# COINS

Conference of Natural and Life sciences.

2016

WWW.THECOINS.EU

# CONTENT

# ABOUT CONFERENCE

The Coins'16 - 11th 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, 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 conference will include:

- lectures of well-known and accomplished scientists;
- presentations of students' research;
- visits to Lithuanian scientific centers and companies;
- cultural and social activities: Welcome reception, national cuisines and dances, Closing event etc.

The Coins'16 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 11th International Student's Conference of Life Sciences (COINS) on behalf of Vilnius University and Faculty of Natural Sciences. 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 – Joint Center of Life Sciences, which is working just a few months.

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
Dean Faculty of Natural Sciences
Vilnius University

Dear participants of The Coins 2016,

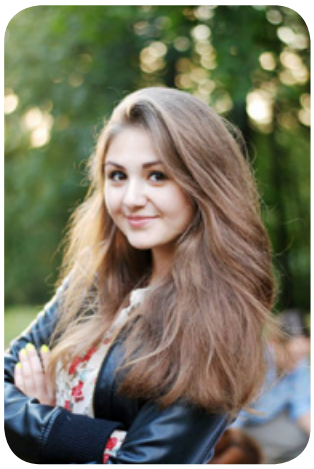
I would like to warmly welcome you all to the International Conference of Natural and Life Sciences. We are delighted to have students and scientists from Lithuania and foreign countries here in Vilnius, all gathered to discuss, learn and share a scientific experience.

This conference is organized for the 11th time and I am confident we'll only proceed further! As long as there are people like you – exceedingly passionate, committed to science and willing to share your research with the scientific community, we will continue to thrive and organize the conference.

The Coins 2016 team will do their best to provide you with an amazing opportunity to get acquainted with well-known scientists, meet new people and have an amazing time together.

I wish you interesting and thought-provoking discussions, an informative conference and a wonderful time spent together. Enjoy everything and take from this conference as much as you can!

So let us go deeper, seek wider and reach higher during The Coins 2016.



Best wishes,
Sabina Gračiova
The Coins 2016 coordinator

# AMBASSADORS

**Prof. Dr. Osvaldas Rukšėnas**
Lithuanian neurobiologist, biophysicist; Dean of Vilnius Univeristy, Faculty of Natural Sciences; Head of Neurobiology and Biophysics Departments; Professor of Biomedical Sciences; President of Lithuanian Association of Neurosciences.

**Juozas Rimantas Lazutka**
Head of the Department of Botany and Genetics of Vilnius University. Teaching Basic Genetics for undergraduate students in molecular biology and genetics, Genetic Analysis for students in genetics. Leading research projects on prostate cancer.

**Andrius Uždanavičius**
Vilnius University Students' Representation president, 3rd year student of Psychology.

# PROGRAMME

## FEBRUARY 29, JLSC (SAULETEKIS AVE. 7)

08:00 - 09:00 Registration/ Coffee break

09:00 - 09:30 Opening Ceremony

09:30 - 11:00 **Panel discussion**

*Personalized medical therapy: dream or reality?*

- Moderator: Mindaugas Zaremba *(Laboratory Member, Department of Protein DNA Interactions, Institute of Biotechnology, Vilnius University)*
- Ričardas Rotomskis *(Head of the Biomedical Physics Laboratory (2004) of National Cancer Institute & Professor (2000) at the Department of Quantum Electronics, Vilnius University, Lithuania.)*
- Giedrius Gasiūnas *(Laboratory Member, Department of Protein DNA Interactions, Institute of Biotechnology, Vilnius University)*
- Algirdas Žiogas *(Head of R&D, Stem Cell Research Centre, Northway Group)*
- Aurelija Žvirblienė *(Head of Department of Immunology and Cell Biology, Institute of Biotechnology, Vilnius University)*

11:00 - 11:45 Coffee break

11:45 - 12:30 **Keynote speaker presentation**

Urtė Neniškytė

*"Molecular signals for synaptic pruning in developing brain."*

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|---|---|
| 12:30 - 13:00 | **Keynote speaker presentation**<br>Dalia Daujotytė<br>*"RNA-Seq as an efficient tool for gene expression profiling."* |
| 13:00 - 13:15 | **Student presentation**<br>Amelija Melech<br>*"Sudden death from positional asphyxia. "* |
| 13:15 - 13:30 | **Student presentation**<br>Anastasiya Volokhova<br>*„Molecular-genetic Heterogeneity of MLL Rearrangements in Pediatric Acute Leukemia."* |
| 13:30 - 13:45 | **Student presentation**<br>Dovilė Barcytė<br>*"Coccomyxa - a dominant planktonic alga in two acid lakes of different origin."* |
| 13:45 - 14:00 | **Presentation**<br>iGEM |
| 14:00 - 15:00 | Lunch break |
| 15:00 - 15:45 | **Keynote speaker presentation**<br>Algirdas Žiogas<br>*"Angiogenesis and tissue-engineered scaffolds: future perspectives in regenerative medicine."* |

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| 15:45 - 16:30 | **Keynote speaker presentation**<br>Dina Petranovic<br>*"Studying Alzheimer's disease using yeast."* |
| 20:00 | Welcome reception |

## MARCH 1, JLSC (SAULETEKIS AVE. 7)

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|---|---|
| 08:00 - 09:00 | Registration |
| 09:15 - 09:45 | **Keynote speaker presentation**<br>Česlovas Venclovas<br>*"The logic of DNA replication in double-stranded DNA viruses: insights from computational analysis of viral genomes."* |
| 09:45 - 11:15 | **Company fair** |
| 11:15 - 12:00 | Coffee break |
| 12:00 - 12:15 | **Student Presentation**<br>Gintarė Grašytė<br>*Linking individual traits and demography with the avian colour polymorphysm* |
| 12:15 - 12:30 | **Student presentation**<br>Oksana Aliieva<br>*„Bacterial Response on Hydrocarbon Biodegradation in Presence of Electric Field."* |

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|---|---|
| 12:30 - 14:00 | Lunch break |
| 14:00 - 14:45 | **Keynote speaker presentation**<br>Giedrius Gasiūnas<br>„Mechanism and applications of *Streptococcus thermophilus* Cas9 protein.“ |
| 14:45 - 15:30 | **Keynote speaker presentation**<br>Danny Porath<br>*"The Quest for Charge Transport in single Adsorbed Long DNA-Based Molecules."* |
| 15:30 - 16:00 | Coffee break |
| 16:15 - 16:30 | **Student presentation**<br>Mikas Ilgūnas<br>*"The development of pathogenic malaria parasite in three experimentally infected common European bird species."* |
| 16:30 - 16:45 | **Student presentation**<br>Kristina Žalnieraitė<br>*"Evaluation of microbiological quality of drinking and public use water"* |
| 16:45 - 17:30 | **Keynote speaker presentation**<br>Mikael Kubista<br>*"Ultrasensitive Molecular Analyses in Research and in Routine."* |

## MARCH 2

## COMPANY VISITS

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| | National Food and Veterinary Risk Assessment Institute |
| | TEVA/Sicor Biotech |
| 08:00-18:00 | National Cancer Institute |
| | Lunch |
| | Thermo Fisher Scientific |

## MARCH 3, JLSC (SAULETEKIS AVE. 7)

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|---|---|
| 08:00 - 09:00 | Registration |
| 09:00 - 09:45 | **Keynote speaker presentation**<br>Sergio Bordel Velasco<br>*"Reverse engineering of evolved microbial strains."* |
| 09:45 - 10:30 | **Keynote speaker presentation**<br>Michael Knop<br>*"Visualisation of protein turn over and degradation and investigation of the ubiquitin-proteasome system using fluorescent protein reporters."* |
| 10:30 - 11:00 | Coffee break |

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| 11:00 - 13:00 | **Poster Presentation Session** |
| 13:00 - 14:00 | Lunch break |
| 14:00 - 14:45 | **Keynote speaker presentation**<br>Ehud Gazit<br>*"Nanotechnology at the forefront of the 21st Century: From Biological Structures to New Materials."* |
| 14:45 - 15:15 | Awards and Certificates |
| 20:00 | Closing Event |

# KEYNOTE SPEAKERS

## Urtė Neniškytė (Italy)

**Postdoctoral Marie Curie Fellow in Dr.C. Gross group, Mouse Biology Unit, EMBL**

**„Molecular signals for synaptic pruning in developing brain."**

The development of a complex nervous system is accompanied by a generation of superfluous neuronal connections that are removed when neural circuits mature. Why are so many synapses lost, what determines which synapses are eliminated, what are the molecular mechanisms involved, and what are the consequences of not getting it right? Synaptic pruning appears to be highly selective process that ensures selective elimination of some synapses and the maintenance of others. Structural and functional refinement of synaptic network is tightly related to the presence of brain immune cells microglia that actively contact and engulf unnecessary synapses. Aberrant or impaired microglial function leads to abnormal synaptic densities and dysfunctional connectivity that causes morphological, functional and behavioral deficits. For example, brain imaging and post-mortem studies suggest the role of deficient synaptic pruning in neurodevelopmental disorders, such as autism and schizophrenia. The reduction of brain volume and reduced density of dendritic spines in schizophrenia is suggestive of over-pruning, whereas increased brain volume and dendritic spine densities may indicate under-pruning in autism. Microglial phagocytic function has been implicated to have a central role in synaptic pruning; however, neuronal "eat-me" signal that discriminates weak and strong

synapses remains to be identified. Using organotypic hippocampal slice cultures and in vivo mouse models we investigate the role of phosphatidylserine as a neuronal surface signal that labels synapses for elimination thus ensuring proper brain development and circuit maturation.

## Dalia Daujotytė (Austria)

**Global Scientific Liaison Manager, Lexogen, Vienna, Austria**

**„RNA-Seq as an efficient tool for gene expression profiling."**

With the rapid development of Next Generation Sequencing (NGS) technologies, RNA--Seq has become the new standard for transcriptome analysis. Although the price per base has been substantially reduced, sample preparation, sequencing, and data processing still remain major cost factors in high--throughput screenings. The Lexogen's QuantSeq technology addresses these issues by providing an easy protocol to generate highly strand--specific NGS libraries close to the 3' end of polyadenylated RNAs within 4.5 hours, requiring only 0.1 – 500 ng of total RNA input and less bioinformatics efforts than standard RNA--Seq. It is the method of choice for fast, affordable and accurate gene expression detection, quantification or 3' UTR studies. The protocol can be readily modified for targeted sequencing and include molecular barcoding to detect PCR artifacts. In this talk we will briefly describe workflows and advantages of RNA--Seq, present QuantSeq technology and specify its advantages over the standard RNA--Seq for gene expression profiling and 3' UTR studies from users' case examples.

**Algirdas Žiogas (Lithuania)**
**Head of R&D, Stem Cell Research Centre, Northway Group**

## "Angiogenesis and tissue-engineered scaffolds: future perspectives in regenerative medicine."

Angiogenesis is intricate and complex multistep process, which mediates the formation of new blood vessels from existing vessels. These newly formed vessel networks are necessary for delivering oxygen, nutrients and metabolites to perfused tissues and also play essential role in tissue regeneration. Therapeutic angiogenesis has enormous potential for clinical application and is being investigated for multiple indications including heart repair, wound healing and numerous others. Recent breakthroughs in regenerative medicine including advances in stem cell technologies and design of innovative biomaterials enabled creation of complex vascularized tissue engineering constructs leading in turn to more advanced modeling of vascular pathophysiology. Currently, the greatest challenges are fabrication of pre-vascularized scaffolds by taking advantage of biomanufacturing techniques, such as design of engineering growth factors, smart biomaterials, cell-based approaches and development of relevant in vitro and in vivo models. We will also present data on the relevant infrastructure required to translate observations and preclinical data into efficient and cost-effective clinical outcomes.

**Dina Petranovic (Sweden)**
**Systems and Synthetic Biology, Department for Biology and Biological Engineering, Chalmers University of Technology, Sweden**

**„Studying Alzheimer's disease using yeast."**

**dina.petranovic@chalmers.se**

Alzheimer's disease (AD) is the most common neurodegenerative disease causing devastating dementia, and ultimately death. Amon other features, it is characterized by deposits of amyloid β-peptide (Aβ) in the amyloid plaques. The exact cause and mechanisms of neuronal death is unknown, and thus it is difficult to develop treatments. Yeast Saccharomyces cerevisiae can provide insights into mechanisms underlying cytotoxicity of Aβ. The Aβ1-42 peptide is the most toxic form of Aβ and shows most aggregation affinity. We constructed yeast strains with stable constitutive expression of Aβ peptides that were directed to the secretory system. When expressed from a constitutive (GPD) promoter and a low copy plasmid, Aβ1-42 caused a 10.7% decrease in growth. In comparison, Aβ1-40 was less toxic and the growth was reduced only by 2%, with the same expression system. When we measured the chronological life span (CLS) in stationary-phase, the Aβ1-42 displayed a drastically reduced ability to maintain viability and therefore a shortened CLS. We also find effects on oxidative stress, mitochondrial damage and respiration capacity.

## Česlovas Venclovas (Lithuania)

**Head of Department of Bioinformatics in Institute of Biotechnology of Vilnius University.**

**„The logic of DNA replication in double-stranded DNA viruses: insights from computational analysis of viral genomes."**

Genomic DNA replication is a complex process that involves multiple proteins. Cellular DNA replication systems are broadly classified into only two types, bacterial and archaeo-eukaryotic. In contrast, double-stranded (ds) DNA viruses feature a much broader diversity of DNA replication machineries. Viruses differ greatly in both the completeness and composition of their sets of DNA replication proteins. This overwhelming diversity raises a number of important questions. Are there patterns of DNA replication systems common to dsDNA viruses regardless of the cellular life forms (archaea, bacteria or eukaryotes) they infect? What is the relationship between viral and cellular DNA replication proteins? These questions cannot be addressed by experiments, but they can be tackled by computational methods. Taking advantage of the accumulated viral genomic data we performed a global computational analysis of viral DNA replication systems. Using sensitive state-of-the-art computational tools, we investigated the diversity and distribution of viral proteins associated with major molecular functions in DNA replication, including replicative DNA helicases, primases, replicative DNA polymerases and their accessory proteins, single-stranded DNA binding (SSB) proteins, nucleases for RNA primer removal, DNA ligases and topoisomerases. Our results show that some proteins are common to viruses infecting all domains of life and likely represent components of the ancestral core set. We also

discovered a clear correlation between genome size and self-sufficiency of viral DNA replication, an unanticipated dominance of replicative helicases and pervasive functional associations among certain groups of DNA replication proteins. Altogether, our results provide a comprehensive view on the diversity and evolution of replication systems in the DNA virome and uncover fundamental principles underlying the orchestration of viral DNA replication.


## Giedrius Gasiūnas

**Laboratory Member, Department of Protein DNA Interactions, Institute of Biotechnology, Vilnius University**


**„Mechanism and applications of Streptococcus thermophilus Cas9 protein."**

Clustered regularly interspaced short palindromic repeats (CRISPR) together with CRISPR associated (cas) genes form an adaptive prokaryotic immune system which provides acquired resistance against viruses and plasmids. CRISPR consists of arrays of short conserved repeat sequences interspaced by unique DNA sequences called spacers. The CRISPR-Cas system functions by acquiring short pieces of foreign DNA as new spacers and subsequently uses them as templates to generate specific small RNA molecules (crRNA) which combined with Cas proteins into effector complexes that trigger degradation of foreign nucleic acid. In Type I and Type III CRISPR systems, nucleoprotein complexes involved in crRNA-mediated silencing of foreign nucleic acids are comprised of large multisubunit aggregates. In Type II systems, the silencing complex consists of a single Cas9 protein, which

binds to crRNA:tracrRNA duplex to mediate sequence-specific cleavage of invasive dsDNA (1, 2).
We isolated the Cas9-crRNA-tracrRNA complex of the S. thermophilus CRISPR3-Cas system and demonstrated that it generates in vitro a double strand break at specific sites in target DNA molecules. DNA cleavage is executed by two distinct active sites within Cas9, to generate site-specific nicks on opposite DNA strands. Sequence specificity of the Cas9-crRNA-tracrRNA complex is dictated by the 42 nt crRNA, which forms a R-loop with target DNA (3). All together our data demonstrate that the Cas9-crRNA complex functions as an RNA-guided DNA endonuclease. The simple modular organization of the Cas9-crRNA complex, where specificity for DNA targets is dictated by a small crRNA and the cleavage machinery consists of a single, multidomain Cas protein, provides a versatile platform for the engineering of programmable RNA-guided DNA endonucleases. These findings pave the way for the development of novel molecular tools for RNA-directed DNA surgery (4–7).

**Danny Porath (Israel)**
**Etta and Paul Schankerman Professor in molecular biomedicine at the Hebrew University**

**"The Quest for Charge Transport in single Adsorbed Long DNA-Based Molecules."**

**danny.porath@mail.huji.ac.il**

DNA and DNA-based polymers have been at the focus of molecular electronics owing to their programmable structural versatility. The variability in the measured molecules and experimental setups, caused largely by the contact problem, has produced a wide

range of partial or seemingly contradictory results, highlighting the challenge to transport significant current through individual DNA-based molecules. A well-controlled experiment that would provide clear insight into the charge transport mechanism through a single long molecule deposited on a hard substrate has never been accomplished. In this lecture I will report on detailed and reproducible charge transport in G4-DNA, adsorbed on a mica substrate. Using a novel benchmark process for testing molecular conductance in single polymer wires, we observed currents of tens to over 100 pA in many G4-DNA molecules over distances ranging from tens to over 100 nm, compatible with a long-range thermal hopping between multi-tetrad segments. With this report, we answer a long-standing question about the ability of individual polymers to transport significant current over long distances when adsorbed a hard substrate, and its mechanism. These results may re-ignite the interest in DNA-based wires and devices towards a practical implementation of these wires in programmable circuits.

## Mikael Kubista (Sweden)

**CEO of Life Genomics AB and TATAA Biocenter.**

**„Ultrasensitive Molecular Analyses in Research and in Routine."**

In 2005 we were first measuring expression of several genes in a single cell. This initiated rapid development of ultrasensitive methods for analysis of biological samples both in research and in routine. In research these methods have been used to study heterogeneity of complex tissues and in tumors, activation on cell level in response to environmental changes, and to reveal mechanisms of asymmetric cell division, while in routine they have

led to non-invasive prenatal testing (NIPT), therapy monitoring of cancer through analysis of circulating tumor cells and cell free DNA, and most recently rejection after organ transplant. In my talk I will present single cell profiling techniques and examples of applications.

## Sergio Bordel Velasco (Sweden)

**Specialist in bioinformatics, Thermo Fisher Scientific, Vilnius**

**„Reverse engineering of evolved microbial strains, one example:"**

By performing an integrated comparative analysis on the physiology and transcriptome of four different S. cerevisiae strains growing on galactose and glucose, it was inferred that the transcription factors Bas1p, Pho2p, and Gcn4p play a central role in the regulatory events causing the Crabtree effect in S. cerevisiae. The analysis also revealed that a point mutation in the RAS2 observed in a galactose-adapted strain causes a lower Crabtree effect and growth rate on glucose by decreasing the activity of Gcn4p while at the same time is at the origin of higher growth rate on galactose due to a lower activity of the transcriptional repressor Sok2p. The role of Gcn4p on the trade-off effect observed on glucose was confirmed experimentally. This was done by showing that the point mutation in RAS2 does not result in a lower growth rate on glucose if it is introduced in a GCN4-negative background.

## Michael Knop (Germany)

**Professor at the University of Heidelberg (ZMBH), Faculty of Medicine**

**„Visualisation of protein turn over and degradation and investigation of the ubiquitin-proteasome system using fluorescent protein reporters."**

Protein homeostasis denotes the summary of all cooperative, competing and integrated processes and pathways in cells that regulate the synthesis, folding, transport, turnover and proteolytic degradation of proteins within and outside the cell. Central to this is the ability of cells to remove proteins upon 'request': Proteolysis can occur as a function of a cellular regulatory process, e.g. during cell division or signalling, and it can occur upon damage or misfolding of a protein, and can target individual proteins, protein species, protein-ensembles (e.g. aggregates or complexes), larger structures including entire organelles, depending on the needs, the state of the cell and the cellular processes. The ubiquitin-proteasome system (UPS) plays a pivotal role in the regulation of selective protein degradation and it is assumed that approx. 10% of all genes in the eukaryotic genome are involved in some of its processes. In this talk I will explain how we employ a novel type of fluorescent protein timer, the so-called tandem fluorescent protein timer (tFT) to visualise protein dynamics and turnover by fluorescence microscopy as well as high throughput plate assays, and how this enables us to systematically map the involvement of every component of the UPS in the degradation of the cellular proteome, with single protein resolution.

## Ehud Gazit (Israel)

**Chair of Nano Biology at the Tel Aviv University**

**„Nanotechnology at the forefront of the 21st Century: From Biological Structures to New Materials."**

Nanotechnology is one of the most fascinating fronts in modern science. The ability to control and manipulate molecules at the nano-scale paves the way for the discovery of new physical and chemical properties as well as exceptional technological applications. It is especially interesting to control the organization of molecular structures by the process of self-assembly in which systems spontaneously organized to form ordered and functional clusters. While most current industrial miniaturization efforts are based on top-down approach, biology is dominated by bottom-up molecular self-assembly events. Inspired from natural self-organization processes, we used minimalistic approach to identify the smallest building blocks of nature that can efficiently form ordered structures with defined architectures (nanotubes, nanopsheres, nanoplate, etc.) and unique mechanical, optical, electrical and piezoelectrical properties. Deposition methods were used to fabricate functional devices in the fields of ultrasensitive sensors, composite materials, energy storage and electrooptics. Another direction of research includes the manipulation of harmful self-assembly into nano-structures that are associated with degenerative human disorders. Nanotechnology provides the tool to develop new chemical entities that can control the formation of pathological molecular species.

# ORAL PRESENTATIONS

## Amelija Melech

## "Sudden Death From Positional Asphyxia."

**AMELIJA MELECH, Gerda Andriuškevičiūtė, Sigitas Laima, Algimantas Jasulaitis**

*Vilnius University, Faculty of Medicine*

**INTRODUCTION:** Death from positional asphyxia emerges because of external breathing suppression when the victim's chest is compressed or deformed. Gravitational and mechanical forces pressures diaphragm by the weight of abdominal organs. Raised diaphragm and difference between abdominal and breathing pressure hinders abdominal and chest breathing. The diagnosis is based on obstruction of normal gas exchange caused by the body position, impossibility to move to another position and exclusion of other causes of death. **AIM:** To present a case report and to rewiev clinical findings, forensic examination, management. **MATERIALS AND METHODS:** Case report description. **RESULTS:** A 51-year-old man was found dead in meadow under the trailer with stuck in the axis clothes from the back in semi kneeling position. During external examination subcutaneous hemorrhages, abrasions and parchment skin lesions on the back, which extended on both armpits and upper arms, squashed wound on the posterior surface of the left upper arm, were visible. Four linear intradermal hemorrhages on the left chest side were directed to the top. Clothing folds coincided with visible external injuries. Bilateral eyelids and conjuctival petechiae were noted. Sudden death signs were visible during internal

examination: petechial hemorrhage under the pericardium and pleura, heart cavities filled with dark liquid blood and clots, edematous lungs and brain. Hyperemia of internal organs were noted. No ethanol was found in the blood sample. The diagnosis of positional asphyxia due to fixed ribcage from the back, which caused suppression of external breathing, was established. **Conclusion:** Positional asphyxia is difficult to diagnose and is a rare cause of the sudden death. Diagnosis is based on circumstances of the incident, specific to asphyxia external and internal findings. Comorbidities may induce death during positional asphyxia. The forensic examination must be started at the incident scene, using special knowledge, which police officers do not have.

## Anastasiya Volokhova

### „Molecular-genetic Heterogeneity of MLL Rearrangements in Pediatric Acute Leukemia."

**VOLOKHOVA A, Stsiogantsava M, Pakhomova I, Valochnik A, Migas A, Kustanovich A**

Acute leukemia is a most widespread malignancy in childhood which results from a variety of alterations in numerous genes important for cell proliferation, differentiation, and cell death at molecular level. Identification and characterization of genetic rearrangements have been proved invaluable for appropriate diagnosis and prognosis, especially in acute leukemia. Rearrangements of the MLL gene (for Mixed Lineage Leukemia, also called KMT2A, ALL-1, HRX, and Htrx) located at chromosome locus 11q23 are commonly involved in adult and pediatric cases of primary acute leukemia and also found in cases of therapy-related secondary leukemia. Overall, 11q23 rearrangements are predictive

of poor clinical outcome, independent of their association with other high-risk clinical features. Analysis of rearrangements MLL gene has clinical significance for allocation of patients with leukemia risk groups in order to optimize the therapy and for minimal residual disease monitoring, i.e. determination of residual tumor cells and prevention of relapse of the disease. At the same time treatment outcome may differ in patients with the same type of MLL rearrangement and may depend on molecular-genetic heterogeneity of MLL-positive tumor clone. The aim of this work was to study the molecular-genetic heterogeneity of tumor clones in the MLL-positive acute leukemia and to evaluate its prognostic significance. To achieve the goal we used such methods as the reverse transcriptase multiplex PCR, semi quantitative real-time PCR, Sanger sequencing. During the work incidence of the most common clinically relevant rearrangements was evaluated, structure of chimeric transcripts involving MLL gene was analyzed and molecular genetic heterogeneity of the MLL-positive leukemic clone was investigated in pediatric MLL-positive acute leukemia.

## Dovilė Barcytė

### *„Coccomyxa* - a dominant planktonic alga in two acid lakes of different origin."

**DOVILĖ BARCYTĖ, Linda Nedbalová**
*Department of Ecology, Faculty of Science, Charles University in Prague, Czech Republic*

The aim of this study was to reveal the taxonomic position and phylogenetic relationships of the dominant planktonic algae in two acid metal-rich lakes (Hromnice Lake and Plešné Lake, Czech Republic) and to investigate their morphology and ultrastructure

under natural and laboratory conditions.
The phylogenetic analyses (18S rRNA and ITS-2) revealed that strain isolated from Hromnice Lake belongs to species Coccomyxa elongata, while Coccomyxa from Plešné Lake was described as a new species C. silvae-gabretae. It is the first evidence that specifically these species are capable to become the dominant phytoplankton alga in the extreme environment of acid lakes with increased supply of phosphorus. There were clear differences in cell morphology under different growth conditions, revealing high phenotypic plasticity of the strains. The ability to change the morphology may help the cells of Coccomyxa to survive harsh conditions in two acid lakes.

## Oksana Aliieva

### „Bacterial Response on Hydrocarbon Biodegradation in Presence of Electric Field."

**OKSANA ALIIEVA, Olena Matvyeyeva**

Bioremediation is proven to be an effective remediation technology for soils polluted by hydrocarbons. Nevertheless, this technique has some features needed to be addressed for successful application: (i) duration of the process and (ii) bioavailability of the pollutants. Electric field application can positively influence bioremediation process due to stimulation of microbial activity, increased mass transfer and better transport of hydrocarbons into microbial cells. Such process is named electrokinetically enhanced bioremediation (EKB). Microorganisms play a crucial role in bioremediation success. That is why it is necessary to understand possible responses of bacterial cells to the presence of electric field

and how this will affect biodegradation process in general. Electric field stimulates migration of bacteria in soil due to electrophoresis and electroosmosis. The electrostatic charge of the cell surface is a net charge resulting from the combined charges of the molecules comprising the cell surface and their counterions. This charge allows bacteria to move under the influence of electric field. Electroosmosis, in turn, causes movement of bacteria with general water flow. One more EK induced change in bacterial performance is associated with cell surface structure. While bacteria respond to exposure to an electric field physiologically, surface properties and even cell shape change. Electric current may affect the orientation of membrane lipids and consequently cell viability. A high electric current can cause irreversible permeabilization of the cell membrane and can even directly oxidize cellular constituents. The present study revealed that a weak electric current induced no significant changes in the cell surface properties of bacteria. However, exposure to DC of more than 20 mA could cause an increase in surface hydrophobicity, the flattening of cells, and the presence of exudate on the cell surface. Thus, it is of critical concern to look for a specific range (a so-called window) of electric current or field strength can be used for bacterial manipulation.

## Gintarė Grašytė

### „Linking individual traits and demography with the avian colour polymorphism."

**Gintarė Grašytė, Saulius Rumbutis, dr. Mindaugas Dagys, dr. Rimgaudas Treinys**
*Nature Research Centre*

Polymorphism - occurrence of several distinct forms within or among populations of species. Polymorphism involves various morphological, physiological and behavioural traits. Colour polymorphism is a widespread phenomenon in many animal taxa, and particularly in vertebrates. About 3.5 % of all avian species shows colour polymorphism and the occurrence of it is frequent in Strigiformes. We studied polymorphic Tawny Owl Strix aluco to test if polymorphism is adaptive and constantly maintained. Between 1985 and 2014, a Tawny Owls nesting in nestboxes was studied in Central Lithuania. Data on demographic variables were collected and nesting individuals were attempted to capture. Individual plumage colour in Tawny Owl varies from reddish-brown to light-grey, hence captured individuals were assigned to the one of the three colour forms (grey, intermediate and brown). Individuals of grey (41%) and intermediate colour forms (37%) were similarly frequent in the study area, while brown individuals were rarest (22%). Frequencies of colour forms were significantly sex dependent at study site scale as well significantly differed among sexes of mated owls. Females and males of different colours were similar in structural size and body condition during breeding phase. Different colour females laid nearly identical number of eggs. In the broods of the intermediate females, however, fledged slightly more juveniles compare to brown and nearly significantly more nestlings compare to grey females. Capture – recapture analysis results support importance of colour form to breeding frequencies: intermediate females breed more frequently (0.51) compare with the brown (0.29) and grey females (0.22). We also found weak support for colour form dependent survival. Survival probabilities were similar for brown (0.76) and intermediate (0.74), but lower for grey females (0.64). During three decades of study, subtle differences in demography of different colour individuals were translated to the population as frequencies of colour forms significantly changed.

## Mikas Ilgūnas

### „The development of pathogenic malaria parasite in three experimentally infected common European bird species."

**MIKAS ILGŪNAS, Dovilė Bukauskaitė, Vaidas Palinauskas, Tatjana Iezhova, Nora Dinhopl, Nora Nedorost, Christiane Weissenbacher-Lang, Herbert Weissenböck Gediminas Valkiūnas**

*Nature Research Center*

Species of avian malaria parasites (Plasmodium) are widespread, but their virulence has been insufficiently investigated. During avian malaria, several cycles of tissue merogony occur, and many Plasmodium spp. produce secondary exoerythrocytic meronts (phanerozoites), which are induced by merozoites developing in erythrocytic meronts. Phanerozoites markedly damage organs, but remain insufficiently investigated in the majority of described Plasmodium spp. Avian malaria parasite Plasmodium (Giovannolaia) homocircumflexum (lineage pCOLL4) is virulent and produces phanerozoites in Domestic Canaries Serinus canaria, but its pathogenicity in wild birds remains unknown. The aim of this study was to investigate the pathology caused by this infection in species of common European birds.

One individual of Eurasian Siskin Carduelis spinus, Common Crossbill Loxia curvirostra and Common Starling Sturnus vulgaris were exposed to P. homocircumflexum infection by intramuscular sub-inoculation of infected blood. The birds were maintained in captivity, and parasitemia was monitored until their death due to malaria. Histological sections of internal organs were prepared using traditional hematoxylin and eosin and inovative ISH methods. All exposed birds developed malaria infection, survived the peak of parasitemia, but suddenly died between 30-38 days post

exposure when parasitemia markedly decreased. Numerous phanerozoites were visible in histological sections of all organs, but they were easier recognisable in the same samples after ISH processing. Blockage of brain capillaries with phanerozoites may have led to cerebral ischemia caused cerebral paralysis and is most likely the main reason of sudden death of all infected individuals. Phanerozoites of Plasmodium homocircumflexum cause death of passerine birds due to marked damage of organs. Mortality was reported when parasitemia decreased or even turned into chronic stage, indicating that the light parasitemia is not always indication of improved health during avian malaria.We recommend application of traditional histological and ISH methods in parallel in investigations of exoerythrocytic development of avian malaria parasites.

**Kristina Žalnieraitė**

**"Evaluation of microbiological quality of drinking and public use water"**

*National Public Health Surveillance Laboratory*
*Microbiological testing department*
*Environmental testing subdepartment*

Underground water is the biggest source of fresh water in the World which takes over 97% of total fresh water resources. Microbiological water quality is a major concern for consumers, water suppliers and health organizations. The use of contaminated water couses a risk for human health and increases possibility of contracting infectious diseases. The aim of this study is to evaluate microbiological quality of drinking and public use water and to investigate contamination of such microorganisms as: coliform bacteria, intestinal enterococci, Escherichia coli, Pseudomonas aeruginosa, Legionella spp., Salmonella spp., Clostridium perfringens, somatic coliphages and its effect for public health.

Investigation of microorganisms in drinking and public use water was made using culture method for microorganisms detection and biochemical methods for identification. The data comparison and evaluation in accordance with applicable safety and quality standards. Pollution sources and microbiological identification of interdependencies. In 2015 were investigated 8471 of water samples. Total it is 20 000 of water researches. Of all the studies we get 1209 positive cases. Also it was investigated 53 positive cases of such pathogens as Legionella spp. Microbiological results analysis show that the count of coliform bacteria, Escherichia coli and intestinal enterococci exceeds the maximum allowable value of the amount in Lithuania HN 24: 2003 "Drinking water quality and safety requirements". The biggest deviations from the norms set out in samples from individual water supply systems, particularly in the dug wells, where the number of coliform bacteria exceeded the limits.

# POSTER PRESENTATIONS

## Agata Mlynska

### 1.„Development and characterization of drug-resistant ovarian cancer cell sublines."

**AGATA MLYNSKA, Egle Povilaityte, Karolina Zilionyte, Vita Pasukoniene**

**INTRODUCTION:** Photodynamic therapy is based on the excitation of cell-accumulated photosensitizer with specific light source, resulting in generation of ROS and free radicals which promote cell death. Clinically approved photosensitizing agents are used for treating cancer or microbial infections. We investigated the phototoxic activity of a novel tricarbocyanine indolenine dye TICS No. 150. Due to its red light absorbance, this dye emerged a promising agent for photodynamic therapy. However, its potential phototoxic antimicrobial effect was not explored yet.

**AIM:** We aimed to examine the phototoxic effect of TICS No. 150 in bacterial and cancer cells and investigate its mechanism of action.

**MATERIALS AND METHODS:** We performed a series of experiments on bacterial Salmonella enterica and eukaryotic prostate cancer cells, which were incubated with photosensitizer TICS No. 150 and irradiated with 720 nm laser diode light.

**RESULTS:** We found out that TICS No. 150 is phototoxic to Salmonella enterica cells in time- and dose-dependent manner. Mutant bacteria with shorter lipopolysaccharide in outer membrane are more sensitive to TICS No. 150 photodynamic effect. TICS No. 150 remains non-toxic until it enters the cell. The sensitivity of Salmonella enterica cells to the dye can be enhanced

by increasing the permeability of outer cell membrane. Moreover, TICS No.150 also showed considerable in vitro phototoxicity to prostate carcinoma cells in time- and dose-dependent manner. TICS No.150 acts by generating singlet oxygen, which causes cell damage and induces apoptosis.
**CONCLUSIONS:** TICS No. 150 is a novel and promising photosensitizer for phototoxic antimicrobial therapy, which is not toxic outside a bacteria and requires some time to reach its targets inside the cell. Lipopolysaccharyde is an effective barrier against photodynamic action of TICS No. 150.

## 2."Phototoxic Antibacterial and Antitumoral Activity of Carbocyanine Dye TICS No. 150."

**AGATA MLYNSKA, Simona Kavaliauskiene, Petras Juzenas, Dalia Kaskelyte, Roaldas Gadonas, Mikhail Samtsov, Aleksandr Lugovsky, Elena Bakiene.**

**INTRODUCTION:** Ovarian cancer is the most common cause of gynaecological-cancer-associated death. Surgery and adjuvant platinum-based chemotherapy remain the main treatment of ovarian cancer patients. Most of patients initially respond well to chemotherapy, however, more than half will relapse within 18 months of diagnosis due to platinum resistance. Drug-resistance is either innate or acquired. Small population of resistant cancer cells may already exist in tumor before treatment, or it may develop during constant treatment with chemotherapeutic drugs. Recently it is becoming clear that epithelial-mesenchymal transtion (EMT) may reflect an adaptation of cancer cells to survive cytotoxic drug activity and may be responsible for chemosensitivity.
**AIM:** We aimed to develop cisplatin- amd doxorubicin-resistant ovarian cell cell line A2780 and evaluate their morphology, cross-resistance and expression of EMT-related genes and proteins

**MATERIALS AND METHODS:** Resistant cell sublines were developed by incremental exposure of A2780 ovarian cancer cell line to either cisplatin or doxorubicin. Resistance and cross-resistance was confirmed by short-term toxicity assays. Relative expression of resistance- or EMT-related genes was measured by qPCR. Surface expression of EMT-related proteins was detected by flow cytometry.

**RESULTS:** Drug resistance was confirmed by the increase of the median lethal dose. A2780Dox appeared cross-resistant to both doxorubicin and cisplatin, whereas A2780Cis is resistant to cisplatin, but not doxorubicin. Both resistant cell lines develop mesenchymal morphology. Moreover, they start to express motility and invasiveness markers CD44, CD73, ESA. EMT-related gene and protein analysis does not fully confirm the occurence of EMT, but displays strong evidence of relation between EMT and acquired drug resistance.

**CONCLUSIONS:** Our results imply that inducing drug resistance in epithelial ovarian cancer cell line may promote EMT and may increase the aggressiveness and metastatic potential of resistant cells.

## Agnė Mataitytė

### "Anaplastic Thyroid Cancer: A Case Report."

**AGNE MATAITYTE[1], Erikas Laugzemys[1], Neringa Pranskeviciute[1,2]**

**Scientific research supervisor: MD. Prof. Virgilijus Beisa[1,2], dr. Anatolijus Ostapenko[2]**

*1.Vilnius University, Faculty of Medicine, Lithuania*

*2.Centre of Abdominal Surgery, Hospital Santariskiu Klinikos, Vilnius, Lithuania*

Introduction. Anaplastic thyroid cancer, though uncommon, is the most lethal of thyroid cancers. The best results are achieved by complex treatment – as far as possible radical surgery, aggressive chemotherapy (doxorubicin as golden standard) and radiotherapy. Mortality reaches 14 – 50%. Median survival rate is 3 to 9 months. This diagnosis is associated with fatal outcomes so treatment's goal is to improve quality of life. Aim. To present a case report on anaplastic thyroid cancer. Materials and methods. Case report description. Results. A 91 year -old woman complained of difficulty in breathing and swallowing, tightness in the neck. She was diagnosed with nodal hyperthyroid goiter 10 years ago, treated with metizole. However, above-mentioned trachea and oesophagus compression symptoms as well as pain occured, there was the risk of tracheal obstruction, skin ulcered as the node increased from 6x6x8cm to 9,8x6,6x13,2cm within 7 weeks. Cytology test before the surgery showed papillary thyroid carcinoma. Neoplasm was removed within the range of healthy tissues. The front right side of the neck muscles, 3cm of internal jugular vein and vagus nerve were also removed. Due to the related severe pathology of the patient, radiotherapy and chemotherapy were contraindicated. The patient died of cardiovascular failure 6 weeks after the surgery. Histological

examination of tissues removed during operation revealed: anaplastic thyroid carcinoma (10%) in papillary thyroid carcinoma (90%), pT4b (macroscopic extra-thyroidal tumor spread). Conclusion. The enlargement of the node made 65% growth ratio whereas the usual goiter growth ratio is estimated at 10 to 20% per year. Such rapid growth is extremely unusual, often results as a consequence of malignancy, ignorance, fear of a surgery, the elderly. Rapidly increasing thyroid node should always raise a suspicion of anaplastic thyroid cancer. Palliative surgery sometimes is an only option, taking into consideration patient's overall condition and age.

## Agnieška Mackoit

### "DNA Methylation Analysis of Angiogenesis-Related Genes ADAMTS12 and FILIP1L in Prostate Cancer."

**AGNIEŠKA MACKOIT, Kristina Daniūnaitė and Sonata Jarmalaitė**

Introduction. Several recent studies have suggested an association between hypermethylation of angiogenesis-related genes ADAMTS12 and FILIP1L and development of various types of cancer including prostate cancer (PCa). The development of novel diagnostic biomarkers' system could enable effective detection of early stages of prostate carcinogenesis. The aim of our study was to analyze promoter methylation of ADAMTS12 and FILIP1L genes of cancerous and non-cancerous (NPT) prostate tissues in order to identify potential epigenetic markers for PCa. Methods. Global DNA methylation profiling by means of microarrays allowed the identification of ADAMTS12 and FILIP1L

genes, which were characterized by aberrant DNA methylation changes in tumors as compared to NPT. Methylation status of the selected genes was validated in 130 PCa, 35 NPT and 17 benign prostate hyperplasia (BPH) samples by methylation-specific PCR. Results. ADAMTS12 and FILIP1L promoter methylation was detected in 109 of 130 (84%) PCa samples for each gene. In NPT, methylation frequencies of ADAMTS12 and FILIP1L were 2 of 35 (6%) and 4 of 35 (11%), respectively. These results showed significant differences of the methylation frequency in PCa versus NPT ($P < 0.001$) or BPH ($P < 0.001$) cases. According to pathological parameters, only methylation of ADAMTS12 gene was significantly associated with BCR ($P = 0.041$) or higher tumor (pT) stage ($P = 0.045$) and was close to significant ($P = 0.057$) in Gleason score 7 versus 6 cases. No significant correlations were observed between ADAMTS12 or FILIP1L methylation status and prostate-specific antigen level, tumor volume or patients' age. Conclusion. Our study revealed a significant difference in methylation frequencies of the ADAMTS12 and FILIP1L genes between prostate cancer and non-cancerous tissues. Further quantitative approach would be helpful to evaluate reliability of the aberrant methylation of these genes as biomarkers for that are able to accurately distinguish indolent and aggressive PCa tumors.

## Aliona Spakova

### "Cross-Reactivity Pattern of Novel Rodent- and Shrew-Borne Characterization and Study of Hantavirus Nucleocapsid Protein-Specific Monoclonal Antibodies and Their Cross-Reactivity."

**ALIONA SPAKOVA[1], Indre Kucinskaite-Kodze[1], Aurelija Zvirbliene[1], Rasa Petraityte-Burneikiene[1], Marc Mertens[2], Sabrina Schmidt[2], Mathias Schlegel[2,4], Bernd Köllner[3], Rainer G. Ulrich[2]**

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**INTRODUCTION:** Hantaviruses belong to the genus Hantavirus, family Bunyaviridae and are carried by small rodents, shrews, moles or bats. Humans are infected by inhalation of aerosolized excreta from infected rodents. Rodent-borne hantaviruses can cause two diseases, hemorrhagic fever with renal syndrome (HFRS) and hantavirus cardiopulmonary syndrome (HCPS) with a high case-fatality rate. The diagnostics of human hantavirus infections is usually based on serological assays. Monoclonal antibodies (mAbs) are important tools in viral clinical diagnostics, but also for virus detection in reservoir pathogenesis studies.
**AIM:** The study aims to characterize 9 different hantavirus nucleocapsid (N) protein-specific mAbs and determine their cross-reactivity pattern.
**MATERIALS AND METHODS:** 9 mAbs were raised against yeast-expressed N proteins of rodent-borne Puumala virus (PUUV), Dobrava-Belgrade virus (DOBV), Tula virus (TULV) and shrew-borne Thottapalayam virus (TPMV). The cross-reactivity patterns of the mAbs was investigated by a commercial immunofluorescence assay (IFA) as well as ELISA and Western blot analysis using yeast-expressed and purified hantavirus N proteins and control N antigens of Rift valley fever virus and Schmallenberg virus.
**RESULTS:** The ELISA and Western blot analysis of 9 mAbs showed different cross-reactivity patterns with N proteins of 15 hantavirus strains, but no reactivity with the two control antigens. The mAbs can be divided into several reactivity pattern groups. Most of the mAbs showed a broad cross-reactivity in ELISA, IFA and WB methods. However, two TPMV-specific mAbs reacted

exclusively with the homologous N protein used for immunization. The IFA investigation confirmed that the anti-PUUV, -TULV and –DOBV mAbs detected also native viral antigen in infected cells.
**CONCLUSION:** The reactivity of the mAbs with native viral antigen makes them promising tools for application in immunohistochemistry on reservoir-derived cryo-tissue samples and for diagnostic applications. Further investigations will have to find out if the mAbs can be used in a competitive ELISA format.

## Aušra Stumbrytė

### "Combined Effect of HPV and SNPs of TP53, MDM2, MDM4, MTHFR, CASP8 Genes in Lung Cancer."

**STUMBRYTE AUSRA, Plioplyte Raimonda, Kunickaite Agne, Gudleviciene Zivile**

**INTRODUCTION:** Lung cancer is the leading cause of cancer morbidity and mortality worldwide. Exposure to carcinogens are considered to be the main cause, genetic variation or HPV may promote the risk of developing lung cancer. HPV is a major risk factor for cervical, anal, vaginal, skin or laryngeal cancer. Infection is local, it develops only in epithelium. However, there were significant findings of HPV infection in breasts, prostate and lung cancers. These findings gave the idea of hematological or lymphogenical transmission of HPV. Main target of HPV E6 is TP53 gene. Degradation of TP53 is caused by ubiquitin protein ligase. MDM2 and MDM4 genes regulate activity of P53. SNP of MTHFR and CASP are related with HPV risk.
**AIM:** To detect correlation between HPV and mentioned genes in lung cancerogenesis.
**MATERIALS AND METHODS:** 88 lung cancer patients from National Cancer Institute were included in the study during 2013-2015 years. HPV screening in tumor samples was performed using PCR with

common Myo9/11 primers and RT-PCR Amplisens HPV screen Kit. We investigated the association between genetic variation of SNP: MDM2 (SNP309G>T), TP53 (Arg72Pro), MDM4 (SNP34091), MTHFR (SNPC677T) and CASP-8 (652 6N ins/del). Polymorphisms were detected by PCR using sequence-specific primers.
**RESULTS:** In 3 samples from 88 patients (3,4%) HPV was detected using RT-PCR. Number of copies varies from 0.98 to 5.12.
Studies of P53, MDM4, MDM2, CASP-8 and MTHFR genes showed results of common SNPs, they were as follow: Arg/Pro- 95,5%, T/T-51,1%, A/A-62,1, ins/del - 63,9%, C/C - 54,9%.
**CONCLUSION:** These results gave us the idea that HPV could spread to the lung epithelium. However, more sensitive tests for HPV detection must be applied.

## Birutė Armokavičiūtė

### "Sosnowskyi Hogweed (*Heracleum sosnowskyi* Manden.): Alellophatic Effect ofIsopsoraleneand8-Methoxypsoralene."

**ARMOKAVIČIŪTĖ BIRUTĖ[2], Butkienė Rita[2], Būda Vincas[2]**
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Sosnowsky's Hogweed is one of the most invasive alien species in Lithuania that rapidly and agressively inhabits the ecosystem by forcing out the local flora species. It is believed that the most hazardous components of Sosnowsky's Hogweed are either phenols (Baležentienė, 2015) or furanocoumarins (Burlėgaitė et al., 2012), which have already been reported as plant growth regulators and also be involved in interactions between plants. This present study aimed to quantative evaluation of isopsoralene and 8-methoxypsoralene that can be found in Sosnowsky's Hogweed leaves, roots and soil that surrounds the plant as well as

to evaluate the allelopathic effect of these compounds on growth and development of 6 model plant species. GC/MS method was used to account for the presence of isopsoralene and 8-methoxypsoralene in Sosnowsky's Hogweed leaves, roots and soil. These were extracted in diethyl ether, and ethanol. To check allelopatchic potential, bioassay was performed on seeds the following plants: Trifolium pratense (Red Clover), Achillea millefolium (Yarrow), Taraxacum officinale (Common Dandelion), Phleum pratense (Timothy-grass), Lolium perenne (Perennial Ryegrass), Festuca arundinacea (Tall Fescue) using water solutions of synthetic isopsoralene (0.02 mg/mL, 0.04 mg/mL) and 8-methoxypsoralene (0.01 mg/mL, 0.02 mg/mL). It was found that increased concentration of either isopsoralene or 8-methoxypsoralene caused the stronger inhibition both of seed germination and roots' growth. Less inhibition was recorded on Monocots rather than Dicots. The most sensitive to these furanocoumarins was T. officinale: isopsoralene at concentration of 0.04 mg/mL suppressed seed germination to 100%, and 8-methoxypsoralene at concentration of 0.02 mg/mL - appr. to 86%. After comparing isopsoralene and 8-methoxypsoralene solutions of 0.02 mg/mL concentration, it was determined that 8-methoxypsoralene cause the stronger inhibition rather than isopsoralene. These findings suggest that naturally occuring amounts of isopsoralene and 8-methoxypsoralene are sufficient for allelopathic effect. Further studies are still required and is in progress.

## Brigita Bartkutė

### "Optimisation of Recombinant Human Carbonic Anhydrase (CA) XIV Expression in *Escherichia coli* Cells."

**BRIGITA BARTKUTĖ, Vaida Juozapaitienė, Daumantas Matulis**

*Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Vilnius University, Lithuania*

**INTRODUCTION:** Carbonic anhydrases are zinc-containing

metalloenzymes that catalyse $CO_2$ hydration reaction. In human are twelve active isoforms of CA that differ in cell localisation and tissue distribution. CA XIV is found in human brain, heart, eyes cells membranes. Non-normal CA XIV expression is related with diseases like Alzheimer's, glaucoma, retinopathy, epilepsy. Therefore, CA XIV can be target for drug design. Large amounts of CA XIV are needed for drug research, but optimal conditions for obtaining it in E. coli is not evident.

**AIM:** The aim of present study is to determinate optimal conditions for production of soluble recombinant CA XIV protein in E. coli cells.

**MATERIAL AND METHODS:** Different lengths of CA XIV cDNA fragments were amplified by PCR and then cloned into pET15b vector with T7 promoter and into modified pET15b vector with tac promoter. Recombinant CA XIV proteins were expressed in Rosetta 2 (DE3), Rosetta-gami 2 (DE3), BL21 trxB (DE3) and Origami B (DE3) E. coli cells. Recombinant proteins expression was induced by 0.5 mM IPTG. Cells incubated at 16 $^0$C temperature, overnight. Target proteins purified from small scale culture by using IMAC with immobilized $Ni^{2+}$ ions. The amounts of purified proteins were measured using Bradford method. Proteins were visualised by SDS-PAGE.

**RESULTS:** Large amounts of recombinant CA XIV were expressed under control of T7 promoter. No considerable differences in level of expression were noticed after performing expression of different lengths of CA XIV under different promoters in chose E. coli strains. The average of purified target proteins from 1 g biomass in all E. coli strains under control of T7 promoter was 0.42 mg, under control of tac promoter – 0.32 mg. More pure recombinant CA XIV proteins were purified from Rosetta 2 (DE3) and Rosetta-gami 2 (DE3) E. coli cells.

**CONCLUSION:** The highest amounts of pure recombinant CA XIV protein was expressed in Rosetta 2 (DE3) cells by using pET15b-6H-CAXIV (16-280) construct, so these conditions can be used for large scale culture protein production.

## Cafer Eken

### "Polyphenol Oxidase Activity in Alfalfa Seedling Inoculated with *Rhizoctonia* spp."

**DUDU DEMİR, Cafer EKEN, Esra ÇELİK & Nurdan ALKAN**

*Department of Agricultural Biotechnology, Faculty of Agriculture, Süleyman Demirel University, Isparta, Turkey.*

Soil borne diseases cause significant losses on crop quantity and quality of many crop species annually. Rhizoctonia is a widespread and ecologically diverse soilborne fungus, causing different types of diseases in many plant species including alfalfa (Medicago sativa). Rhizoctonia species have been traditionally identified based on the cell nuclear condition (multinucleate, binucleate and uninucleated strains).

Polyphenol oxidases (PPOs) are ubiquitous copper-containing enzymes that are widely occurring enzymes among plants. PPOs are involved in the oxidation of polyphenols into quinones (antimicrobial compounds) and lignification of plant cells that contribute to the formation of defense barriers against pathogens.

The study was conducted with the aim to determine the effect of indigenous isolates of a multinucleate (Rhizoctonia solani AG-4) and nineteen isolates of binucleate Rhizoctonia on PPO activity in alfalfa (cv. Gea) seedling under in vitro conditions. The activity of PPO enzyme was determined in inoculated and uninoculated control alfalfa plants after ten days from inoculation. There was a significant increase in the activity of PPO after treatment of alfalfa seedling with isolates of Rhizoctonia. Among Rhizoctonia isolates, highest induction of PPO activity were recorded with pathogenic R. solani AG-4.

In presented study increased amounts of PPO were also observed in plants that were challenged with Rhizoctonia spp. PPO has a role in catalyzing phenolic oxidation in limiting disease development. PPO may therefore be involved in induction of defense resistance against plant diseases.

## Domas Linkevičius

### "Excitability Changes of a Model Hippocampal CA1 Pyramidal Neuron Under ACh Modulation."

**Domas Linkevičius, Bruce Graham, Aušra Saudargienė**

Even though the field of computational neuroscience has advanced leaps and bounds since its inception, the brain has proven to be one of the most difficult object to study so far. Increasing efforts have been put to model the structure of the brain at an ever-finer levels. One of the primary examples of such efforts, modeling of a neuron, is the subject of this study.

Our aim in this study was to model a hippocampus CA1 pyramidal neuron. However, while most model studies have generally focused on including the most prominent ion channels and synapses, we have also accounted for the modulation of ion channels by neurotransmitter acetylcholine (ACh). The effects of acetylcholine have been shown to be critical in consolidation of memory, prominent in reward circuits and Alzheimer's disease. In the present study we used a compartmental model of a hippocampal CA1 pyramidal neuron with ACh modulation. Based on the existing data, we have defined two ACh concentrations, high (10□M) and low (0.001□M), at which we ran simulations and measured both excitability and dendritic spine calcium concentration of the neuron. The model was realized using NEURON 7.4 modeling environment. We found that high concentrations of ACh significantly change quantitative behavior of the neuron, however some qualitative changes arise as well. Of the most important results, we report an increase in dendritic spine calcium influx as well as the number of spikes the neuron outputs in response to stimulation. These results show that, through the suppressory effects on such potassium currents as IAHP

and IA , ACh increases excitability of the model neuron. Furthermore, an increase in dendritic spine calcium influx confirms that ACh, in concert with other molecules, such as CaMII kinase, is essential.

## Dovilė Saulenaitė

### "Cobalt Influence on Barley Double Mutants' Germination, Florescence and Organ Development."

**DOVILĖ SAULĖNAITĖ**

**INTRODUCTION**: Cobalt is a heavy metal. Its amount in soil is increasing, due to growth of chemical, oil industries and other anthropological factors, therefore, it is important to research the effect of cobalt on barley (Hordeum vulgare L.), which is the fourth most used culture of monocots in the world.

**AIM:** Experiments were held in order to analyse the influence of cobalt on organ development and to identify changes in morphology and germination in different mutants and their hybrids.

**MATERIALS AND METHODS**: Single mutants Lemma hooded (Lh), Hooded (H), tweaky spike (tw2) (formed by chemical mutagenesis) and their hybrids N6, B6, N11, N13, N17, N19, N21 were used. "Auksiniai II" (AII) was used as a wild type. Kernels were soaked in cobalt(II) chloride solution (60, 120, 180, 300 mM). Barley were cultivated in the field and in the greenhouse. Morphological analyses were made using stereomicroscope Motic SMZ-143.

**RESULTS:** Germination was mostly corrupted in the Lh mutants. A significant increase of lodicule transformations and formation of ectopic structures were observed mostly in the N6, N11, N13 hybrids. Phenotypic spectre showed an increase in plants with small inflorescences and structures called "crowns" as well as those which have long internodes and varied leafy structures. Morphoses were found in Lh and H mutants. Pigmentless spots that negatively

correlated with the $CoCl_2$ concentration were observed in AII.

**CONCLUSION:** Germination is retarded and normal organ development is disrupted in barley after cobalt chloride exposure. Lemma hooded mutants and their hybrids are affected the worst probably due to the BKn3 gene duplication that leads to additional meristems. Interestingly no fatal changes were found even after the exposure to 300 mM $CoCl_2$. More research regarding cobalt absorbtion must be conducted.

## Dudu Demir

### "Characterization and Purification of Polyphenol Oxidase Enzyme from Apple Varieties in Eğirdir (Isparta), Turkey."

**Esra ÇELİK[1] & Dudu DEMİR[1],**

*1. Suleyman Demirel University, Faculty of Agriculture, Department of Agricultural Biotechnology, Isparta, Turkey*

Apples (Malus domestica) are one of the most popular fruit crops in the world. Apple and its derivatives are a great source of nutrients due to high levels of bioactive compounds. Polyphenol oxidase (PPO) is a group of oxidoreductase enzyme consists of copper at active center. PPO is also known as phenolase, tyrosinase, catecholase, catekoloxidase, o-difenoloxidase, mono phenoloxidases and kresolase. PPO catalyzed enzymatic browning reactions occurred in fruits and vegetables. Apple production in Turkey is take first place at Eğirdir district of Isparta province. Golden Delicious, Starking Delicious and Granny Smith apple varieties are more grown in Eğirdir (Isparta). In this study, PPO has been purified using the affinity gel comprised of Sepharose 4B-L-tyrosine-p-amino benzoic and characterized from these apple varieties.

KM and Vmax values of the purified enzymes were determined by

Lineweaver-Burk method. The values of Vmax/KM showed that Starking Delicious has the greatest PPO activity, on the other hand, Golden Delicious showed the least activity toward catechol substrate. The activity of PPO in the apple depends mostly on the varieties of apple.

The work was supported by the TÜBİTAK, Project No: 115Z794.

## Egidijus Šakalys

### "Peculiarities Of Sports Injuries Of Throwing And Sprint Events In Track And Field Athletics."

**EGIDIJUS ŠAKALYS, Linas Rekus**

**INTRODUCTION:** Track and field athletics is a popular sport worldwide. Sports injuries have an important impact to athletes sport and daily life. Sadly, but most of athletes don't have full recovery after some injuries, which leads to trauma recurrence or even higher severity injuries. There is distantly research on injury rate, prevalence, type and severity affecting athletes of track and field. Understanding injury profile, planning sports safety and choosing more beneficial treatment and rehabilitation for athlete, it is important to analyze the localization, severity degree, recurrence of injury.

**AIM:** Research aim is to analyze the most injuried body parts/ localizations, recurrence of same injury, chronic pain/unheald trauma/ unheald injury, severity degree and compare differences in sprint and throwing cohorts of track and field athletics.

**MATERIALS AND METHODS:** 33 athletes of track and field were given questionnaires developed using standardized methodology validated by the IOC and implemented by the IAAF during international track and field competitions.

**RESULTS:** There were 33 athletes and they had 57 traumatic injury cases. Results from study showed that in both events of sport the lower extremity injuries statistically significantly dominated comparing with

upper extremity, head and trunk. Injuries by anatomical region were: lower extremity 67%, upper extremity 12%, head and trunk 21%. Most of all were injured: hamstring 23%, inguinal 10,5%, lumbar 13% area. Analyzing the severity of the injuries, was noticed that moderate and mild injuries were dominating and interestingly low severity injuries appear to lead to a higher risk of reinjury than high severity.

**CONCLUSION:** Physicians, coaches and athletes of sprint and throwing cohorts preparing programmes of training must focuse on the lower extremity, especially hamstrings, as mostly injuried body part, to prevent future injuries.


## Gintarė Sauliutė


### "Heavy Metal Accumulation Patterns in Body Tissues of Ecologically Different Fish Species."


**SAULIUTĖ GINTARĖ[1], Svecevičius G.[1]**

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Fishes absorb metals directly from water via gills and from the diet via the gut (direct and trophic routes). The direct uptake route is most important because gills are the main target-organ for metal bioaccumulation and toxicity in fish. Majority of studies into metal toxicity and accumulation in fish are focused on the effects of particular metals. However, in the natural environment, fish are exposed to multimetal mixtures, which toxicity level as a rule differs from those of single ones as their impact can be additive, more than additive (synergistic), or less than additive (antagonistic). The purpose of the present study is to investigate competitive metal-metal interactions during the bioaccumulation process in the body tissues (gills, liver, kidneys and muscle) of ecologically different fish species at environmentally-relevant metal concentrations. In order to carry

out these objectives an investigation under controlled experimental conditions should be performed, concurrently using several fish species of a similar age and size, including those bioindicatory and commercially important (e.g. Atlantic salmon, roach and perch). We believe it is appropriate to use the mixture consisting of at least 5-6 metals, which are representative for the waters of a given geographic area and are officially regulated therein. Selected test-metals should represent all major categories: essential, non-essential, non-toxic and non-essential toxic. Exposure duration should be determined experimentally in order to reach stable-state. Comparative studies of individual metal and multimetal mixture exposures should be performed because under different conditions the metal uptake mechanisms involved may be different. Test-organs/tissues should be selected in accordance with their suitability and importance for conducting environmental risk assessment (ERA). Consequently, this study could inspire the improvement of ecotoxicologically-relevant water quality standards for metal mixtures covering both toxicological and bioaccumulation processes in aquatic organisms. This study is funded by Research Council of Lithuania, Project No. 108/2015.

## Goda Milinavičiūtė

### "Recombinant Production And Thermodynamics Of Inhibitor Binding To Human Histone Deacetylases 6 And 8."

**GODA MILINAVIČIŪTĖ, Justina Kazokaitė, Daumantas Matulis**

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**INTRODUCTION:** The histone deacetylases (HDAC) are enzymes that deacetylate lysine residues in histones as well as in several other non-histone proteins. The deacetylation of histones induces a condensed and transcriptionally inactive DNA. There are 18 HDAC

family members in human genome. They are divided into 4 different groups according to their catalytic pockets and mechanisms of action. Several HDAC isoforms, such as HDAC6, potentially play a role in neurodegenerative diseases and cancer. HDAC6 deacetylates histones and many other substrates. For example, HDAC6 was shown to take part in the microtubule network by acting as a specific α-tubulin deacetylase. HDAC8 is mostly expressed in smooth muscle and the function is related with muscle contractility. In addition, HDAC8 has been shown to be important for the growth of human tumor cell lines. Therefore, both HDAC6 and HDAC8 are potential drug targets.

**AIM:** The aim of my research is to produce recombinant HDAC6 and HDAC8 and investigate inhibitor binding affinity to them.

**MATERIALS AND METHODS:** The gene of human recombinant HDAC6 was cloned into four different plasmids containing N-terminal His-tag, C-terminal His-tag and N-terminal GST-tag. All of the constructs were expressed in various E. coli strains. Unfortunately, efforts to purify the recombinant HDAC6 by affinity and ion exchange chromatographies have not yet succeeded. Human recombinant HDAC8 with a C-terminal His-tag was expressed in E. coli strain and purified by affinity chromatography. The thermodynamics of interaction between HDAC8 and inhibitors such as Tubastatin A and Belinostat, which is clinically used for the treatment of cancer, were determined by the fluorescent thermal shift assay (FTSA). In addition, the thermal stability of HDAC8 was determined at various pH and in the presence of buffers and salts by FTSA.

**RESULTS**: Tubastatin A exhibited a dissociation constant of 14.29 µM for HDAC8. Belinostat bound HDAC8 with 7.69 µM affinity. HDAC8 was the most stable in sodium phosphate buffer at pH 6.0-7.0.

**CONCLUSIONS:** The recombinant human HDAC8 was successfully produced and characterized. The production of recombinant HDAC6 and the search for novel inhibitors are in progress. It is important for the design of new compounds with desired affinity properties.

## Ieva Rauluševičiūtė

### "DNA methylation of genes, selected by microarray analysis, in prostate cancer."

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**Kristina Daniūnaitė[1], Sonata Jarmalaitė[1]**
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Prostate cancer (PCa) is one of the most common type of cancer in Lithuania and worldwide. It is essential to focus on the disease diagnosis and treatment. Nowadays, the most popular test for PCa diagnostics is evaluation of prostate specific antigen (PSA) level in blood. However, it lacks specificity. Therefore, novel molecular biomarkers, like DNA methylation, are under investigation.
In the present study, a set of genes was selected from analysis of the global DNA methylation profiling by means of microarrays in 9 pairs of PCa and non-cancerous (NPT) prostate samples. Promoter methylation of the selected genes was investigated in 130 PCa and 35 NPT in total by qualitative methylation-specific PCR (MSP). Moreover, 17 benign prostate hyperplasia (BPH) cases were included as an additional control.

Genes CCDC181, PRKCB, NAALAD2, ZMIZ1 and NEK9 were methylated in 91%, 73%, 60%, 90% and 0% of PCa and 9%, 0%, 7%, 33% and 0% of NPT, respectively. The differences of the methylation frequencies between PCa and NPT were statistically significant ($P < 0.001$) for all analysed genes except NEK9. Aberrant promoter methylation of CCDC181 and PRKCB in PCa also differed significantly from BPH (0 % for both genes; $P < 0.001$). PRKCB and NAALAD2 were more frequently methylated in cases with biochemical disease recurrence ($P = 0.029$ and $P = 0.015$, respectively). Moreover, tendencies between methylation of PRKCB promoter and Gleason score or pathological tumor stage

(pT) were close to significant (P = 0.072 and P = 0.059, respectively). No correlations were observed between genes' methylation and PSA, tumor volume, prostate mass or patients age.

In conclusion, our study identified genes CCDC181, PRKCB, NAALAD2 and ZMIZ1 as novel biomarkers for diagnostic of PCa and prognosis of progression. Further quantitive methylation analysis in body fluids would substantially contribute to the evaluation of diagnostic potential of these biomarkers for noninvasive detection of PCa.

## Ieva Savickytė

### "Non-Syndromic Hearing Loss In Lithuania: Mutations And Variants In CDH23, TMIE, GJB2 Genes."

**IEVA SAVICKYTĖ, Violeta Mikštienė**

Hearing loss is one of the most common genetically inherited diseases. More than 50 per cent of hearing loss or impairment cases are congenital and most often these traits are inherited as autosomal recessive. To date, more than 70 genes were associated with non-syndromic hearing loss, including GJB2, which variants or mutations are now most frequently reported as the cause of autosomal recessive non-syndromic hearing loss (ARNSHL), and also SLC24A6, CDH23, TMIE, TMPRSS3 and other genes. The contribution of these genes to ARNSHL is strongly researched amongst various populations around the world. However, such a study has never been carried out in Lithuania before, therefore, this research aims to evaluate and describe the mutations and variants in a group of genes, including GJB2, TMIE, CDH23, found in 60 Lithuanian individuals with ARNSHL. To achieve this goal, we sequenced regions of these genes by Sanger sequencing. Until now, two variants (c.-35_-31delCGAGG (rs377387074)

and c.-35_-31insCGAGG (rs560521778)) were found in CDH23 gene, exon 1, which were previously reported, but not described. Either deletion or insertion was found in 43 individuals out of 60. Research is still undergoing with the hope to find more variants or mutations in other genes. Overall, it is important to conduct clinical studies related with hearing loss in Lithuania and new findings will possibly have implications on earlier detection of deafness in Lithuania, as well as further support evidence of the importance these genes have on hearing loss.

## Indrė Čeidaitė

### "The Evaluation Of Trophic State Of Balsys Lake (Lithuania) Utilizing Spectrometric Phytoplankton Analysis."

**INDRĖ ČEIDAITĖ, Virginija Kalcienė**

**INTRODUCTION:** The emerging eutrophication problem increasingly affects natural aquatic systems. The analysis of phytoplankton is widely used to assess trophic state of aquatic ecosystems. Phytoplankton pigment chlorophyll a only is often used in such researches. However variations of other photosynthetic pigments also can be used to analyze biological productivity and changes of community structure in aquatic systems.

**AIM:** The goal of this research was to evaluate the trophic state of Balsys lake (Lithuania) utilizing spectrometric phytoplankton analysis.

**MATERIALS AND METHODS:** Balsys lake was selected for this study, since it belongs to landscape reserve of Green lakes and is heavily used for recreation during summer. The ethanolic extracts of phytoplankton were analyzed according their absorbance and fluorescence excitation spectra. Chlorophyll a concentration where determined using spectrophotometric method (ISO 10260:1992) with minor changes.

**RESULTS:** It was determined that the mean concentration of chlorophyll a in Balsys lake was 2,03 µg/l. Such pigment concentration is characteristic for natural deep and mesotrophic lakes. The absorption and fluorescence emission values of samples showed seasonal and natural variability of phytoplankton biomass and species diversity in Balsys lake. The highest values of absorption and fluorescence were observed in autumn samples. The absorption and fluorescence emission peaks indicating pigment chlorophyll a were registered in all seasonal samples. The peaks connected mainly with pigment phycocyanin and chlorophyll c were determined spectrophotometrically in all samples, but weren't registered using fluorimetric technique. However the peaks, which are characteristic for phycoerythrin were found by both methods in summer and autumn samples.

**CONCLUSION:** The identified absorption and fluorescence emission peaks in phytoplankton of Balsys lake indicates main pigments, which are typical to phytoplankton groups (Diatomophyta, Cryptophyta, Dynophyta, Chrysophyta, Cyanobacteria) of mesotrophic lakes.


## Inga Šileikaitė

### "Investigation of biocatalyst synthesis encoded by metagenomic gene cluster."

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Biocatalysis (enzymatic catalysis) is described as the employment of enzymes to implement chemical transformations. This research field is increasingly applied in industry due to the benefits of enzymes in comparison with chemical catalysts, such as mild conditions of

reaction environment and high stereo-, regioselectivity [1].
The amount of various microorganisms and their species in nature is abundant, however only 1 % of microorganisms can be cultivated at laboratory conditions, what also limits the finding of new and novel biocatalysts. Thus, many efforts are directed towards development of new alternative methods for discovery of biocatalysts from nature. One of such alternatives is metagenomics, which is based on extraction of total DNA from natural sample and followed by construction and analysis of DNA libraries. It is an alternative method for investigation of non-cultivated microorganisms [2].
It is known that enzymes are encoded by single genes. Nevertheless, recent studies have indicated that enzymes could also be encoded by gene clusters. Gene cluster is described as group of related genes that are responsible for biosynthesis of biologically active molecules. Most studies of gene clusters are focused on discovery of pharmaceutical molecules, for example, glycopeptide based antibiotics [3]. However, gene clusters may play an important role also in biocatalysis.
The object of our study is gene cluster of metagenomic origin which has exposed lipolytic activity. Sequencing analysis of gene cluster revealed that it is composed of six genes. Current research is focused on development of proof of concept of evidence of gene cluster and it's functional mechanism. Functional analysis of separate genes and variants of gene groups on selective agar medium with tributyrin has indicated that only gene cluster as an entire unit is responsible for the defined lipolytic activity. More detailed data on investigation of gene cluster will be presented during poster session.

## Justas Dainys

# „Energetic Demand Of Eel Spawning Migration: Do Stocked Eels From Lithuania Acumulate Enough Energy Resources For Spawning Migration To The Sargasso Sea."

**JUSTAS DAINYS[1], Linas Ložys[1]**

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In a response to decline in eel stock many European countries are stocking eels to seas and inland waters in order to enhance the production of adult eels escaping to the sea for spawning migration. However, the degree of contribution to the spawning stock of these eels is poorly known. It is not known yet do stocked eels accumulate enough energy resources (lipids) for successful ~7500 kilometre long spawning migration from Lithuanian to Sargasso Sea. Migrating silver eels do not feed during migration; therefore they rely for their energy completely on fat stores which are accumulated during sedentary yellow eel stage in coastal or inland waters.

Current study was carried out to answer the question whether eels translocated from coasts of Western Europe and stocked in Lithuania accumulate enough energy for gonadal development and spawning migration. Fulton's condition factor is considered to be one of the measures of silver eel quality. Fish with higher condition factor are considered healthier, having more energy reserves for normal activities, growth and reproduction. However, results of our study suggest that Fulton's condition factor has no significant correlation ($p>0.05$) with fat content (%) in studied eels. This suggests that eels with higher Fulton's factor do not necessarily have accumulated more lipids. However, swimming potential and fat content significantly ($p<0.05$) correlates with ocular index (OI) and silvering stage according

to EELREP (2005). Average swimming potential of studied silver eels (stages S4, S5 and SMII) is estimated to be 7631 km (average 26.7%), while eels of the last silvering stage (SMII) had highest fat content (average 31.9%) and highest swimming potential (9046 km on average). The results of this study demonstrates that eels are able to perform spawning migration from Lithuania and cover more than 7500 kilometres in this way contributing to successful spawning and recruitment.

## Justina Kazokaitė

### "Fluorinated benzenesulfonamide anticancer inhibitors of carbonic anhydrase IX exhibit lower toxic effects on zebrafish embryonic development than ethoxzolamide."

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**INTRODUCTION:** The toxic effects of two recently discovered inhibitors (VD12-09 and VD11-4-2) that selectively and with extraordinary strong, picomolar, affinity bind to human carbonic anhydrase (CA) isoform IX were investigated on zebrafish embryonic development. CA IX has been recently introduced as an anticancer target since it is highly overexpressed in numerous human cancers but nearly absent in normal tissues.

**AIM:** to assess the toxicity in zebrafish of inhibitors VD12-09 and VD11-4-2.

**MATERIALS AND METHODS:** Morphological changes in zebrafish treated by the compounds were studied by light-field microscopy and histological analysis. Homology models of zebrafish CA II and CA IX were built to identify the conserved amino acid residues in the active site of zebrafish CAs.
**RESULTS:** The toxicity studies here showed that the LC50 values at 120 hours post-fertilization (hpf) were 13 µM for VD12-09, 120 µM for VD11-4-2 and 9 µM for ethoxzolamide (EZA), a non-selective CA inhibitor commonly used as a drug in clinic. Thus EZA was the most toxic of the three compounds. The zebrafish embryos exposed to LC50 doses of VD12-09 and VD11-4-2 showed fewer phenotypic abnormalities compared to the embryos exposed to the corresponding dose of EZA. Histochemical studies did not show any gross morphological changes in the embryos treated with VD12-09 and VD11-4-2 unlike EZA.
**CONCLUSIONS:** The results of our study indicate that the compounds exhibited 10-fold lower toxicity and induced fewer side effects in zebrafish than EZA. Therefore, the exposure to VD11-4-2 and VD12-09 at concentrations below LC50 did not lead to deleterious effects on the zebrafish embryonic development and thus both inhibitors may be further developed as drugs.

## Kristina Stuopelytė

### "Detecting Prostate Cancer: Urine-circulating MiRNAs."

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**Introduction:** Prostate cancer (PCa) is the second most prevalent oncologic disease and the fifth leading cause of cancer-related death among men world-wide. Prostate-specific antigen (PSA) screening for PCa diagnosis has high false positive and false negative rates. Therefore, there is a need to find novel more PCa-specific biomarkers to replace or at least to combine with PSA testing. Cell-free miRNAs circulating in bodily fluids might reflect the situation in tumour and serve as the non-invasive diagnostic or even prognostic tools.

**Aim:** The main purpose of our study was to analyse miRNA expression profile in PCa tissue in order to identify candidate miRNAs for non-invasive PCa detection in urine.

**Materials and methods:** Initial screening of 754 miRNAs was performed using TaqMan Low Density Arrays on 42 cancerous and 12 non-cancerous prostate tissues (NPTs). Levels of selected miRNAs were analysed in urine specimens from two independent cohorts of patients with PCa (N=215 in total) and compared with cancer-free controls (N=85 in total) by means of quantitative reverse transcription PCR.

**Results:** Over 100 miRNAs were found to be deregulated in PCa as compared to NPTs. 4 miRNAs were selected for the analysis in urine specimens. The abundance of miR-148a and miR-375 in urine was identified as the specific biomarker of PCa. The tandem of those miRNAs was highly sensitive and specific for PCa in both cohorts (AUC=0.79 and 0.84). Also, it strongly improved the diagnostic power of the PSA test (AUC=0.85) including the grey diagnostic zone (AUC=0.90).

**Conclusion:** Urine-circulating miR-148a and miR-375 can serve as the non-invasive biomarkers for sensitive and specific detection of PCa.

## Linas Kunigėnas

### "Cell culture models for investigation of stemness in cancer."

**Linas Kunigėnas, Vaidotas Stankevičius, Kęstutis Sužiedėlis**

**INTRODUCTION:** Colorectal cancer is one of the most frequently diagnosed cancers in the world. Despite advances in cancer treatment, conventional therapies cannot entirely control tumour tendency to recur and metastasize. To approach better treatment novel tumour models should replace traditional monolayer cell cultures that poorly represent tumour microenvironment in vivo. Current hierarchical cancer concept consider presence of cancer stem cells: undifferentiated, tumour inducing and therapy resistant cells. Thus it is important that modern cancer research models address stemness of cancer cells.

**AIM:** To compare the expression of stem cell and epithelial-mesenchymal transition (EMT) markers in colorectal cancer cell lines cultured between two-dimensional monolayer forming adherent conditions and three dimensional cultures grown in commercial protein mixture resembling extracellular matrix (Matrigel) or spheroids grown in suspension.

**MATERIALS AND METHODS:** Colorectal cancer cell lines DLD1 and HT29 were grown for experiments in aforementioned culture conditions. Total RNA were collected 2 and 6 days following seeding. Gene expression was evaluated using RT-PCR.

**RESULTS:** Spheroids grown in suspension showed more differently expressed stem cell and EMT markers than cells grown in matrigel compared to two dimensional cell culture. Also, stem cell and EMT gene expression was more robust 2 days after seeding, especially of EMT genes.

**CONCLUSION:** Following seeding of cancer cell line cells into three-

dimensional cultures, expression of stem cell and EMT markers increase. Greatest expression changes occur in three dimensional cell culture without attachment to extracellular matrix. Analysis performed indicates the potential of three-dimensional spheroid cultures to study stemness of cancer cells.

## Lukas Valančauskas

### Identification of plant protein interacting partners using proximitydependent biotin identification (BioID).

**Lukas Valancauskas, Kotryna Kvederaviciute, Irute Meskiene, and Alois Schweighofer**

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All life forms employ regulation of their biological processes on the molecular level. This regulation includes operation of proteins specifically acting in signaling pathways to trigger cellular responses. Components of cell signaling pathways interact with each other to transmit generated signals. However, these interactions are often transient, thus making the identification of the involved protein complexes technically challenging. Here we apply a method, previously reported for mammalian cells, that enables marking possible protein interactors in vivo in plant cells. This method employs the E.coli biotin ligase BirA to mark interactors/near neighbors of the BirA-protein fusion with biotin. Afterwards biotinylated proteins can be purified by affinity chromatography and identified using mass spectrometry

## Mažena Mackoit

### "First-Principles Calculations of Point Defects in h-BN."

**MAŽENA MACKOIT, Audrius Alkauskas**

Two-dimensional (2D) materials containing hole defects that are made by ejection of atoms from the sheet were extensively studied for many applications as atomically thin nanopores, which addressed possibility of hole defects as DNA sequencing, gas sensors and purifiers at lab-scale. It was shown that h-BN may exhibit superior durability and insulating properties in high-ionic strength solution compared with graphene and h-BN nanopore device for DNA sensor was created. h-BN band gap value was determined to be 5.955 eV by means of optical spectroscopy, so it is expected to host optically active defects that have ground and excited states within the gap. This year scientists were able to measure quantum emission from localized defects in h-BN monolayers even at room temperature. However, it is still not clear what defects are a probable source of the measured emission. Ab initio calculations, which are based on density functional theory (DFT), could bring a lot of valuable information about defects: they can predict whether a desired defect is likely to form in a given material and will be it stable once formed, etc. In this study we investigate boron vacancy, which is one of the dominating defects in h-BN. Boron vacancy defect has $C_{3v}$ point group symmetry. In neutral charge state, VB has 3 unsaturated dangling bonds. These defect states can be ionized by adding/removing electrons to it. Spinpolarized calculations were performed to examine neutral and positive charge states of this defect. Total spin states were determined and formation energies of these charged defects were calculated with ab-initio methods. Additionally first attempts to qualitatively estimate excited states were made.

## Milda Stankevičiūtė, Živilė Cibulskaitė

## "Nanoparticle And Heavy Metal Toxicity Mechanisms In Fish During Ontogenesis: An Interdisciplinary Project."

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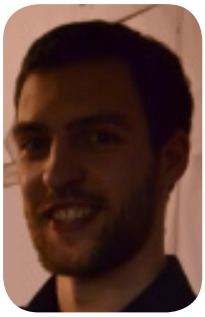
Aim of the project is complex using toxicological, genetic, cytological, physical, chemical, biochemical and mathematical methods to investigate nanoparticles (NP) and their constituent heavy metal (HM) toxicity on fish during ontogenesis, determine their stability,

access routes, distribution and accumulation (bioaccumulation) in fish at all development stages, determine the mechanisms of toxicity and accumulation in assessment metallothioneins (MT) expressing and to create NP and HM operation empirical model in fish during ontogenesis. During the study NP and HM consisting of them toxicity as well as toxicant access routes, their distribution and accumulation in the fish body tissues of different ontogeny will be compared. The investigation should determine the effective concentration of MT in fish tissues at different stages of development and biological evaluation of their role in the interpretation of NP and HM toxicity, as well as different

mechanisms of accumulation in fish body tissues during ontogenesis will be evaluated. In addition, clastogenic (DNA damage)/aneugenic (abnormal chromosome segregation) effects and elimination of cytogenetic damage by the apoptosis will be assessed. Summarizing the results of the research, empirical model of NP and HM operation in fish during ontogenesis will be developed. Implementation of the project will have a theoretical and practical significance, because it will help to solve NP and HM ecotoxicity and embryotoxicity problems not only in fish, but also in organisms of different phylogenesis, including humans. These results will throw the light how NP physicochemical properties are associated with their toxicity and genotoxicity to the body, and promote the safe production technologies of NP. This work is funded by Research Council of Lithuania, Project No. 108/2015.

## Mindaugas Binkis

### "Reprogramming and Carcinogenesis: Two Nuts of the Same Tree."



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Introduction: Induced pluripotent stem cells, also called iPSCs, might be obtained via viral transduction of four transcription factors – OCT3/4, KLF4, SOX2 and c-MYC. Most often this approach is associated with potential in clinical use. However the epigenetic modifications that occur during reprogramming might lead to cancer development. Aim: Identification of marker genes and pathways during the process of reprogramming likely related to the changes leading to the process

of carcinogenesis. Materials and Methods: iPS cells were derived from fetal human colon epithelial cells (FHC), using four transcription factors. Global gene expression was analyzed using Agilent whole human genome microarray, 4x44K. GSEA (Gene set enrichment analysis v2.2) tool was used to perform analysis of our microarray data. Results: Transcriptome analysis of iPSCs have shown that genes associated with cell cycle, DNA replication and spliceosome are the most enriched. In addition to this, analysis of transcription factors associated genes have shown enrichment of E2F family associated genes, such as MELK, NR3C2, DCTPP1 and HIRA. By performing a network construction of top enriched E2F family and functionally enriched genes, such as cell cycle associated, we were able to identify hub genes having the highest node degree and functional clusters, some of which containing both the hub genes and top enriched TF's gene – MELK. Conclusions: The results of this analysis marks possible pathways and genes responsible for the significant changes in cell cycle and more detailed insight into understanding the processes related to carcinogenesis which might occur during the induction of pluripotency.

## Olga Meščeriakova-Veliunienė

### "Female Mortality From Reproductive System Cancers In Lithuania: Critical Points In Time And Place."

**OLGA MEŠČERIAKOVA-VELIULIENĖ, Snieguolė Kaselienė, Ramunė Kalėdienė, Skirmantė Sauliūnė**

**INTRODUCTION.** Female mortality from reproductive system cancers in Lithuania is probably the highest among all European Union member states, and in Lithuania, these cancers are the leading cause of all cancer deaths in women. In 2014, deaths attributable to these diseases in the overall structure of female deaths due to malignant

tumors accounted for 33.28%.

**AIM.** To analyze the trends in mortality from reproductive system cancers among females aged 30 years and more and to determine urban/rural inequalities by cut points over the period of 1995-2014.

**MATERIALS AND METHODS.** Information on deaths from reproductive system cancers (ICD-10 codes C50-C58) among females aged 30 years and more in 1995-2014 was obtained from Statistics Lithuania. Mortality rates were age-standardized using the European standard. The regression model (jointpoint analysis) was used to identify the best-fitting points, wherever a statistically significant change in mortality occurred. Coefficients of regression multiplied by 100 were presented as average annual changes, which were considered statistically significant at $P<0.05$ level.

**RESULTS.** Female mortality from reproductive system cancers was 1.13 times lower in rural than urban areas in 1995 ($P<0.05$). Such a trend was observed until 2000 when mortality rates in urban and rural areas became equal, and in 2010, mortality in rural areas was 1.21 times bigger than that in urban areas. During 1995-2014, female mortality from reproductive system cancers decreased by 1.0% per year on average ($P<0.05$). Female mortality varied unevenly – there was one statistically significant cut point in 2011. Female mortality, declining by 0.69% per year on average during 1995-2011, in 2011-2014 decreased annually by even 3.96% on average ($P<0.05$). Changes in female mortality from reproductive system cancers in urban and rural areas during 1995-2014 were similar, showing a decrease of 1.23% and 0.61%, respectively, on average, and no statistically significant cut points were documented.

**CONCLUSION.** Female mortality from reproductive system cancers is decreasing, and an especially sharp decline was observed in 2011-2014. This allows making an assumption that the reproductive system cancer prevention programs for females running in Lithuania are effective.

## Povilas Matusevičius

## "Biocatalyst for the synthesis of polyol from natural oil: lipoxygenase expression in heterologous systems."

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Biocatalysis is the chemical process through which enzymes perform transformations of organic compounds. Enzymes are more proficient and environmentally friendly catalysts compared to chemical catalysts. Thus, industrial interest in various enzymes is constantly increasing. One of such industrially potential enzymes is lipoxygenase (LOX). It can be used in different industrial applications ranging from food processing to oleochemical production. Currently, the main commercial LOX application is co-oxidation of carotenoid pigments. In addition, LOX can produce fatty acid hydroperoxides, which could be used as precursors in synthesis of a wide variety of oleochemicals. LOXs are found in animals, plants, fungi and bacteria. However, currently only plant LOX is commercially available. Little research have been done on bacterial lipoxygenases, which features may differ from other LOXs. This shows importance of bacterial LOX investigation.

The aim of our work was to express LOX of bacterial origin in E.coli. We have extracted DNA from P.aeruginosa strain, which had LOX gene. LOX gene was amplified using PCR method, ligated with pLATE11 vector and transformed into E.coli DH5α and BL21(DE3) cells. Functional analysis on agar-starch plates indicated several clones with LOX activity. Analysis of protein expression by SDS-PAGE gel revealed that LOX was highly overexpressed, but in the insoluble fraction. Further research was focused on testing of other heterologous expression systems in order to obtain soluble enzyme.

## Raimonda Kubiliūtė

### "Epithelial-to-Mesenchymal Transition Contributes to Doxorubicin Resistance in MX-1 Breast Cancer Cell Line."

**RAIMONDA KUBILIŪTĖ,**
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Epithelial-to-mesenchymal transition (EMT) is a pivotal event in cancer progression as epithelial cancer cells lose cell-cell junctions and acquire mesenchymal phenotype and metastatic features. Moreover, EMT of tumor cells confers to the gain of cancer stem cell-related features and chemoresistance through elevated expression of drug transporters (ABCB1, ABCC1), drug metabolizing aldehide dehydrogenases (ALDHs), cytochrome P450s and other enzymes.

**Aim.** The study aims at analysis of alteration of gene expression and epigenetic regulation in doxorubicin resistant MX-1 breast cancer cell line.

**Materials and methods.** Research object was MX-1 cell line and doxorubicin resistant sublines: MX-1/D, MX-1/T, MX-1/TD where resistance was induced by doxorubicin (D), tetraphenylphosphonium (T) or both (TD). Global gene expression profiling was performed using gene expression microarrays. The alteration of individual genes expression was assessed by RT-qPCR. DNA methylation status was determined by methylation-specific PCR and pyrosequencing.

**Results.** All doxorubicin resistant cell sublines experienced EMT and gained expression of some mesenchymal cell markers, such as COL1A1, FN1, ALDH1A3, also cancer stem cell-specific marks, like CXCL8, FZD7, DLL1, and others. In addition, increased

expression of certain ABC transporter genes, especially ABCB1, and drug metabolizing enzymes, such as ALDH1A3, ALDH4A1, ALDH6A1, CYP51A1, was determined. Increased expression of ABCB1 was validated with RT-qPCR and from 190-fold to 4 million-fold higher expression level was established, while ABCC1 expression increased 5-fold only. Moreover, 2.5-fold increase in NANOG expression was found. Upon selection to doxorubicin resistance, DNA methylation changes have occurred in RARB, TERT, SOX2 genes. Pyrosequencing results revealed markedly decreased methylation intensity in ABCB1 promoter of MX-1/T and MX-1/TD sublines, but not MX-1/D. In addition, increased expression of methylcytosine dioxygenase gene TET2 in doxorubicin resistant cells was observed.

**Conclusion.** Epithelial-to-mesenchymal transition contributes to doxorubicin resistance in MX-1 cells. Decreased methylation intensity of ABCB1 may be related to increased expression of TET2.


## Rasa Šimanauskienė


### Tree Cover Fragmentation In Lithuanian Raised Bog Habitats During 20th Century


*Vilnius University*


Increased knowledge about peatland development and response to climate change are crucial issues as peatlands are globally important landscape elements containing enormous amounts of stored carbon. About 10% of Lithuania is covered by peatlands, of which 44% have been colonized by woody vegetation. Open raised bog habitats of European importance are under threat of extinction, because of anthropogenic pressure. Moreover, effective precipitation has been decreasing in the region, resulting in a hydrological regime less favourable for open raised bog habitats. It leads to one of the most relevant problems of modern times – the fragmentation of living organism habitats that is directly influencing the decline of the biodiversity. Therefore, the main aim of this study is to evaluate

the changes of tree cover fragmentation in raised bog habitats using remote sensing techniques.

The study of two raised bogs – Aukštumala and Rėkyva is based on analysis of historical maps, aerial photos and LANDSAT ETM images, covering the entire peatlands and their surroundings. The visual interpretation of aerial photos as well as the multispectral analysis of LANDSAT 7 satellite telemetrical data reflected the main tendencies of fragmentation process in studied areas.

The main results of the study show an increase of raised bog trees over the 20th century. Currently smaller areas (small mosaic) that are closely huddled next to each other dominate in average, comparing with former forest spatial structure (large mosaic) that existed before the drainage. Having analyzed changes in formation of woody vegetation in raised bogs, two periods of the establishment of tree cover are clearly distinguished: natural and anthropogenic. In particular the latter (anthropogenic) has accelerated dryness and overgrowth of open raised bog areas with woody vegetation. However, the final conclusions on causality for abundance of wooden vegetation are subject to assessing the potential impact of climate change on the raised bog habitats.

## Roberta Misgirdaitė

### "MiRNA Expression Profile of Osteogenically Differentiated Human Mesenchymal Stem Cells."

**ROBERTA MISGIRDAITĖ[1], Kristina Daniūnaitė[1] and Sonata Jarmalaitė[1]**

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**Introduction.** Mesenchymal stem cells (MSCs) have the ability to differentiate into multiple lineages and show strong regenerative properties. MSCs differentiation potential is determined by various

factors including microRNAs (miRNAs) which are a class of endogenous non-coding single-stranded RNA molecules of 18-24 nucleotides. MiRNAs play important regulatory roles in cell proliferation, differentiation, and development.

**Aim.** The main purpose of our study was to investigate miRNA expression profile of undifferentiated and differentiated into adipogenic and osteogenic lineage adipose tissue-derived (ADSCs) and synovial membrane-derived mesenchymal stem cells (SM-MSCs).

**Methods.** TaqMan-based Low-Density Array (TLDA) cards set (Life Technologies) and microarrays (Agilent Technologies) were used to analyze global miRNA expression profile of ADSCs and SM-MSCs, while selected miRNAs (miR-20a, miR-210, miR-335, and miR-4284) and PODXL gene, a predicted miR-4284 target, were investigated by real-time PCR (RT-qPCR). Cell transfections with miRNA-4284 inhibitor and mimic were performed to validate the association between miRNA and its target mRNA expression in undifferentiated ADSCs.

**Results.** Microarray results revealed 39 miRNAs ($P < 0.05$) deregulated during osteogenic differentiation in both ADSCs and SM-MSCs. Six out of 39 miRNAs were also deregulated in TLDA-based analysis. Expression changes of selected miRNAs were confirmed by RT-qPCR: miR-210 (13.8x) and miR-335 (38.7x) were down-regulated, while miR-20a (1.6x) and miR-4284 (2.0x) – up-regulated ($P < 0.05$) during MSCs differentiation. Hyperexpressed miR-4284 (598.4x , $P = 0.0075$) caused significant downregulation (3.5x , $P < 0.0001$) of its predicted target – the PODXL gene.

**Conclusion.** We identified essential miRNAs, which regulate stemness (miR-210 and miR-335) and differentiation (miR-20a and miR-4284) state of human MSCs. Also, we confirmed the PODXL gene as a target of miR-4284, possibly involved in osteogenic differentiation of MSCs.

## Roberta Valskienė

### "Environmental Genotoxicity Assessment in Chemical Munitions Dumping Zones in the Southern Baltic Sea."

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Environmental genotoxicity was investigated in herring (Clupea harengus) collected at 19 study stations located in environs of chemical munitions dumping zones in the southern Baltic Sea. The levels of micronuclei (MN), nuclear buds (NB), nuclear buds on filament (NBf), nucleoplasmic bridges (BNb) and blebbed (BL) nuclei in peripheral blood erythrocytes were used as genotoxicity endpoints. The frequencies of MN, NB and NBf in herring caught close to chemical munitions dumping zones were higher compared with those from the reference stations. The frequency of nuclear abnormalities (NAs) was distinctly increased in herring caught at five of eight stations in Gdansk basin and two of eleven stations in Bornholm basin. These stations were located close to chemical munitions dumping zones where fish evidently suffer from higher exposure to genotoxic pollutants.

## Rūta Kuleševičiūtė

### "Determination Of Total Phenolic, Total Flavanoid Contents And Antioxidant Activity In *Rhaponticum carthamoides* DC. Iljin Extraction Using Spectrophotometric Method In Different Vegetation Phases."

**RŪTA KULEŠEVIČIŪTĖ[1], Ona Ragažinskienė[1],**

**Violeta Bartkuvienė**[1]
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**Introduction:** Since ancient times people were using various herbs to heal illnesses, regain strength and improve health. One of many valuable herbs is Rhaponticum carthamoides (Rhaponticum carthamoides DC. Iljin) also known as Maral root, Leuzea carthamoides, Cnicus carthamoides. Rhaponticum is poorly examined, however researches which were made had shown that plant has lots of beneficial qualities.
**Aim:** To investige in which vegetation phase is the highest amount of total phenolic, total flavonoid content and antioxidant activity of Rhaponticum carthamoides DC. Iljin extractions using spectrophotometric method.
**Materials and method:** Rhaponticum (rootstock, blossom and leafs) was collected and prepared in Vytautas Magnus University Botanic Garden (2015april-june). There was analysed 5 phases: intensive growth, budding, blossoming, massive blossoming, ending of blossoming and frudification. For extract preparation it was used methanol (50%).Total amount of phenolic, flavonoid contents and antioxidant activity was determined by spectrophotometric method. For determination of total phenolics extracts samples were mixed with Folin – Ciocalteu reagent and sodium carbonate solution. For determination of total flavonoids extracts samples were mixed with hexamine and aluminum chloride solutions, acidify with acetic acid. Samples were measured at 760nm (phenolics), 407nm (flavonoids) and 515nm (antioxidant activity). Antioxidant activity was determined using the DPPH free radical method. We used standart calibration curve of rutin to quantify the amount of total phenolics, flavonoids and antioxidant activity. The data were processed using Microsoft Excel.
**Results:** The results show that the biggest amount of phenolics was found in blossoming (56.83mg/g, ($p<0.05$)).The highest amount of flavonoids and antioxidant activity was found in ending of blossoming

and frudification (34.05mg/g, ($p<0.05$); 87.11% ($p<0.05$)).The value of $p<0.05$.

**Conclusions:** This study showed that Rhaponticum has the biggest amount of phenolics in blossoming; flavonoids and antioxidant activity was found in ending of blossoming and frudification.

## Severina Marija Pociūnaitė

### "Analysis of Salicylic acid in *Arabidopsis thaliana*."

**Severina Marija Pociunaite, Kotryna Kvederaviciute, Alois Schweighofer and Irute Meskiene.**

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Plant immunity and pathogen resistance in plants is associated with accumulation of plant hormone salicylic acid (SA), which is controlled by mitogen-activated protein kinases (MAPKs). MAPKs play an important role in eukaryotic cell signalling, including plants, where MAPKs mediate plant responses to pathogens. MAPKs are regulated by protein phosphatases, such as Arabidopsis protein phosphatases of type 2C (AP2Cs), which dephosphorylate and thus inactivate MAPKs. Thus, AP2C may affect plant pathogen immunity, e.g. SA production, but this has not been studied so far. The aim of our study is to determine if AP2Cs control SA accumulation in plants. Usually, SA amounts are detected using HPLC technology, here, measurement of SA amounts produced by the Arabidopsis plants was adapted as bioassays using bacterial reporter – a simple and low cost method.

## Sima Garberytė

## "Evaluation of predictive markers in the ovarian cancer microenvironment and peripheral blood."

**Sima GARBERYTĖ[1,2], Agata MLYNSKA[2,3], Karolina ŽILIONYTĖ[2], Birutė INTAITĖ[4], Vita PAŠUKONIENĖ[2]**

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*3. Department of Biochemistry and Molecular Biology, Vilnius University*
*4. Department of Oncogynecology, National Cancer Institute*

**INTRODUCTION:** Ovarian cancer (OC) is the sixth most common cancer and the third most common cause of women's death from cancer in Lithuania. The main reasons for the high mortality of OC are late diagnosis and resistance to chemotherapy. Identification of new predictive markers and their use as anti-cancer therapeutic targets for ovarian cancer may optimize OC diagnostics and therapy.

**AIM:** To identify predictive stemness, resistance and immunosuppression markers in human ovarian tumor microenvironment as well as in the peripheral blood and to determine the interactions among them. MATERIALS AND METHODS: The study population consisted of samples from patients with advanced epithelial ovarian cancer (FIGO stage IIIC/IV). Data about PB lymphocytes, stemness and resistance marker expression were obtained using the LSR II flow cytometer (BD Biosciences). IDO expression was detected by ELISA method. Finally, the correlation among these characteris-tics was evaluated.

**RESULTS:** We observed differences in stemness, resistance and immunosuppression marker expression in patients. Our results showed that of all the tested markers immunosuppressive enzyme IDO was proved to be versatile, because its expression correlated with increased expression of MDR transporters ABCC1 and ABCG2,

with expanded population of immunosuppressive lymphocytes CD4+CD25+FoxP3+ and marker of stemness CD44.

**CONCLUSION:** There are significant relationships between different elements of immunosuppres-sion, stemness and drug resistance in ovarian tumors. Further investigations are necessary to vali-date the significance of the relationships in larger study populations and to prove the prognostic and predictive value of selected multi-biomarker panel.

## Sofija Semeniuk

### "The evaluation of ethylene and its biosynthesis pathway compounds' production in *Arabidopsis thaliana* by gas chromatography."

**Sofija Semeniuk[1], Kotryna Kvederaviciute[1], Ricardas Paskauskas[2], Alois Schweighofer[1], Irute Meskiene[1]**

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Ethylene is one of the many plant hormones which participates in plant's developmental processes such as mitosis, root and stem elongation, leaf abscission etc., as well as in immune responses to different biotic and abiotic challenges – these may be responses to pathogen infections of mechanical damage of the plant. It is synthesised from amino acid methionine, which is transformed to S-Adenosyl-L-methionine (SAM). SAM is then converted to an ethylene precursor 1-aminocyclopropane-1-carboxylic acid (ACC), which is in turn converted to ethylene by an enzyme 1-aminocyclopropane-1-carboxylate oxidase (ACO). The pathway of ethylene biosynthesis is regulated by mitogen-activated protein kinases (MAPK), specifically MPK6, which activates and stabilizes ACS through phosphorylation.

In this research, three Arabidopsis thaliana plant lines (aco, mpk6 and wt) were used to evaluate three different compounds which occur during ethylene biosynthesis – evaluate ACC concentrations, ACO activity and general ethylene production in 5 weeks old and 8 weeks old plants.

## Tomas Makaras

### "Behavioral response patterns in fishes exposed to polluting substances."

**TOMAS MAKARAS, Gintaras Svecevičius**

Behavior of certain fish species is a highly sensitive biomarker of environmental water pollution. Fish behavior parameters might be appropriate for assessing the ecological conditions of natural hydro ecosystems in general. A number of studies performed in order to evaluate effects of individual pollutants (e.g. heavy metals, organic and inorganic compounds) on fish behavior at lethal and sublethal levels. Actually, in natural environments fish are exposed to multicomponent pollution. Only a few studies have focused on determination of sublethal effects of complex effluents based on comparative analysis of behavioral parameters of various fishes under the controlled laboratory conditions. It is essential to investigate the effects of distinct multicomponent effluents consisting of mixture of contaminants at environmentally relevant concentrations. Moreover, fishes from different geographical regions can significantly differ in sensitivity to the same aquatic pollutants. Therefore, behavior of common fish of Lithuania (Roach, Perch, Dace, Three-spined Stickelback) should be investigated under the effect of multicomponent effluents. The obtained data will be compared with behavioral responses of rainbow trout (standard species commonly used in aquatic toxicity tests) and standard ecotoxicological test data (Daphnia immobilization tests ISO 6341: 2012). These studies will have great practical significance

in a development of biological early-warning-system (BEWS). Using fish behavioral response results under Lithuanian condition. This experimental study will be carried out according to the BEWS development and their application principles. Fish behavioral responses, sensitivity and appropriateness for the method of the prototype development for ecological and economical reduction should be conducted. The data obtained could help to solve many practical problems of aquatic toxicology related to fish behavioral test standardization according to ISO and OECD. This study is funded by Research Council of Lithuania, Project No. 108/2015.

## Vidmantas Vaičiulis

### "Dependence Of Morbidity From Cardiovascular Disease On Meteorological Factors."

**DR.VIDMANTAS VAIČIULIS,**
**Prof. Ričardas Radišauskas**

**Introduction:** Ambient air, climate, and geomagnetic activity play a crucial role in the earth life system, and public welfare, human health, epidemiologic conditions, etc. depend on these factors. Aim: To determine and evaluate the dependence of morbidity rates due to cardiovascular disease on sudden changes in meteorological parameters among Kaunas dwellers. **Materials and methods:** The study population comprised all Kaunas dwellers from 25 years, who were diagnosed AMI. We analyzed the parameters of mean atmospheric temperature, pressure, and minimal relative air humidity when the changes in them between two or more days in a row were an increase

or a decrease by ≥5°C, ≥10 hPa, and ≥15%, respectively. The association of sudden changes in meteorological parameters with AMI morbidity was evaluated by using a Poisson regression model.

**Results:** Evaluation of a ≥5°C decrease in the mean temperature between two (or more) adjacent days, showed that such a change in temperature increased the risk of AMI the most in the autumn and winter among women aged ≥65 years (1.6 times and 1.4 times, respectively). Women aged 55–64 had a 2.7-fold greater risk. After a sharp increase in air humidity by ≥15%, the greatest risk of AMI, i.e., 1.6 times, was determined in the spring among women aged 55–64 years. In the winter, the risk of AMI increased by 1.7 times, in the 25–54-year-old male group. Variation in the atmospheric pressure, the highest risk of AMI was documented in the spring among 55–64-year-old men (2 times greater) and in the summer among women aged more than 65 years (1.8 times greater).
**Conclusion:** Sharp changes in the meteorological parameters during a short period were associated with the greatest increase in the number of AMI events in the male and female groups from 55 years of age.

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# 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.

# VILNIUS UNIVERSITY JOINT LIFE SCIENCES CENTER

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

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

The JLSC 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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