor physics and electronics; whereas the project 'Santara Valley' focuses on biotechnology, bio-pharmacy, molecular medicine, innovative medical technologies, information technology, ecosystems and safe environment.

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