

# The (Extra)ordinary **COINS 2021**



## Book of Abstracts

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# Foreword

## Dear Colleagues,

I am very happy to introduce The (Extra)ordinary COINS 2021!

The global pandemic, which is caused by SARS-CoV-2 virus, has a major impact on the scientific community and students. The past year challenged all of us to switch from normal everyday life to computer and internet dependent routine. Many aspects of our lives have gone online, which has become an enormous challenge for a big proportion of people.

As organizers of the conference, we faced many obstacles as well. However, this year we saw an opportunity for The COINS to unite Lithuanian scientific community across the globe. Amid the worldwide challenges and changes we gathered to discuss scientific victories and the strength of Lithuanian leadership in a global context of scientific research.



I am very glad that the challenges we are facing right now have opened plenty of opportunities for discussions and improvement.

Sincerely,  
Coordinator of The COINS 2021  
Daniel Šematovič

# The (Extra)ordinary COINS 2021



## February 13<sup>th</sup>, Workshop: How to Make the Best Poster?

The cycle of The (Extra)ordinary COINS 2021 started with a workshop on how to get ready for the poster session. Workshop consisted of theoretical and practical parts. During theoretical part laureate of multiple poster sessions Vilius Malūnavičius shared his experience and tips on making an outstanding poster and analysed some examples. Putting knowledge into practice, lecture was followed with a workshop that provided an opportunity for the participants to create a poster based on the tips shared in the first part of the event. All the questions during the workshop were answered by Jurgita Jurgelevičiūtė, Kotryna Čekuolytė and dr. Renata Gudiukaitė.

## February 27<sup>th</sup>, Conference: Leadership of Lithuanian Scientists in Global Context of Life Sciences

The second event of The (Extra)ordinary COINS 2021 – a virtual conference “Leadership of Lithuanian Scientists in Global Context of Life Sciences” that embraced the leadership of Lithuanian scientists in the field of life sciences.

Our goal was to give students the opportunity to get acquainted with Lithuanian scientists’ work and achievements. For this we had two outstanding lectures by Dr. Virginijus Šikšnys and Dr. Ingrida Olendraitė. Virginijus Šikšnys, the Chief Scientist and Head of the Department of Protein-DNA Interactions at the Vilnius University Institute of Biotechnology, presented how basic research in the mechanisms of CRISPR-Cas immunity springboarded development of genome editing tools for engineering biology and highlighted the future challenges and perspectives. Ingrida Olendraitė, a virologist and a postdoctoral research associate at the University of Cambridge, gave a lecture on the diversity of RNA viruses and their non-canonical molecular mechanisms.

Our second goal was encouraging the scientific community to discuss the development of science in Lithuania and the perspectives of Lithuanian scientists on a global scale. To achieve this we held a panel discussion, in which Dr. I. Olendraitė, Dr. V., Šikšnys shared their thoughts from the scientists perspective, then Dr. Gintaras Valinčius, biochemist and the Vilnius University Life Science Center director, and Agnė Vaitkevičienė, Executive Director at Lithuanian Biotechnology Association and the Chief Operating Officer at Cureline Baltic, added from the organisational university and business sides respectively, and finally the Director of Innovation and Industry Department in the Ministry of the Economy and Innovation of the Republic of Lithuania Ričardas Valančiauskas represented the Lithuanian Government position towards the encouragement and the leadership of the Lithuanian life sciences scientists.



**And now it's time for the long-awaited poster session!**

# Our Partners

## Turning ideas into innovations



Agency for Science, Innovation and Technology (MITA) is a national innovation agency, responsible for implementation of innovation policy in Lithuania.

To contribute to the ambitious goal for Lithuania, that is, to become one of the leading life sciences countries in the region by 2030, in October 2019 MITA launched a new initiative called Promotion of Life Sciences Industry Development. Although the Lithuanian life sciences sector is already well advanced, MITA aims to go beyond this and to further strengthen the great potential of this industry. The focus of this new initiative is to create favorable conditions for start-ups, to attract foreign investment and consolidate the position of Lithuania as a life science center in the international arena.



**Lina Kieselė, PhD**

Innovation manager



**Deividas Petrulėvičius**

Innovation consultant



**Kęstutis Andriulaitis**

Innovation consultant



**Rasa Kulvietienė**

Innovation manager



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Agency for Science, Innovation and Technology (MITA)

A. Goštauto str. 12, 01108 Vilnius, Phone: +370 (5) 26644708, E-mail: [info@mita.lt](mailto:info@mita.lt)

## 1. EFFECT OF IONIC STRENGTH ON THIOFLAVIN-T AFFINITY TO AMYLOID FIBRILS AND ITS FLUORESCENCE INTENSITY

Kamile Mikalauskaite<sup>1</sup>, Mantas Ziaunys<sup>1</sup>, Tomas Sneideris<sup>1,2</sup>, Vytautas Smirnovas<sup>1</sup>

<sup>1</sup>Institute of Biotechnology, Life Sciences Center, Vilnius University, LT-10257 Vilnius, Lithuania

<sup>2</sup>Department of Chemistry, University of Cambridge, Cambridge CB2 1EW, UK

[kamile.mikalauskaite@gmc.vu.lt](mailto:kamile.mikalauskaite@gmc.vu.lt)

Conformational changes in proteins and their aggregation in the form of amyloid fibrils have been implicated in many neurodegenerative disorders, such as Alzheimer's, Parkinson's diseases or mammalian spongiform encephalopathy [1]. Currently, there is a lot of research carried out in order to examine how aggregation of amyloid proteins occurs and how various environmental factors may influence the processes of protein aggregation. It is observed that temperature, pH, ionic strength and type of shaking change not only the kinetic parameters of aggregation, but also the conformational structure of the protein, thus forming fibrils of different strains [2,3]. The fluorescent dye thioflavin-T is one of the most widely used dyes in amyloid protein studies [4]. In this work, it was investigated whether ionic strength of the solution affects the binding of thioflavin-T to amyloid fibrils using absorbance and fluorescence spectroscopy.

Insulin (200  $\mu$ M protein dissolved in 100 mM NaCl 100 mM phosphate buffer (pH 2.4), incubation 24 h, 60  $^{\circ}$ C), lysozyme (200  $\mu$ M protein dissolved in 2 M GuHCl 50 mM phosphate buffer (pH 6), incubation 24 h, 60  $^{\circ}$ C, with shaking, using glass beads), mouse prion-protein (0.5 mg/ml protein dissolved in 0.5 M GuHCl 50 mM phosphate buffer (pH 6), incubation 24 h, 60  $^{\circ}$ C,  $\alpha$ -synuclein (200  $\mu$ M protein dissolved in PBS, incubation 24h, 60  $^{\circ}$ C, with shaking, using glass beads) amyloid fibrils were prepared. After aggregation, the fibrils were centrifuged, resuspended into distilled water and concentrated to 400  $\mu$ M). The absorbance of the samples was measured after mixing the fibrils, ThT and NaCl solutions (final protein conc. 100  $\mu$ M) in the range from 300 to 600 nm. Excitation-emission matrices (EEM) of each sample were then scanned using an excitation and emission range from 435 to 500 nm. The samples were then centrifuged for 10 min at 9000 rpm and the supernatant absorbance in the range from 300 to 600 nm was measured.

A direct dependence between the amount of ThT molecules bound to fibrils and the solution's ionic strength was observed. At higher ionic strength, more dye molecules are attached to the amyloid fibrils, which increases the sample fluorescence intensity (Fig. 1). Similar ionic strength effects were observed using all four amyloid proteins.

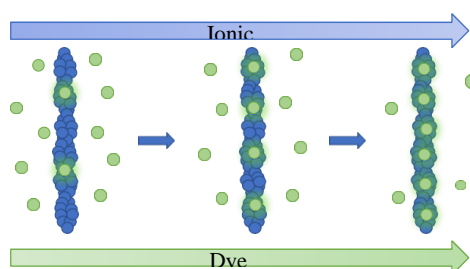


Figure 1. Higher ionic strength leads to increased fluorescent dye binding.

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## 2. STUDIES OF MIR-7, MIR-10B, MIR-30B AND MIR-181A EXPRESSION IN DIFFERENT GRADES OF GLIOMA

Rasa Kavaliauskaite<sup>1</sup>, Rytis Stakaitis<sup>1</sup>, Giedre Miniotaite<sup>2</sup>, Paulina Vaitkiene<sup>2</sup>

<sup>1</sup>Laboratory of Molecular Neurooncology, Neuroscience Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania

<sup>2</sup>Laboratory of Molecular Neurobiology, Neuroscience Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania

[rasa.kavaliauskaite@stud.lsmu.lt](mailto:rasa.kavaliauskaite@stud.lsmu.lt)

Gliomas are the most common primary malignant brain tumors in adults. Despite scientific advances and combination therapy, patients with glioblastoma, the most malignant grade of glioma, have a mean survival of less than 15 months [1]. As glioblastomas are highly invasive and heterogeneous tumors, biomarkers are needed for more accurate diagnosis and treatment. One such marker could be non-coding RNA molecules. There is a growing interest in micro-RNA, a class of the non-coding RNA. These molecules are only 19-25 nucleotides in length and have a high regulatory power - by inhibiting transcription and/or promoting the target gene mRNA degradation, they are involved in transcription, translation and cell signaling [2].

The aim of this study was to identify changes in the expression of short non-coding RNA (micro-RNA) in different grade gliomas and to evaluate their associations with clinical patient data. Micro-RNA molecules selected for study were: miR-7, miR-10b, miR-30b, miR-181a. All these molecules were studied in the patients' postoperative glioma tissues. MiR-7 and miR-30b were additionally studied in patients' blood exosome samples. For expression evaluation, RNA was isolated from glioma tissue and blood exosome samples, converted to cDNA, and amplified using a RT-PCR reaction. The calculated miRNA expression levels were assessed according to 4 parameters: glioma grade, patient sex, age group, and the overall survival time. Statistical analysis of quantitative data was performed using GraphPad Software Inc. Prism 8.0. The results showed that the expression levels of miR-7, miR-10b, miR30b, and miR-181a differed significantly between different grade gliomas. No links between miRNA expression and sex were observed. The expression of miR-10b, miR-30b, and miR-181a was different between age groups, and patient overall survival had a connection with miR-10b and miR-30b expression levels. Exosomal miR-7 and miR-30b expression levels had no significant links with the parameters mentioned above, and there was no correlation with both miR-7 and miR-30b expression in tumor tissues.

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### 3. GENE EXPRESSION IN HROG36 GLIOBLASTOMA CELLS EXPOSED TO TEMOZOLOMIDE (TMZ) UNDER DIFFERENT OXYGEN CONCENTRATIONS

Giedrė Miniutaitė<sup>1</sup>, Deimantė Kulakauskienė<sup>2,3</sup>, Zbigniew Balion<sup>2,4</sup>, Aistė Jekabsone<sup>2,5</sup>, Paulina Vaitkienė<sup>1</sup>

<sup>1</sup>Laboratory of Molecular Neurobiology, Neuroscience Institute, Lithuanian University of Health Sciences, Lithuania

<sup>2</sup>Laboratory of Pharmaceutical Sciences, Institute of Pharmaceutical Technologies, Lithuanian University of Health Sciences, Lithuania

<sup>3</sup>Department of Drug Chemistry, The Faculty of Pharmacy, Lithuanian University of Health Sciences, Lithuania

<sup>4</sup>Laboratory of Biochemistry, Neuroscience Institute, Lithuanian University of Health Sciences, Lithuania

<sup>5</sup>Institute of Cardiology, Lithuanian University of Health Sciences, Lithuania  
[giedre.miniutaitė@lsmu.lt](mailto:giedre.miniutaitė@lsmu.lt)

Glioblastoma is the most commonly diagnosed and aggressive type of brain cancer, accounting for 80% of primary malignant brain tumours of the central nervous system (CNS) and 60% of all brain tumours in adults [1]. It is characterized by low tumor oxygenation, a phenomenon named hypoxia, which is mainly the result of an increase of cancer cell proliferation that overcomes the limit of the blood supply [2]. Some studies have showed that hypoxia promotes resistance to drugs that usually target cancer cells in a proliferative state, maintaining cancer stem cells in a quiescent state [3]. In glioblastoma in vitro models, Ahmed et al. showed, under hypoxic conditions, an enhancement of resistance to cisplatin, temozolomide (TMZ) and etoposide [4].

The aim of this study was to investigate gene (BAD, HIF-1 $\alpha$ , etc.) expression in HROG36 glioblastoma cells which were exposed to TMZ under normoxia and hypoxia conditions. Firstly, HROG36 glioblastoma cells were grown in DMEM/F12 culture medium with 10% fetal bovine serum and 1% penicillin-streptomycin solution. To monitor the effects of temozolomide, the cell line was treated with 100  $\mu$ M of drug and incubated for 48 hours under normal - normoxia conditions in a 37°C and 5% CO<sub>2</sub> incubator and in the absence of oxygen - in a 37°C hypoxia chamber at up to 93% N<sub>2</sub>, 5% CO<sub>2</sub> and 2% O<sub>2</sub> gas. After two days of incubation, the cells were harvested from the culture dish, separated from the culture medium by centrifugation, and frozen at -80 °C for gene expression analysis. Then RNA isolation and cDNA synthesis were performed. Next, these cDNA samples were used in real-time PCR analysis. The obtained data was processed and its statistical analysis was performed.

The results showed that BAD was downregulated in hypoxia conditions. Also, BAD expression was higher in hypoxia-TMZ cells than hypoxia-control cells which shows that TMZ promotes this gene expression. Furthermore, BCL-2 gene expression was upregulated in normoxia-TMZ and hypoxia-control cells which shows that TMZ and hypoxia promotes this gene expression. However, this gene was downregulated in hypoxia-TMZ cells compared with hypoxia-control cells. Also, the results revealed that LAMP2 and PHLPP1 gene expression was downregulated in hypoxia-TMZ cells compared with normoxia-TMZ cells. In order to better understand the molecular mechanisms occurring in cells, more studies on the expression of genes involved in apoptosis should be included in further research, as well as their protein levels and possible modifications that regulate protein activity.

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## 4. DEEP BRAIN STIMULATION AND miRNA EXPRESSION ALTERATIONS IN PARKINSON'S DISEASE

Augustina Mišeikaitė<sup>1</sup>, Giedrė Miniotaitė<sup>1</sup>, Aistė Pranckevičienė<sup>2</sup>, Andrius Radžiūnas<sup>3</sup>, Vytenis Deltuva<sup>3</sup>, Paulina Vaitkienė<sup>1</sup>

<sup>1</sup>Laboratory of Molecular Neurobiology, Neuroscience Institute, Medical Academy, Lithuanian University of Health Sciences, Lithuania

<sup>2</sup>Laboratory of Behavioral Medicine, Neuroscience Institute, Medical Academy, Lithuanian University of Health Sciences, Lithuania

<sup>3</sup>Department of Neurosurgery, Medical Academy, Lithuanian University of Health Sciences, Lithuania  
[augustina.miseikaite@lsmu.lt](mailto:augustina.miseikaite@lsmu.lt)

Parkinson's disease (PD) is the second most common and progressive neurodegenerative disease with a mean onset age of 55 years. PD is characterized by a progressive loss of dopaminergic neurons. Deterioration of the dopaminergic system leads to severe motor symptoms including resting tremor, rigidity, bradykinesia and postural instability. However, non-motor symptoms of PD are recognized as being important for the impact on quality of life as well as motor [1]. Current treatments for PD afford symptomatic relief, but a disease modifying or neuroprotective agent capable of halting the progression of the disease is not yet available. The lack of a robust biomarker with high sensitivity and specificity has limited the progress towards the development of effective therapeutics for PD. Deep brain stimulation (DBS) has become widely used and is generally preferred over ablative procedures because of its adaptability and reversibility and the possibility of performing bilateral interventions [2]. However, deep brain stimulation raises concerns regarding infections and hardware complications, the implicit risks of surgery, some specific contraindications (eg. elderly age, psychiatric disorders), and the cost of the equipment, which puts it beyond the reach of many patients worldwide [3]. There is a need for molecular markers that could contribute to better treatment selection and outcome prediction. Some of these molecular markers could be miRNA [4]. The purpose of this pilot study is to determine the expression of miRNA in blood samples from patients with Parkinson's disease who have received Deep brain stimulation implantation surgery and check whether there are any differences.

The study protocol and consent procedure were approved by the Ethics Committee for Biomedical Research of the Lithuanian University of Health Sciences (LUHS). Written informed consent was obtained from each study patient before inclusion in the study. Consecutive patients admitted at the Department of Neurosurgery, Hospital of LUHS, Kaunas, for treatment of Parkinson's disease in a period from October 2015 until March 2021 were invited to participate in this study.

The expression levels of miR7, miR21, miR30b, miR155 and miR181a were evaluated by RT-PCR in 20 PD patients, who underwent DBS implantation surgery, samples and 27 PD controls. Statistical analysis was performed to find correlations between expression levels of exosomal miRNA in serum from PD patients before DBS implantation surgery (B-DBS) and after (A-DBS) and PD controls (PDC). The miR30b expression was significantly higher in A-DBS than in B-DBS and in PDC than in B-DBS ( $p < 0.05$ ). Also, the miR7 expression was significantly higher in B-DBS than in PDC ( $p < 0.0001$ ). However, the miR21, miR155 and miR181a expression was not significantly related to PD status before and after DBS implantation surgery and controls.

In conclusion, the different miRNA expression levels in PD blood samples showed patients heterogeneity and indicate a potential role of miRNA in PD pathogenesis, but the corresponding details require intensive research.

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## 5. THE DYNAMICS OF SIZE OF NISIN-LOADED PECTIN-CHITOOLIGOSACCHARIDES PARTICLES

Jolita Pachaleva<sup>1</sup>, Ruta Gruskiene<sup>1</sup>, Alma Bockuviene<sup>2</sup>, Jolanta Sereikaite<sup>1</sup>

<sup>1</sup>Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania

<sup>2</sup>Department of Polymer Chemistry, Vilnius University, Vilnius, Lithuania

[jolita.pachaleva@stud.vgtu.lt](mailto:jolita.pachaleva@stud.vgtu.lt)

Quality of food and nutritiousness take a valuable place in today's healthy life. Synthetic additives and various physical methods are commonly used to ensure a longer shelf-life of food products. Typical preservation methods have different side effects for food products and human health. New, natural antibacterial agents are promising alternatives for old preservation methods. Nisin is a natural small 3510 Da antibacterial peptide produced by *Lactococcus lactis* and is a commonly used natural additive for dairy and canned food products. This bacteriocin has the number E234 and is generally recognized as safe (GRAS) [1].

Other food additives, components and physical treatments during food processing affect the antibacterial activity of nisin. Various encapsulation methods expand nisin application in food and extend its activity in food products for a longer time. Biopolymers of different types and properties are used as a protective layer for bacteriocin [2]. The type of biopolymers and the number of layers affect the physical properties of the particles e.g. a size. The size of particles has an impact on the stability of particles [3].

This study is aimed to determine the dynamics of size distribution of nisin-loaded particles during the storage at 4°C. For nisin-loading, three different types of anionic pectin biopolymer, i.e. high methoxy pectin (HMP), low methoxy pectin (LMP) and pectic acid (PecA) were used. The complexation process was performed at different pH in the range of 4.0–5.0. Prepared complexes were additionally coated using different amounts of chitooligosaccharides (Fig. 1). The final concentration of nisin and pectin was 0.1 mg/ml and 0.2 mg/ml, respectively. The concentration of cationic chitooligosaccharides was in the range of 0.025–0.3 mg/ml.

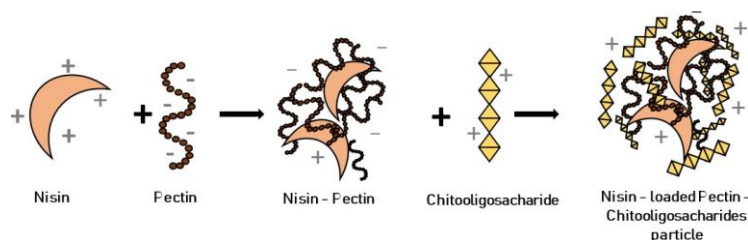


Figure 1. Nisin-loaded pectin-chitooligosaccharides particle

The dynamic light scattering method was used to determine the size of three-component particles. The measurements were made by using Zetasizer NanoZS device. Measurements were performed first day and every week for one month after complexation. The size of particles depended on the type of pectin and concentration of chitooligosaccharides, and the hydrodynamic radius was in the range of 110 to 330 nm. Generally, there was no significant change in the particle size over time. Natural nisin-loaded pectin-chitooligosaccharides particles can prolong the shelf life of food products without harmful effects on the quality and commercial appearance of the food products.

**Acknowledgements:** This research was funded by the European Social Fund under the No 09.3.3-LMT-K-712. "Development of Competences of Scientists, Other Researchers and Students through Practical Research Activities" measure. Grant No 09.3.3-LMT-K-712-22-0039.

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## 6. INVESTIGATION OF ENZYMATIC NANOTUBES DERIVED FROM BACTERIOPHAGE RAK2 SELF-ASSEMBLING PROTEIN GP041

Greta Labutytė<sup>1</sup>, Vytautas Petkevičius<sup>1</sup>, Algirdas Noreika<sup>1</sup>, Rūta Stanislauskienė<sup>1</sup>, Rolandas Meškys<sup>1</sup>, Vida Časaite<sup>1</sup>

<sup>1</sup>Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Saulėtekio av. 7, Vilnius, Lithuania  
[greta.labutyte@gmc.stud.vu.lt](mailto:greta.labutyte@gmc.stud.vu.lt)

Controlled self-assembly systems based on viruses or virus proteins are applied for a variety of applications, such as the development of biosensors, manufacture of nanoelectronic materials and vaccines, gene and drug delivery devices, and tissue engineering [1]. The binding of enzymes to virus-based particles is an attractive method for the development of enzyme nano-carriers. The surface of the majority of bacteriophage tails (a contractile structure employed for recognition and attachment to a host cell) is composed of tail sheath proteins. These proteins can self-assemble both in vivo and in vitro into tubular structures of variable lengths called polysheaths [2].

We aimed to construct novel nanostructured material based on a self-assembling tail sheath protein gp041 from the Klebsiella-infecting bacteriophage vB\_KleM-RaK2. Nanotubes were constructed by genetically fusing amidohydrolase YqfB [3] from Escherichia coli to the C-terminus of the truncated gp041 protein with a flexible linker. Constructed chimeric protein was successfully synthesized in E. coli cells and in vivo self-assembled into nanotubes. The modeling studies show that the C-terminus of gp041 and thus the fused YqfB is directed to the inner space of the tube being formed. The transmission electron microscopy analysis showed that the length of the chimeric nanotubes (the length ranged from 130 to 490 nm) was similar to the length of ones formed by the unmodified gp041, and the diameter of the chimeric nanotubes ( $50 \pm 2.7$  nm) was about 10 nm larger than that consisting of an unmodified gp041. Remarkably, chimeric nanotubes possessed an enzymatic activity that was confirmed by hydrolysis of N4-acetyl-2'-deoxycytidine to 2'-deoxycytidine. The specific activity of chimeric nanotubes, pH optimum, and temperature stability was comparable to that of free YqfB. In conclusion, the results indicated that gp041-based hybrid proteins self-assemble in vivo into the catalytically active nanotubular structures.

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# 7. RESPONSES OF BIOCHEMICAL BIOMARKERS IN BIVALVE MUSSELS (*UNIO PICTORUM*) FROM NEMUNAS RIVER (LITHUANIA)

Reda Nalivaikienė<sup>1</sup>, Virginija Kalcienė<sup>2</sup>, Aleksandras Rybakovas<sup>1</sup>, Veslava Jakutaitė<sup>2</sup>, Laura Butrimavičienė<sup>1</sup>

<sup>1</sup>Institute of Ecology, Nature Research Centre, Lithuania

<sup>2</sup>Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania

[reda.eglinskaite@gamtc.lt](mailto:reda.eglinskaite@gamtc.lt)

The River Nemunas is under a major threat of contamination related to municipal and industrial effluents, as well runoff from roads, urban and agricultural areas [1, 2]. In order to evaluate the impact of pollution on the ecosystem, various biomarkers are being used as sensitive early warning tools. Due to widespread distribution, sedentary lifestyle, filtration capacities leading to bioaccumulation of contaminants freshwater bivalve mussels are good bioindicators, which perfectly represent environmental status [3]. Bivalve mussels of the family Unionidae are important components of river ecosystem [4]. The objective of this study was to assess polycyclic aromatic hydrocarbons (PAH) metabolites and antioxidant capacity in *Unio pictorum* mussels collected at different sites of the Nemunas River.

*Unio pictorum* specimens were collected in 2020 (summer and autumn seasons) at five Nemunas River sites. The first sampling site (N1) was located near Alytus bridge and was upstream of City industrial and municipal effluents input. Four other sites (N2, N3, N4 and N5) were in different distances downstream from Alytus City effluent release site. Metabolites of polycyclic aromatic hydrocarbons (PAH) in *U. pictorum* haemolymph was analysed using a semi-quantitative fixed wavelength fluorescence method [5]. Naphthalene-type, pyrene-type and benzo[a]pyrene-type PAH metabolites in mussels' haemolymph were determined using appropriate pairs of excitation and emission wavelengths. The total antioxidant capacity of *U. pictorum* was evaluated using the ferric reducing antioxidant power (FRAP) assay [6].

Preliminary results indicate that the highest levels of pyrene-type and benzo[a]pyrene-type PAH metabolites, indicating products of combustion processes and naphthalene-type PAH metabolites specific to petrogenic origin were detected in *U. pictorum* mussels, which were collected in study site (N3) located a few kilometres downstream from the Alytus City effluents input in autumn. Whereas measured levels of PAH metabolites were lower at other sites (N4 and N5) downstream from points of discharges of Alytus City effluents. The lowest levels of antioxidant capacity were detected in mussels collected at site (N2) close to effluents input in summer. This study revealed that measurement of PAH metabolites in freshwater bivalves by semi-quantitative fixed wavelength fluorescence method can be used for PAH contamination monitoring in aquatic environment.

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## 8. MOLECULAR IDENTIFICATION OF *SARCOCYSTIS* PARASITES IN RODENTS

Rakauskaitė, P., Stirė, V., Rudaitė-Lukošienė, E., Balčiauskas, L., Butkavikas, D., Prakas, P.

Nature Research Centre, Vilnius, Lithuania;  
[paulinarakauskaite@stud.gmc.vu.lt](mailto:paulinarakauskaite@stud.gmc.vu.lt)

Members of the genus *Sarcocystis* are worldwide distributed protozoan parasites. Currently, over 200 species of *Sarcocystis* are known to infect reptiles, birds and mammals. These parasites are characterised by an obligatory two-host prey-predator life cycle. Asexual multiplication with a formation of sarcocysts in muscles or CNS occur in the intermediate host, and sexual stages with a formation of oocysts/sporocysts develop in the definitive host. Some of *Sarcocystis* species are harmful to domestic and wildlife animals. The high *Sarcocystis* species diversity was disclosed in rodents. It is known that rodents act as intermediate hosts of about 40 *Sarcocystis* species. The last research on *Sarcocystis* parasites in rodents was performed in Lithuania 20 years ago. Morphologically *Sarcocystis* species are described and differentiated in the intermediate host. However, it is difficult to detect *Sarcocystis* in rodents due to the low prevalence of these parasites and the small amount of muscle material. Morphological analysis commonly is insufficient for the discrimination of *Sarcocystis* species in closely related hosts. Besides, there is a lack of molecular studies on *Sarcocystis* parasites in rodents. Therefore, molecular methods for the identification of *Sarcocystis* species in rodents should be developed. The aim of the present study was to optimize molecular-based method for the identification of *Sarcocystis* species from rodents.

In September–November 2020, 288 rodents (94 *Apodemus flavicollis*, 73 *Microtus arvalis*, 61 *Microtus glareolus* and 60 *Apodemus agrarius*) were collected from various parts of Lithuania. Skeletal muscles of animals were examined for the presence of *Sarcocystis*. In order to reduce time and financial costs, each specimen from the same location and belonging to one host species was grouped into sample consisting of 6–11 individuals. Muscle tissues were digested with pepsin, followed by DNA extraction and amplification of the partial mtDNA cytochrome c oxidase subunit I (cox1) region. By nested PCR the obtained 657 bp fragments were sequenced and compared using nBLAST with those of various *Sarcocystis* species deposited in NCBI GenBank. *Sarcocystis* spp. were found in three groups. Two group of animals originated from Utena and represented *M. glareolus*, while the third group was from Žiežmariai and represented *A. flavicollis*. *Sarcocystis* sp. from *M. glareolus* showed 99.2% identity to *S. ratti*, and *Sarcocystis* sp. *A. flavicollis* shared 99.8% identity with *S. lutrae* and *S. lari*. Thus, two *Sarcocystis* species were found in examined samples. The molecular approach applied in the present study is useful for the identification of *Sarcocystis* parasites in rodents. Future morphological and molecular research are needed for the characterization of detected *Sarcocystis* spp.

## 9. *IN VITRO* INTESTINAL BARRIER MODEL DEVELOPMENT AND APPLICATION FOR ELECTROLYTE ABSORPTION STUDIES

Karina Jelkinaitė<sup>1</sup>, Dalė Baranauskienė<sup>2</sup>, Aistė Jekabsone<sup>1,3</sup>

<sup>1</sup>Laboratory of Pharmaceutical Sciences, Institute of Pharmaceutical Technologies, Faculty of Pharmacy, Lithuanian University of Health Sciences, Kaunas, Lithuania

<sup>2</sup>Laboratory of Toxicology, Neuroscience Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania

<sup>3</sup>Laboratory of Preclinical Drug Investigation, Institute of Cardiology, Lithuanian University of Health Sciences, Kaunas, Lithuania  
[jelkinatekarina@gmail.com](mailto:jelkinatekarina@gmail.com)

**Introduction.** The human intestine is a dynamic barrier that has an important role in the absorption of essential nutrients, water, electrolytes and vitamins [1,2]. Every day from 100 to 800 mmol of sodium (Na<sup>+</sup>), chloride (Cl<sup>-</sup>), and potassium (K<sup>+</sup>) pass through the intestinal lumen [3]. The electrolyte absorption disorders may underlie important diseases, and *in vitro* models made from human intestinal cells can help to better understand mechanisms involved in electrolyte absorption processes.

**Aim.** To create human intestine *in vitro* model from human intestinal epithelial cell line, that replicates intestinal barrier function, and apply it to electrolyte absorption studies.

**Materials and Methods.** The immortalized human colon epithelial cells HCEC-1CT were grown in DMEM on cell culture inserts. The integrity of the *in vitro* intestinal barrier was monitored by transepithelial resistance (TEER) using voltmeter Millicell ERS-2. Nine electrolyte solutions (mineral content varying from 0,2 to 15 g/l) were diluted with cell medium at ratio 1:4 and applied on cells in the insert chamber. Cell metabolic activity was assessed using PrestoBlue™ after 4, 24, and 48 hours in a multimode microplate reader Infinite 200 Pro M Nano Plex. Electrolytes Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> from the samples above and below the *in vitro* intestinal barrier were determined by inductively coupled plasma mass spectrometer ICP-MS NexION 300D.

**Results.** The TEER values increased with each day after cell seeding, and the highest value of 179 Ω 6,5 Ω cm<sup>2</sup> were observed on day 7 of culture. The electrolyte solutions did not significantly affect HCEC-1CT cell metabolic activity after 4 h of incubation. However, the solutions of low mineralization (0,2 g/l, 0,7 g/l, 1 g/l of electrolytes) significantly decreased metabolic activity after 24 h, and the effect remained after 48 h. The electrolyte solutions of intermediate concentration (1,2 g/l, 1,6 g/l) significantly increased metabolic activity after 24 h of exposure. However, after longer exposure up to 48 h this effect was no longer detected. It is noteworthy that a high concentrations (15 g/l, 13 g/l, 3 g/l), electrolyte solutions increased metabolic activity of the cells both after 24 h and 48 h of exposure. Electrolyte absorption through barrier from the apical into the basolateral compartment was observed for Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup>. Significant transportation through the barrier was observed when high concentration electrolyte solutions (15 g/l, 13 g/l) were applied for 4 and 24 hours of exposure. Low concentrated solutions (0,2 g/l, 0,7 g/l, 1 g/l) did not have a significant impact on electrolyte transport through *in vitro* intestinal barrier. The highest amounts passed from the apical into the basolateral compartment was observed for Na<sup>+</sup> and K<sup>+</sup>.

**Conclusions.** Developed intestinal barrier model *was* successfully applied for electrolyte absorption evaluation. Electrolyte solutions containing Na<sup>+</sup>, K<sup>+</sup>, Ca<sup>2+</sup> and Mg<sup>2+</sup> stimulate metabolic activity of intestinal epithelial cells in a concentration-dependent manner, and this is related with stimulation of electrolyte transport through the intestinal *in vitro* barrier.

**Acknowledgements.** The research was supported by Lithuanian Business Support Agency via measure "Intelektas" granted to "Birštono mineraliniai vandenys ir Ko" UAB, grant agreement No. J05-LVPA-K-01-0220.

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# 10. HYPOXIA REDUCES SENSITIVITY TO TEMOZOLOMIDE AND ACTIVATES CATALASE EXPRESSION

Deimantė Kulakauskienė<sup>1</sup>, Dovydas Gečys<sup>1,2</sup>, Zbigniew Balion<sup>1,3</sup>, Daiva Majienė<sup>3</sup>, Aistė Jekabsone<sup>1,2</sup>

<sup>1</sup>Institute of Pharmaceutical Technologies, Lithuanian University of Health Sciences, Lithuania

<sup>2</sup>Institute of Cardiology, Lithuanian University of Health Sciences, Kaunas, Lithuania

<sup>3</sup>Neuroscience Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania

[deimante.kulakauskiene@lsmu.lt](mailto:deimante.kulakauskiene@lsmu.lt)

Glioblastoma multiforme (GBM) is the most common and aggressive primary astrocyte-derived brain tumor in adults. GBM is characterized by ability to penetrate the surrounding brain tissue making it complicated to remove all tumour parts by surgical resection. Moreover, some of GBM cells are prone to develop resistance to radiochemotherapy with DNA alkylating agent temozolomide (TMZ) [1], and this causes GBM relapse in the TMZ-resistant, untreatable form [2]. The mechanisms of TMZ resistance still are not well understood, however, some data show it can be related to hypoxia, the condition that usually develops in the core of solid tumours [3]. To survive in low oxygen environment, cancer cells avoid cellular death pathways by changing their gene expression [4]. Recently, it was found that TMZ-resistant GBM cells can survive much higher levels of ROS due to upregulation of ROS-converting enzymes, changes in balance of apoptosis induction and chaperone mediated autophagy genes [5]. The aim of this work was to investigate whether hypoxia might reduce TMZ-induced cytotoxicity to human GBM cells, and how it is related with the changes in expression of genes related to response to hypoxia, ROS conversion, apoptosis and chaperone mediated autophagy.

HROG-36, human GBM cell line, was grown in DMEM/Ham's F12 cell culture media supplemented with 10% FBS and 1% penicillin-streptomycin solution (10 000 U/mL). TMZ (100  $\mu$ M) was applied on cultured GBM for 48 hours under normoxia (37 °C, 5% CO<sub>2</sub>) and hypoxia (37 °C, 93% N<sub>2</sub>, 5% CO<sub>2</sub>, 2% O<sub>2</sub>) conditions. GBM metabolic activity after exposure with TMZ was evaluated by PrestoBlue. Changes in expression of HIF1 $\alpha$ , Catalase (CAT), Hspa8, Phlpp1 and Bcl-2 family genes were evaluated using real-time polymerase chain reaction.

We demonstrate that hypoxia significantly reduced the size of TMZ-induced damage to GBM cells. Hypoxia significantly activated expression of HIF1 $\alpha$  and CAT, and the elevated level of expression of these genes remained after treatment with TMZ under hypoxic conditions. However, no significant changes in apoptosis modulating Bcl-2 family genes (BAD, BAX, Bcl-2) expression were observed between TMZ-treated GBM cells in normoxic and hypoxic conditions. Moreover, hypoxia did not affect expression of autophagy-related genes Hspa8 and Phlpp1 in TMZ-treated cells.

Our findings show, that hypoxia decreases sensitivity of HROG36 cells to TMZ, and this might occur due to higher resistance to oxidative damage via CAT activity, but not due to the changes in Bcl-2 family-dependent apoptosis or chaperone mediated autophagy.

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# 11. THE EFFECT OF pH, IONIC STRENGTH AND DENATURANTS ON AGGREGATION AND SELF-REPLICATION OF SUPEROXIDE DISMUTASE 1

Dominykas Veiveris<sup>1</sup>, Vytautas Smirnovas<sup>1</sup>

<sup>1</sup>Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania

[dominykas.veiveris@gmc.stud.vu.lt](mailto:dominykas.veiveris@gmc.stud.vu.lt)

The conformational changes of proteins and their aggregation to insoluble amyloid aggregates are linked to many neurodegenerative disorders, including Alzheimer's, Parkinson's diseases or amyotrophic lateral sclerosis (ALS) [1]. Despite numerous studies and many years of researches, there are still very few compounds that are used as anti-amyloid drugs and none of them is capable of completely curing any neurodegenerative disease. One of the reasons why researchers struggle to find effective anti-amyloid compounds is conformational and morphological diversity of protein aggregates. It is known, that same protein molecules can form different strains of amyloid fibrils [2,3]. Various environmental factors play a key role in forming protein aggregates with distinct structural characteristics. The shape of amyloid conformers determines their specific self-replication tendencies and aggregation rate in general [3]. In this work we tested how primary aggregates that were grown under different pH solutions affect aggregation rate of Superoxide dismutase 1 (SOD1), which is one of the major causes of ALS. Also, we tested SOD1 aggregation kinetics under distinct ionic strength and denaturing conditions.

Primary SOD1 aggregates („seeds”) were prepared under pH 3.0, 4.0, 5.0, 6.0, 7.0, 8.0 conditions (200  $\mu$ M SOD1, 100 mM NaCl (pH range 3–8), 5  $\mu$ M tris(2-carboxyethyl)phosphine (TCEP), incubation 72h, with shaking). SOD1 aggregation was performed in pH 3–8 100 mM NaCl solutions, using 5% „seeds” from each primary pH solution (Fig. 1). The effect of ionic strength on SOD1 aggregation was tested under 0 M, 0.05 M, 0.1 M, 0.2 M, 0.5 M, 1 M NaCl conditions, pH 8, 5  $\mu$ M ThT. To test denaturing conditions, 0 M, 0.2 M, 0.4 M, 0.6M, 0.8 M GuHCl samples were used (100 mM NaCl, pH 8, 5  $\mu$ M ThT). The curves of aggregation kinetics were created by measuring fluorescence of ThT (excitation 440 nm, emission 480 nm) and aggregation half-times were calculated.

The results show that „seeds” grown in acidic pH solutions accelerated SOD1 aggregation more rapidly. However, „seeded” SOD1 aggregation occurred more quickly in higher pH solutions. Ionic strength showed a positive effect on SOD1 aggregation time, while high GuHCl concentrations inhibited the aggregation process.

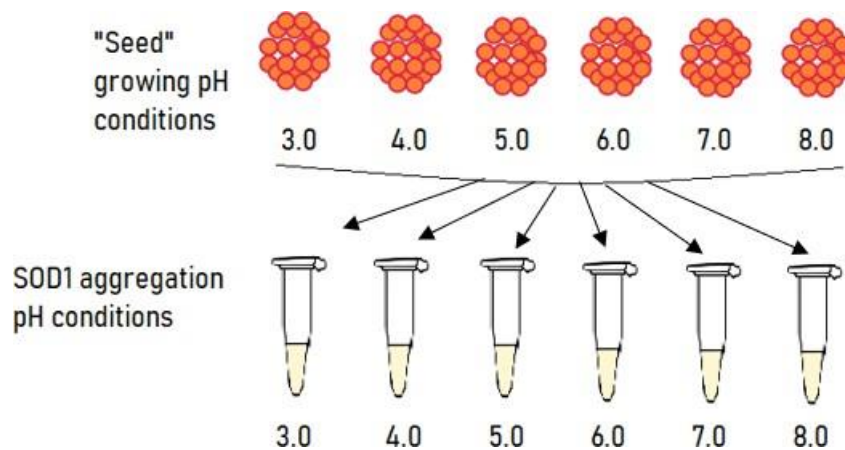


Figure 1. „Seeds” formed under distinct pH conditions were put into six different pH SOD1 aggregation mixtures.

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## 12. ALTERED EXPRESSION OF MIRNAS IN LUNG TUMORS

Neda Zenkevičiūtė<sup>1</sup>, Kamilė Normantaitė<sup>1</sup>, Julija Šimienė<sup>1</sup>, Rytė Rynkevičienė<sup>1</sup>,  
Eglė Balčiūnaitė<sup>1</sup>, Kęstutis Sužiedėlis<sup>1</sup>

<sup>1</sup>Department of Molecular Oncology, National Cancer Institute, Lithuania  
[neda.zenkeviciute@gmail.com](mailto:neda.zenkeviciute@gmail.com)

Lung cancer is one of the leading causes of cancer deaths worldwide. Every year lung cancer is diagnosed to more than a thousand people in Lithuania. Smoking increases the risk, though lung cancer can occur to those who never smoked. There are 2 major types of lung cancer: non-small cell lung cancer (NSCLC) and small cell lung cancer (SCLC). NSCLC accounts for 80%-85% of all lung cancers.

MiRNAs are single-stranded 20-25 nucleotide long non-coding RNA molecules that regulate gene expression by translation termination or mRNA degradation. MiRNAs are involved in various biological processes such as gene regulation, apoptosis, cell growth, proliferation, and differentiation. Depending on the type of cancer, miRNAs can act as oncogenes, tumor suppressors, or metastasis regulators. Although regulation of miRNAs expression may be either a cause or a consequence of cancer, functional studies have shown that they may play an important role in tumor initiation and growth. To date, both in vitro and in vivo studies indicate, that dysregulation of the expression of some miRNAs can be closely related to the pathogenesis of lung cancer<sup>1</sup>. Certain miRNAs could serve as diagnostic biomarkers, thus further investigations are necessary to better understand the role of specific miRNAs in the progression of lung cancer.

Previous investigations in the laboratory identified a number of miRNAs that are potentially associated with lung cancer metastasis<sup>2</sup>. Six different miRNAs (miRNA-355, miRNA-877, miRNA-500a, miRNA-328, miRNA-196a, and miRNA-195) were selected for the expression analysis in the lung cancer biopsy specimens. Samples from patients aged between 48-83 years old were used in the study.

Firstly, the total RNA purification procedure was performed. After purification, possible genomic DNA impurities were removed by treatment of the samples with DNase. Next, miRNA-specific copy DNA (cDNA) was synthesized. Quantitative reverse transcription PCR (RT-qPCR) was performed to assess the expression of miRNAs. The expression of miRNAs in malignant lung tumors was compared with normal lung tissues. In addition, qPCR samples were analyzed by DNA electrophoresis to evaluate the specificity of the PCR reaction. Finally, a statistical analysis of the data was performed. Our results revealed significant changes in the expression of miRNA-877 and miRNA-196a in cancer samples compared to the non-cancerous tissues. Furthermore, we observed a decrease in the expression of miRNA-196a in the recurrent NSCLC specimens.

Taken together, our results suggest that miRNA-877 and miRNA-196a may serve as future diagnostic biomarkers for lung cancer.

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# 13. THE STUDY OF RNA m5C WRITER NSUN5 REGULATION IN HUMAN GLIOMAS

Ramunė Karpavičiūtė, Raminta Bagdanavičienė, Giedrius Steponaitis

Laboratory of Molecular Neurooncology, Neurosciences Institute, Lithuanian University of Health Sciences, Lithuania

[ramune98@gmail.com](mailto:ramune98@gmail.com) or [giedrius.steponaitis@lsmuni.lt](mailto:giedrius.steponaitis@lsmuni.lt)

**Background.** Astrocytoma is a tumor of the human brain-derived from astrocytic-like glial cells. The disease is associated with poor patient prognosis and short survival. The improvement of early diagnostics of the disease by incorporating novel molecular markers emerging at the early stages of tumor development would contribute to improving the outcome of the disease. Epitranscriptome is a recently studied element of post-transcriptional regulation of gene expression. It includes a multitude of RNA modifications, most of which biological functions are still unclear. Epitranscriptome processes also involve hundreds of proteins and microRNA molecules that regulate the attachment, removal, and recognition of these modifications. One of the most frequent and relevant RNA modification is C5-methylation of cytosine (m5C), which was shown to have an important role in gene regulation. The modification can be carried out by few proteins called writers, however, the most common writer of m5C is RNA methyltransferase NSUN5. Recently few pilot studies revealed that NSUN5 might be involved in glioma development nevertheless, there is a lack of information about NSUN5 level regulation in gliomas. Thus, the AIM of the study was to elucidate if RNA 5mC mark writer NSUN5 is controlled by gene promoter methylation and/or via miRNA interference in human gliomas.

**Methods.** In total 55 astrocytomas of grade II-IV were used for the study. Gene expression of NSUN5 was analyzed by quantitative RT-PCR using SYBR-green. TaqMan assay applied for microRNA level measurement, and MS-PCR used for NUSN5 promoter methylation analysis. Expression was calculated applying dCT method. RNA m5C modification in total RNA was measured applying "EpiQuik™ m6A RNA Methylation Quantification Kit".

**Results.** The study results did not reveal a significant connection between NSUN5 promoter methylation and NSUN5 gene expression in astrocytoma specimens, ( $p=0.118$ ,  $n=39$ ). The expression of microRNA-574, and microRNA-124, which were selected based on target prediction at miRDB database revealed a weak significant correlation with NSUN5 expression level:  $r=0.403$ ,  $p=0.011$ , and  $r=-0.305$ ,  $p=0.042$ , respectively. M5C level was not associated with NSUN5 mRNA level, still we found strong correlation between miR-574 expression and m5C level ( $r=0.748$ ,  $p>0.001$ ). We also did not find any significant association between analyzed molecular features of tumor specimens and patient clinicopathological data, except in the case of miR-574, the lower expression of which was associated with a better prognosis of patient survival (LogRank,  $\chi^2=9.21$ ,  $p=0.0024$ ).

**Conclusions.** The pilot study revealed that NSUN5 gene expression inversely correlates to miRNA-574 level indicating possible NSUN5 regulation via miRNA interference, thus following functional study would be useful to validate pilot data. Methylation analysis did not show analyzed CpG site methylation impact to NSUN5 mRNA level. To clarify if NSUN5 level could be controlled via promoter methylation whole CpG island screening should be accomplished. It should be mentioned, that all of grade II-III specimens NSUN5 promoter was found to be methylated, which could as well indicate relevant biological value.

Interestingly, the m5C modification level of total RNA showed a strong correlation to miR-574 expression level and such crosstalk for the first time recorded in the present research is worth deeper exploring. Moreover, miR-574 expression was associated with glioma patient survival and tumors IDH1 mutation status what also indicates the possible biological role of the molecule in gliomagenesis pathways.

# 14. IDENTIFYING INSULIN FIBRIL CONFORMATIONAL DIFFERENCES BY THIOFLAVIN-T BINDING CHARACTERISTICS

Mantas Ziaunys<sup>1</sup>, Andrius Sakalauskas<sup>1</sup>, Vytautas Smirnovas<sup>1</sup>

<sup>1</sup>Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania  
[mantas.ziaunys@gmc.vu.lt](mailto:mantas.ziaunys@gmc.vu.lt)

Amyloidogenic protein aggregation into insoluble fibrillar aggregates is linked to several amyloidoses, including neurodegenerative Alzheimer's and Parkinson's diseases [1]. Despite many years and countless studies, there are still very few effective anti-amyloid drugs available and most potential compounds fail to pass all clinical trials. One of the main reasons for these failures is the complex nature of amyloid aggregates. It is known that the same protein molecule may form fibrils with distinct conformational and morphological characteristics. Each of these fibril types also possess specific self-replication tendencies and may respond differently to potential anti-amyloid compounds. In many cases, fibril identification is done by employing atomic force microscopy, where results are highly dependent on sample deposition techniques, as well as infrared spectroscopy, which requires relatively high concentrations of the protein sample. In recent years it has been observed that distinct protein fibrils have specific amyloidophilic dye binding [2] characteristics (affinity, fluorescence intensity), which could potentially be used to identify unique amyloid aggregates.

In order to examine whether an amyloid-specific dye – thioflavin-T [3] could be used to differentiate between conformationally-different fibrils, insulin was aggregated into four distinct fibril types, under four environmental conditions. Human recombinant insulin powder was dissolved in four types of solutions (1. 20% acetic acid, containing 100 mM NaCl; 2. 100 mM sodium phosphate buffer (pH 2.0); 3. 100 mM sodium phosphate buffer (pH 2.4), containing 100 mM NaCl; 4. PBS (pH 7.4)) and incubated at 60°C. After fibrils were formed, they were initially examined by atomic force microscopy and Fourier-transform infrared spectroscopy, to verify morphological and structural differences. Afterwards, the samples were mixed with a range of thioflavin-T concentrations and their absorbance spectra, as well as excitation-emission matrices were scanned.

The results show that each type of aggregate has unique bound-dye fluorescence properties, such as maximum excitation and emission wavelengths, as well as fluorescence quantum yield. Considering that such a dye-based examination requires minimal amounts of fibrils and is relatively quick to perform, it may be used as an alternative method to identify amyloid fibril conformation differences.

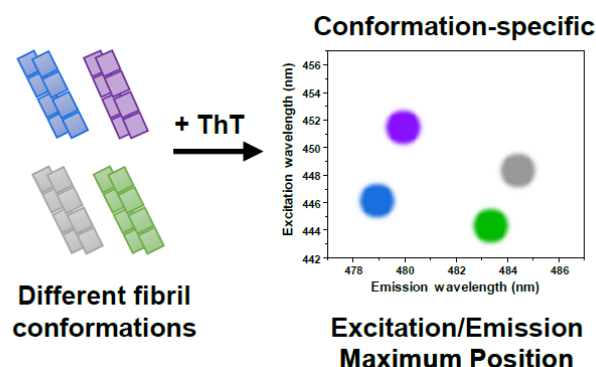


Figure1. Different insulin fibril conformations have specific ThT binding and fluorescence characteristics.

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# 15. PULSED ELECTRIC FIELD EFFECT ON NUCLEAR FACTOR NF- $\kappa$ B PROMOTER CONTROLLED REPORTER GENE EXPRESSION IN CHO-K1 CELL LINE

Auksė Kazlauskaitė<sup>1,2</sup>, Justina Kavaliauskaitė<sup>1</sup>, Arūnas Stirklė<sup>1</sup>

<sup>1</sup>Laboratory of Bioelectronics, State Research Institute Center for Physical Sciences and Technology, Saulėtekio avenue 3, Vilnius, Lithuania

<sup>2</sup>Bioscience Institute, Life Sciences Center, Vilnius University, Saulėtekio avenue 7, Vilnius, Lithuania  
[aukse.kazlauskaite@gmc.stud.vu.lt](mailto:aukse.kazlauskaite@gmc.stud.vu.lt)

It is widely known that pulsed electric field (PEF) causes permeabilization of cell plasma membrane and electrophoretic movement of DNA or transfer of other impermeable molecules, but less is known about PEF effects on gene expression. Milisecond and nanosecond duration PEFs were already showed to modulate gene expression in vitro and in vivo but more research is needed [1, 2]. In this context, PEF could be potentially used as a non-invasive, biophysical regulator of gene expression in gene therapy based treatments as well as gene expression regulator in genetic circuits for synthetic biology applications in mammalian cells.

The aim of this work was to determine the effect of microsecond pulsed electric field ( $\mu$ sPEF) on the NF- $\kappa$ B/SEAP reporter system expression in *Cricetulus griseus* ovarian tissue (CHO-K1) cell line. NF- $\kappa$ B/SEAP plasmid contains NF- $\kappa$ B response promoter, which regulates transcription of secreted alkaline phosphatase (SEAP) gene. Thus, if signalling pathway activation occurs, the NF- $\kappa$ B dimer can bind to the promoter and activate the transcription of SEAP. SEAP protein is subsequently involved in the colorimetric reaction after which yellow product is formed. The amount of the product was evaluated by measuring the absorbance at 405 nm. Therefore, induction of NF- $\kappa$ B/SEAP reporter system directly correlates with the activity of SEAP. To test  $\mu$ sPEF effect on NF- $\kappa$ B/SEAP reporter system, we chose to treat transfected cells with 8, rectangular, 1 Hz, 100  $\mu$ s duration and different voltage pulses based on published data [3]. Results are presented in relative SEAP expression 24 and 48 hours after  $\mu$ sPEF treatment. Additionally, metabolic cell activity after  $\mu$ sPEF treatments was assessed using XTT based assay and is reported as percentage relative to untreated control cells. Control experiments with known physical NF- $\kappa$ B/SEAP reporter system inducer – ultraviolet light (UV), were also conducted.

Control experiments showed that UV doses of 4,14 J/(cm<sup>2</sup>) and 20,7 J/(cm<sup>2</sup>) increased SEAP expression 2.5 fold in comparison to untreated cells and validated the suitability of reporter system to evaluate  $\mu$ sPEF effects. Our results showed that 24 hours after  $\mu$ sPEF SEAP expression was lowered and the highest electric field of 0.4 kV/cm caused the highest reduction in SEAP expression as well as cell viability. Conversely, 0.3 kV/cm  $\mu$ sPEF resulted in 1.2 fold increase of SEAP expression and cell viability 48 hours after pulsation.

We conclude that no reporter system activation was achieved 24 hours after  $\mu$ sPEF but an increase in SEAP expression can be detected 48 hours after pulsation.

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## 16. HEPN-MNT TOXIN-ANTITOXIN SYSTEM: THE HEPN RIBONUCLEASE IS NEUTRALISED BY OLIGOAMPYLATION

Songailiene Inga<sup>1,4</sup>, Juozapaitis Jonas<sup>1,4</sup>, Tamulaitiene Giedre<sup>1</sup>, Ruksenaite Audrone<sup>1</sup>, Šulčius Sigitas<sup>2</sup>, Sasnauskas Giedrius<sup>1</sup>, Venclovas Česlovas<sup>1</sup>, Siksnys Virginijus<sup>1,3</sup>

<sup>1</sup>Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania

<sup>2</sup>Laboratory of Algology and Microbial Ecology, Nature Research Centre, Vilnius, Lithuania

<sup>3</sup>lead contact, siksnys@ibt.lt

<sup>4</sup>Contributed equally to the presented work

[jonas.juozapaitis@gmc.vu.lt](mailto:jonas.juozapaitis@gmc.vu.lt)

Prokaryotic toxin-antitoxin systems (TA) are usually two gene modules composed of a toxin, capable of interfering with key cellular processes, and its neutralizing antidote, the antitoxin. Under normal cell growth conditions antitoxin inhibits its cognate toxin and the cell remains not affected. Dysregulation of antitoxin and toxin levels leads to active toxin that often occurs under cellular stress conditions [1, 2]. Some TA systems are known to stabilise mobile genetic elements such as plasmids. However, many TA modules are encoded in the cell chromosome and functions of these systems are more diverse and, sometimes, less clear [1, 2]. Antiviral defense was proposed as a primary physiological role of the *E. coli* hok/sok TA system [3], although other cellular functions of TA systems such as stress response, bio-film formation and persistence are also under debate [2, 4].

Here, we focus on the HEPN-MNT TA encoded in the vicinity of a I-D CRISPR-Cas system in cyanobacterium *Aphanizomenon flos-aquae*. We show that HEPN and MNT form a type II TA system. HEPN (higher eukaryotes and prokaryotes nucleotide-binding domain) acts as a toxic RNase, which cleaves off 4 nt from the 3'-end in a subset of tRNAs, thereby interfering with translation. Such toxin RNase specificity has not been known until now. Also, we find that the MNT (minimal nucleotidyltransferase) antitoxin inhibits HEPN RNase through covalent di-AMPylation (diadenylation) of a conserved tyrosine residue in the active site loop. Furthermore, we present crystallographic snapshots of the di-AMPylation reaction at different stages that explain the mechanism of HEPN RNase inactivation. DiAMPylation results in relocation of tyrosine residue, which moves away from the HEPN catalytic cleft into the protein periphery. This change flips the catalytic histidine away from the catalytic center, accounting for the loss of HEPN catalytic activity. Finally, we propose that HEPN-MNT system functions as a cellular ATP sensor that monitors ATP homeostasis and at low ATP levels releases active HEPN toxin.

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## 17. TYPE III CRISPR-CAS EFFECTOR COMPLEX EXPRESSION OPTIMISATION AND SINGLE MOLECULE STUDIES

Konstanty Keda<sup>1</sup>, Irmantas Mogila<sup>1</sup>, Patrick Irmisch<sup>2</sup>, Brighton Samatanga<sup>2</sup>,  
Ralf Seidel<sup>2</sup>, Gintautas Tamulaitis<sup>1</sup>

<sup>1</sup>Institute of Biotechnology, Life Science Center, Vilnius University, Saulėtekio av. 7, LT-10257 Vilnius, Lithuania

<sup>2</sup>Peter Debye Institute for Soft Matter Physics, Universität Leipzig, 04103 Leipzig, Germany  
[konstanty.keda@gmc.stud.vu.lt](mailto:konstanty.keda@gmc.stud.vu.lt)

CRISPR-Cas systems provide adaptive defense against bacteriophages by employing effector complexes which destroy viral nucleic acids. Effectors recognize virus genetic material by utilizing CRISPR RNA (crRNA) molecules, which are transcribed from CRISPR loci and are complementary to the parts of virus DNA or RNA. CRISPR-Cas systems are classified into six types based on their effector complex composition [1]. Type III CRISPR-Cas effector complex consists of proteins from five Cas families bound to crRNA. When complex recognizes cognate RNA transcribed from virus DNA, the Cas10 protein performs hydrolysis of the DNA matrix and produces cyclic oligoadenylates, which activate additional effectors. The transcript is degraded by Csm3 protein which diminishes Cas10 activity [2]. Despite the functions of *Streptococcus thermophilus* CRISPR-Cas effector complex (StCsm) proteins were found [3], it has not been elucidated how the complex recognizes and binds to target RNA and how recognition of target molecule regulates Cas10 protein. To study StCsm regulation a single molecule approach is preferred. However, previously produced StCsm samples were not homogenous, because of inefficient complex maturation in a heterologous *Escherichia coli* host [4] and not suitable to make precise measurements of binding kinetics. In this study we optimized complex purification step to acquire a homogenous StCsm sample which was used to measure the complex binding and cleavage of immobilized fluorescent RNA and DNA. Initial results explaining StCsm regulation will be presented.

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# 18. FLAVONE OXIDATION DERIVATIVES ACT AS INHIBITORS OF AMYLOID-BETA AGGREGATION

Andrius Sakalauskas, Vytautas Smirnovas

Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania  
[Andrius.Sakalauskas@gmc.vu.lt](mailto:Andrius.Sakalauskas@gmc.vu.lt)

Protein accumulation into highly dense structure aggregates is associated with multiple neurodegenerative disorders [1]. Alzheimer's disease (AD) was recognized to be the most prevalent condition that affects over 35 million people worldwide and is projected to increase up to 76 million by 2030 [2]. Typically, Alzheimer's disease onset at the age of 60 or later, with leading symptoms of progressive decline in memory and other distinct cognitive capabilities [3]. The pathology of this disease is linked to the aggregation of amyloid-beta ( $A\beta$ ) and the formation of Tau neurofibrillary tangles.  $A\beta$  aggregates are found at the very early stage of the disease, thus the inhibition of this peptide formation and aggregation has profound attention of scientists [4]. Over the years, various compounds were suggested for treatment, however, the effort to find disease-modifying drugs were not rewarding leaving more than 99.5% of clinical trial unsuccessful [5]. Yet, in this research, we focus on the flavones as the potential inhibitors of  $A\beta$  aggregation. Flavones are natural antioxidants and exhibit neuroprotective and anti-inflammatory effects. The flavones belong to the group of acetylcholinesterase inhibitors, which is shown to have the best positive effect on the treatment of AD symptoms [6]. Although many protein aggregation experiments in vitro using flavone derivatives show positive results, the in vivo results are not flattering. This may be related to a different oxidation mechanism at physiological conditions in vivo and in vitro leading to environmentally distinct oxidation products [7], [8].

In this study, we show the link between flavone hydroxy-groups position and the oxidation derivatives that possess  $A\beta$  and insulin amyloid aggregation inhibition effect. The insulin amyloid aggregation was added to this research to analyze flavone inhibition potential at the conditions where auto-oxidation does not occur, thus both non-oxidized and oxidized flavone particles were tested. The absorbance spectrum data reveal that not all tested flavones undergo auto-oxidation at the experimental conditions. The  $A\beta$  and insulin aggregation kinetic data show the extreme change of aggregation halftime after compound auto-oxidation. In addition, the atomic force microscopy images display the morphology of the fibrils formed during the experiment.

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## 19. STUDY OF THE INTERACTION OF HSP70 WITH UNILAMELLAR VESICLES

Akvilė Milašiūtė, Rima Budvytytė, Gintaras Valinčius

Institute of Biochemistry, Vilnius University Life Sciences Center  
[akvile.milasiute@gmc.stud.vu.lt](mailto:akvile.milasiute@gmc.stud.vu.lt)

Cells produce a various heat shock proteins in response to stress. 70-kDa heat shock protein (Hsp70) is one of the best known heat shock proteins. Hsp70 participates in folding and assembly of newly synthesized proteins, refolding of misfolded and aggregated proteins. Hsp70 expression is increased during stress conditions, and their function is vital for the recovery of cells after the insult [1]. Hsp70 is mainly localized in subcellular compartments, for example, cytosol, endoplasmic reticulum and mitochondria. In addition, Hsp70 is detected in extracellular space where this protein appear to play a different function from their chaperone activity [2].

Extracellular Hsp70 do not contain a consensus signal for their secretion via classical ER-Golgi compartment. Therefore, they are likely export by an alternative mechanism but the exact Hsp70 translocation mechanism across the plasma membrane remain to be elucidated.

The aim of this work was to investigate Hsp70 interaction with various compositions unilamellar vesicles. In this study, Hsp70 was added to DOPC, DOPC/Chol (60:40) and DOPC/DOPE/Chol/DOPS (20:30:20:30) vesicles solutions and the change in fluorescence intensity due to calcein release from vesicles was detected. Results show that the incubation time of Hsp70 and unilamellar vesicles was 10 min. The smallest Hsp70 concentration which disturbs the integrity of membrane was 0,25 nM. Furthermore, fluorescence intensity of calcein release was about 1.5 times bigger at higher (>2nM) Hsp70 concentrations and vesicles contained negatively charged phospholipids (Fig. 1).

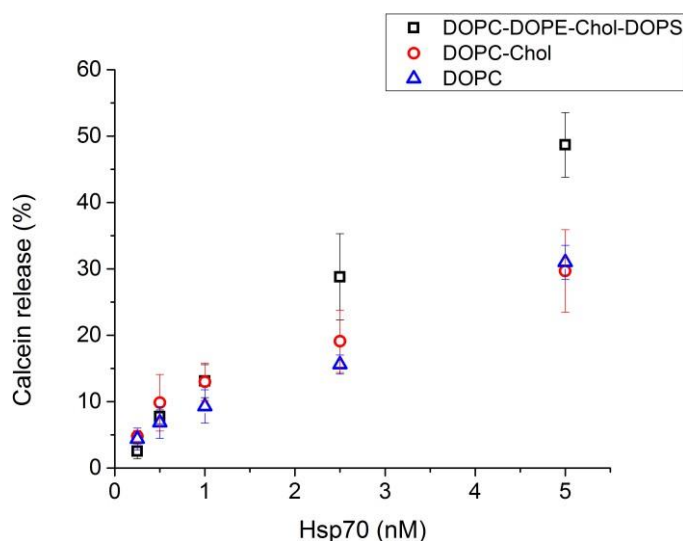


Fig. 1. Hsp70 interaction with unilamellar vesicles. Calcein release from vesicles was similar at lower Hsp70 concentrations (<1 nM). At higher (>2 nM) Hsp70 concentrations, calcein release was 1.5 times bigger in case of negatively charged vesicles. Incubation time – 10 min.

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## 20. PRODUCTION OF RECOMBINANT CYTOSOLIC MOUSE CARBONIC ANHYDRASES

Edvardas Timoščenka, Aurelija Mickevičiūtė, Lina Baranauskienė

Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Life Sciences Center,  
Vilnius University, Lithuania

[edvardas.timoscenka@gmc.vu.lt](mailto:edvardas.timoscenka@gmc.vu.lt)

The metalloenzyme carbonic anhydrase (CA) catalyzes one of the most ubiquitous biochemical reactions – the bicarbonate conversion to CO<sub>2</sub> and H<sub>2</sub>O. Carbonic anhydrases are involved in the regulation of pH, CO<sub>2</sub> transport in blood during respiration, electrolyte homeostasis, and some metabolic pathways. All mammals have only  $\alpha$ -class CAs with fifteen isoforms present in humans. Twelve of the human CA isoforms are catalytically active. CA is confirmed as drug target since some of the isoforms are related to several diseases, such as glaucoma or some types of cancer [1]. In pre-clinical trials, *Mus musculus* is often used as a model organism for testing drug effects. There are thirteen catalytically active mice CA (Car) isoforms. In addition to different organ/tissue distribution and catalytic activity levels, they also differ in their cellular localization. Car I, Car II, Car III, Car VII and Car XIII are cytosolic, Car IX, Car XII, and Car XIV have a transmembrane fragment, Car IV and Car XV are linked to the membrane via glycosylphosphatidylinositol anchor with the catalytic domain in the extracellular side, Car VA and Car VB are mitochondrial isoforms, while Car VI is secreted.

This study's aim was to determine Car cloning strategies, their expression, and purification, and in order to identify its stability and functional activity fluorescent thermal shift assay was used. Cloning of the recombinant Car proteins was firstly done with PCR of DNA, then restriction enzymes were used to generate sticky ends, and DNA was ligated into a plasmid. After the creation of the construct, protein expression conditions were found. The recombinant Car was purified using IMAC and then protein functional activity was evaluated using fluorescent thermal shift assay[2] by binding CA-specific small molecule ligands. Figure 1A presents optimized results of Car XIII expression and purification, while 1B shows its binding to dichlorophenamide by fluorescent thermal shift assay.

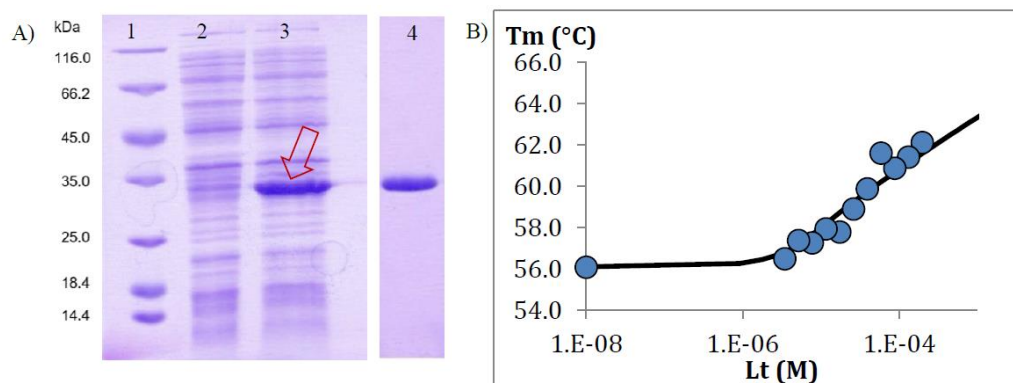


Figure 1. Car XIII expression, purity and functional activity. A) SDS-PAGE of Car XIII: lane 1 – MW markers, 2 – bacterial lysate before induction, 3 – bacterial lysate after induction, 4 – purified Car XIII. B) fluorescent thermal shift assay results of Car XIII binding to dichlorophenamide – symbols correspond to experimental data and line present fitting using  $K_d$  determination model.

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## 21. CHARACTERISATION OF LOW MOLECULAR WEIGHT BIOMARKERS OF INFLAMMATION AND CANCER

Giedrė Karzaite<sup>1,2</sup>, Mahboubeh Eskandari<sup>1</sup>, Tautgirdas Ruzgas<sup>1</sup>

<sup>1</sup>Malmö University, Department of Biomedical Sciences, Per Albin Hanssons 35, SE-214 32 Malmö, Sweden

<sup>2</sup>Vytautas Magnus university, Department of Biological, Vileikos str. 8-802, LT-44404, Kaunas, Lithuania

[giedre.karzaite@gmail.com](mailto:giedre.karzaite@gmail.com)

Increased amino acid catabolism is essential for tumor growth and immune regulation in cancer. This is especially true for the kynurenine metabolites of tryptophan, which exert diverse biological effects and have been investigated as markers of tumor progression and therapeutic effect. The kynurenine pathway has been associated mostly with immune tolerance and tumor escape [1]. Approximately 95% of the free L-Trp in the body is metabolised down the Kyn pathway, generating several biologically active metabolites, including kynurenine (Kyn), kynurenic acid, 3-hydroxykynurenine (3-HK), 3-hydroxyanthranilic acid (3-HAA) [2]. Analysis of plasma kynurenine/tryptophan levels in patients with cancer affirms that the IDO (indoleamine 2,3-dioxygenase) pathway is activated in multiple tumor types [3]. IDO (indoleamine 2,3-dioxygenase) is a member of a unique class of mammalian haem dioxygenases that catalyse the oxidative catabolism of the least-abundant essential amino acid, L-Trp (L-tryptophan), along the kynurenine pathway [2].

In this research the main tasks were to review low molecular weight (LMW) biomarkers of inflammation and cancer, which are derivatives of amino acids, e.g., derivatives of histidine, tryptophan, arginine, etc; to evaluate methods enabling transdermal detection of amino-acid derived LMW biomarkers of inflammation and cancer; to experimentally assess the relevance of some of these methods for detection of skin inflammation and cancer, e.g., skin penetration assays and cell culture methods.

Our results showed that the 3-HKYN and 3-HAA are relatively stable in the pH range 5.5-7.4. If we are going to make extraction of these compounds from skin at basic solutions we might need to study stability at pH 10 and higher. It is possible to measure penetration of these compounds through skin after lipid extraction from skin using electrochemical methods. Detection limits by electrochemistry are 1  $\mu$ M or higher.

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## 22. NEW LINEAR AZOL(IN)E CONTAINING ANTIMICROBIAL PEPTIDE IDENTIFICATION IN THERMOPHILIC BACTERIUM

Justas Martūnas<sup>1</sup>, Alisa Gricajeva<sup>1</sup>, Arnoldas Kaunietis<sup>1</sup>

<sup>1</sup> Department of Microbiology and Biotechnology, Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania  
[justas.martunas@gmc.stud.vu.lt](mailto:justas.martunas@gmc.stud.vu.lt)

Bacteriocins are ribosomally synthesized antimicrobial peptides produced by various bacteria. These antimicrobial peptides are usually stable at high temperatures and over a wide pH range [1]. Bacteriocins have huge potential as both food preservatives, and as next-generation antibiotics targeting the multiple-drug resistant pathogens. It has been suggested that the majority of bacterial species synthesize bacteriocins. The increasing number of reports of new bacteriocins with unique properties indicates that there is still a lot of to learn about this family of peptide antibiotics. Our goal is identification and characterization of novel bacteriocins encoded in thermophilic bacteria. In this study we have identified novel post-translationally modified bacteriocin, belonging to the subclass of linear azol(in)e containing peptides (LAPs), which was encoded in *Parageobacillus toebi* bacterium. Here we present cloning and expression of this bacteriocin in *Escherichia coli*. Following the expression, we will purify and characterize its antibacterial effect on various *(Para)Geobacillus* spp. and other bacteria. Moreover, we will investigate its stability in various temperatures and pH values.

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## 23. CHARACTERIZATION OF PARACOCCLUS SP. INFECTING BACTERIOPHAGE KLEP18-1

Jurgelytė R.<sup>1</sup>, Šimoliūnienė M.<sup>1</sup>, Skapas M.<sup>2</sup>, Meškys R.<sup>1</sup> and Šimoliūnas E.<sup>1</sup>

<sup>1</sup>Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Centre, Vilnius University, Saulėtekio av. 7, Vilnius, Lithuania

<sup>2</sup>Center for Physical Sciences and Technology, Saulėtekio av. 3, Vilnius, Lithuania

*Paracoccus* is a biochemically versatile genus of bacteria, that have substantial biotechnological potential, especially in bioremediation, since some species can conduct denitrification and utilize various toxic organic compounds, and several are opportunistic human pathogens. Despite extensive knowledge of these bacteria, little is known about the viruses of *Paracoccus* spp. So far, only a limited number of active phages infecting these bacteria have been identified.

We present here the report on the characterization of *Paracoccus*-infecting bacteriophage vB\_PmaP\_KLEP18-1 (KLEP18-1), which was isolated from sulfate-type gypsum karst lake Ramunėlis located near Biržai, Lithuania. *Paracoccus marcusii* strain KR4M-18 was used as the host for phage propagation and phage growth experiments. Transmission electron microscopy images showed that KLEP18-1 is a podovirus with an isometric head (~ 62 nm in diameter) and short tail (~ 15 nm in length). The efficiency of plating test revealed that phage could form plaques in the temperature range from 4 to 38 °C with an optimum temperature for plating around 18 °C. KLEP18-1 forms plaques with a clear center (~ 4 mm in diameter) surrounded by constantly, albeit slowly, growing opaque halo zone which could reach ~ 6.6 cm in diameter within a period of two months. The sensitivity to environmental factors experiments showed that KLEP18-1 is a chloroform-sensitive phage – after one hour of incubation with 1/10 of chloroform at 37 °C, only ~ 3.5 % of phages remain infective. Similarly, only ~ 1.34 % of phages remain infective after heating 1 hour at 50 °C, while freezing has less negative effect to KLEP18-1 – after 48 hours of incubation at -20 °C, ~77.1 % of phages remain infective.

Thus, the data presented here will expand our knowledge of morphological and physiological characteristics of *Paracoccus*-infecting phages, which have been studied insufficiently to date.

## 24. ISOLATION AND IDENTIFICATION OF BACTERIA FROM GYPSUM KARST LAKES

Petrikonytė P.<sup>1</sup>, Šimoliūnienė M.<sup>1</sup>, Šulčius S.<sup>2</sup>, Meškys R.<sup>1</sup> and Šimoliūnas E.<sup>1</sup>

<sup>1</sup>Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Saulėtekio av. 7, Vilnius, Lithuania

<sup>2</sup>Laboratory of Algology and Microbial Ecology, Nature Research Centre, Akademijos str. 2, Vilnius, Lithuania

[patricija.petrikonyte@gmc.stud.vu.lt](mailto:patricija.petrikonyte@gmc.stud.vu.lt)

In the northern part of Lithuania, a karst region is located. Active karst phenomena occurring because of the leaching of gypsum and dolomite rocks lead to the formation of gap holes, which are then transformed into small water bodies. Bottom sediments and water in such lakes are dominated by  $\text{Ca}^{2+}$  and  $\text{SO}_4^{2-}$  ions. Gypsum karst lakes with sulfate type water are very rare not only in Lithuania, but also throughout Europe. Gypsum karst lakes and their microbial communities are one of the least studied environments in Lithuania. This means many species or evolutionary relationships are under sampled and unidentified in these sulfate rich environments. Therefore, it is important to isolate and identify more microorganisms in these karst lakes to broaden understanding of biodiversity and microbial relationships.

In this study we present a number of bacteria isolated from water samples of sulfate-type gypsum karst lake Ramunėlis located near Biržai, Lithuania. Based on the results of partial 16S rRNA sequencing, bioinformatic and phylogenetic analysis, 28 culturable bacterial isolates were identified including bacteria from the genus: *Acetobacteroides* (1), *Acinetobacter* (5), *Aeromonas* (3), *Bacillus* (1), *Deinococcus* (1), *Exiguobacterium* (2), *Fictibacillus* (1), *Macrococcus* (1), *Massilia* (1), *Microbacterium* (1), *Micrococcus* (1), *Paracoccus* (1), *Planococcus* (1), *Pseudomonas* (4), *Staphylococcus* (3) and *Trichococcus* (1). Phylogenetic analysis showed an interesting spectrum of bacteria variety stemming from strictly anaerobic bacteria like *Acetobacteroides hydrogenigenes* to bacteria mostly found in animal intestine *Staphylococcus pasteurii*. Thus, the data presented in this study will not only expand our knowledge of the diversity of bacteria but also leads for a better understanding of almost unexplored communities of bacteria in the unique sulfate-type gypsum karst lakes.

This research was funded by Research Council of Lithuania (Grant No. S-MIP- 20-38).



## 25. IMMOBILIZATION AND APPLICATION OF GD-95RM LIPASE FOR CONTINUOUS FLOW TRANSESTERIFICATION REACTION

Agnė Savickaite<sup>1</sup>, Gabija Šimkutė<sup>1</sup>, Renata Gudiukaite<sup>1</sup>

<sup>1</sup>Institute of Biosciences, Life Sciences Center, Vilnius University, Sauletekis ave. 7, LT-10257 Vilnius, Lithuania

[agne.savickaite@gmc.stud.vu.lt](mailto:agne.savickaite@gmc.stud.vu.lt)

Microbial lipases and esterases comprise one of the most important groups of industrial enzymes due to their unique ability to hydrolyze fatty acid ester bonds in aqueous environment and to perform a reverse reaction in non-aqueous conditions. Lipolytic enzymes find diverse applications in hydrolysis of fats and oils, food, detergent, pharmaceutical and cosmetics industries [1]. The ability to produce high energy products like biofuel and value-added compounds, such as fragrance or flavor fatty acid esters, mono- and diacylglycerols and to synthesize valuable chiral intermediates, building blocks and products with high enantiopurity via energy-efficient and ecologically friendly way makes lipolytic biocatalysts an important tool for sustainable biotechnology. However, the industrial applicability of free enzymes is limited due to their high cost, low operational stability and challenges for their recovery and reuse [2]. Thus, enzymatic immobilization can bring several benefits, such as easy separation of enzymes from the product, production of high purity compounds, compatibility with a wide range of applications, process minimization, making this method more profitable, feasible and energy-efficient [3]. One of the most effective enzyme immobilization techniques is enzyme entrapment in calcium alginate hydrogel.

This study highlighted immobilization of GD-95RM lipase [4] via encapsulation method. To achieve the most stable structure 3 % of sodium alginate and 150 mM of CaCl<sub>2</sub> was used. The results clearly showed that immobilized GD-95RM lipolytic enzyme displayed significantly higher activity at 65 °C – 85 °C temperatures compared with the free enzyme. Furthermore, after immobilization thermostability of enzyme increased drastically. Entrapment in calcium alginate hydrogel also improved the ability of GD-95RM to hydrolyze p-NP stearate, a long acyl chain substrate. The analysis of continuous transesterification of avocado oil with ethanol as a substrate confirmed that encapsulated GD-95RM lipase is a useful approach to produce fatty acid alkyl esters. Principal scheme of the research is shown in Fig. 1.

This research is important for the future development of cost-effective immobilized lipolytic enzyme systems that can be applicable in industry for transesterification, esterification or hydrolysis reactions..

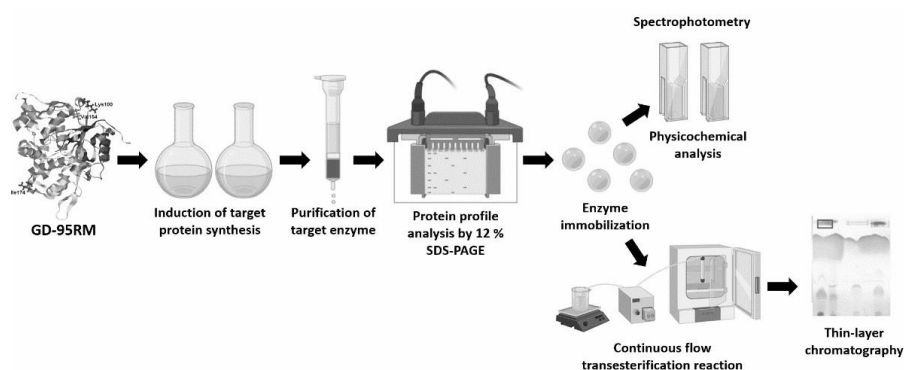


Figure 1. Principal scheme of research.

**Acknowledgment.** This research was funded by the European Social Fund under the No 09.3.3-LMT-K-712 "Development of Competences of Scientists, other Researchers and Students through Practical Research Activities" measure, Grant No. 09.3.3.-LMT-K-712-16-0020.

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## 26. DEVELOPMENT OF CONTROLLED GENE EXPRESSION SYSTEM FOR *(PARA)GEOBACILLUS* SPP. BACTERIA

Aistė Kairyte<sup>1</sup>, Inga Matijošytė<sup>2</sup>, Arnoldas Kaunietis<sup>1</sup>

<sup>1</sup>Department of Microbiology and Biotechnology, Institute of Biosciences, Life Sciences Center, Vilnius University, Saulėtekio ave. 7, LT-10257 Vilnius, Lithuania

<sup>2</sup>Sector of Applied Biocatalysis, Institute of Biotechnology, Life Sciences Center, Vilnius University, Saulėtekio ave. 7, LT-10257 Vilnius, Lithuania  
[aiste.kairyte@gmc.stud.vu.lt](mailto:aiste.kairyte@gmc.stud.vu.lt)

Thermostable enzymes, such as cellulases, xylanases, proteases, pectinases, etc. have wide biotechnological and industrial applications. Most of these proteins are currently produced in mesophilic bacteria using heterologous gene expression systems due to a lack of versatile expression system for thermophilic bacteria. This often leads to different post-translational modifications or incorrect folding and, thus, protein properties are affected. These obstacles could be overcome if thermostable proteins were produced by thermophilic bacteria. Until now most attempts were unsuccessful due to a lack of genetic tools necessary for thermophilic expression, including thermostable reporter proteins, selection markers, plasmid vectors and others. However, these genetic tools are being rapidly developed which allows further successful attempts to construct an expression system for thermophilic bacteria.

The aim of our work is to create a regulated gene expression system for *(Para)Geobacillus* spp. bacteria. *(Para)Geobacillus* are thermophiles that usually have higher reaction rates at elevated temperatures and often are susceptible to genetic modifications, which makes them a suitable host for thermophilic expression.

To achieve this, pMSN4-GFP vector was developed. It was constructed by inserting genes of geobacillin I two component induction regulation system (*geoR*, *geok*), promoter (*PgeoA*), reporter protein (sfGFP) and terminator (*PgeoT*) into pNW33N plasmid. sfGFP was inserted between *PgeoA* promoter and *PgeoT* terminator. Its expression is regulated by adding subinhibitory amount of geobacillin I. Added geobacillin I binds to a histidine-protein kinase (GeoK) which is then autophosphorylated. Subsequently, phosphate group is transferred to a response regulator (GeoR) which in turn leads to an activation of *PgeoA* promoter. Finally, activated *PgeoA* induces the expression of sfGFP or another protein in its place. Geobacillin I two component induction regulation system and sfGFP were chosen because of their stability at higher temperatures (55 °C).

pMSN4-GFP vector was later successfully inserted into *Parageobacillus thermoglucosidasius* DSM 2542 electrocompetent cells by electroporation method. Geobacillin I produced by *Geobacillus thermodenitrificans* DSM 465 was used for pMSN4-GFP expression system induction. However, sfGFP fluorescence was not detected. Therefore, we will use recombinant geobacillin I which was synthesized in *E. coli* [1] for further induction efficiency assessment.

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## 27. INVESTIGATION OF THE IMPACT OF GLYCOSYLATION DEFECTS ON SECRETION OF RECOMBINANT PROTEINS IN YEAST *SACCHAROMYCES CEREVISIAE*

Andrius Burdulis<sup>1</sup>, Danguolė Žiogienė<sup>1</sup>, Alma Gedvilaitė<sup>1</sup>

<sup>1</sup>Department of Eukaryote Genetic Engineering, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania  
[andrius.burdulis@gmail.com](mailto:andrius.burdulis@gmail.com)

Protein glycosylation is an enzymatic polysaccharide attachment to protein amino acids, and it is one of the most prevalent protein modifications in eukaryotes. Glycans attached to proteins are important for their stability, interactions, correct folding and have significant impact on protein secretion efficiency [1]. Core steps of protein glycosylation in the endoplasmic reticulum (ER) are conserved and similar in all eukaryotes. Dolichol kinase (DK), encoded by the essential SEC59 gene in *Saccharomyces cerevisiae*, resides in the membrane of ER where it phosphorylates dolichol. Synthesized dolichol phosphate is then used as a carrier on which the core oligosaccharide is assembled in a chain of enzymatic reactions performed by glycosyltransferases of the Alg family and then transferred on protein by oligosaccharyltransferase (OST) complex. Glycosylated proteins are then transported from the ER to Golgi apparatus where they are further modified and diverse glycoproteins, important for various cellular functions are formed [2].

Until now there are only few known yeast DK mutants. *S. cerevisiae* DK mutant sec59-1 shows impaired glycosylation and secretion of carboxypeptidase Y (CPY) and invertase whereas *Kluyveromyces lactis* MD2/1-9 mutant also has glycosylation deficiencies but secretes recombinant proteins more efficiently than wild-type cells [3, 4, 5].

The aim of this research was to investigate the impact of deficiencies or mutations of enzymes that participate in the core glycosylation steps in the ER, on protein glycosylation and secretion in yeast *S. cerevisiae*. The state of protein N-glycosylation was evaluated by analyzing CPY glycosylation profile and secretion efficiency of selected model proteins in generated *S. cerevisiae* sec59 mutant strains as well as in null mutants of non-essential alg (alg3, alg5, alg6, alg8, alg9, alg10 and alg12) and ost5 genes. Our results demonstrate that DK mutations, as well as deletion of non-essential alg and ost genes in yeast *S. cerevisiae* enhanced the secretion of recombinant  $\alpha$ -amylase and Kex2 peptidase to various degrees and this increase in secretion efficiency was related to reduced CPY glycosylation.

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## 28. MULTI-STEP PROCESSING OF SINGLE-BACTERIA USING SEMI-PERMEABLE CAPSULES

Greta Leonavičienė<sup>1</sup>, Karolis Leonavičius<sup>1</sup>, Rolandas Meškys<sup>2</sup>, Linas Mažutis<sup>1</sup>

<sup>1</sup>Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania

<sup>2</sup>Institute of Biochemistry, Life Sciences Center, Vilnius University, Lithuania

[greta.stonyte@bti.vu.lt](mailto:greta.stonyte@bti.vu.lt)

Modern molecular biology research increasingly relies on high-throughput analytical methods to process complex samples at a single-cell or single-molecule resolution. Compartmentalization of individual cells, DNA, enzymes, or other biomolecules in water-in-oil droplets (or other forms of microscopic compartments) can assist this effort by enabling massively parallel analysis with a throughput orders of magnitude higher when compared to the microtiter plate platform. However, many biological methods are built on sequential sample processing in order to initiate, modify, or terminate a reaction. These multi-step operations are difficult to implement in droplet, or other types of emulsion-based assays. Sequential sample processing can become very challenging in microbiology assays when encapsulated bacteria, or their genetic material, has to be processed through a series of independent reactions. For example, for the amplification and analysis of genetic material of microorganisms, it may be necessary to perform cell lysis, a step that might be inhibitory or incompatible with subsequent enzymatic step(s). Although some solutions, such as droplet fusion, droplet reinjection, splitting, and sorting, enables multi-step procedures however, the required expertise and complexity of fluidic operations often limits the broader use of such approaches.

To circumvent some of the inherent limitations of microdroplet systems, we combined advantages offered by droplet-based and hydrogel-based systems to create capsules containing a thin, semi-permeable shell. The shell acts as a passive sieve — retaining encapsulated, large molecular weight compounds while allowing smaller molecules (such as proteins) to diffuse through. We used an aqueous two-phase system (ATPS) composed of dextran and acrylate-modified polyethylene glycol to generate the biocompatible hydrogel particles and showcased a few examples of sequential reactions on encapsulated species. Specifically, we demonstrated the capsule use for single genome amplification of Gram-positive and Gram-negative bacteria, and the expansion of individual *Escherichia coli* clones into isogenic microcolonies for later screening for biodegradable plastic production.

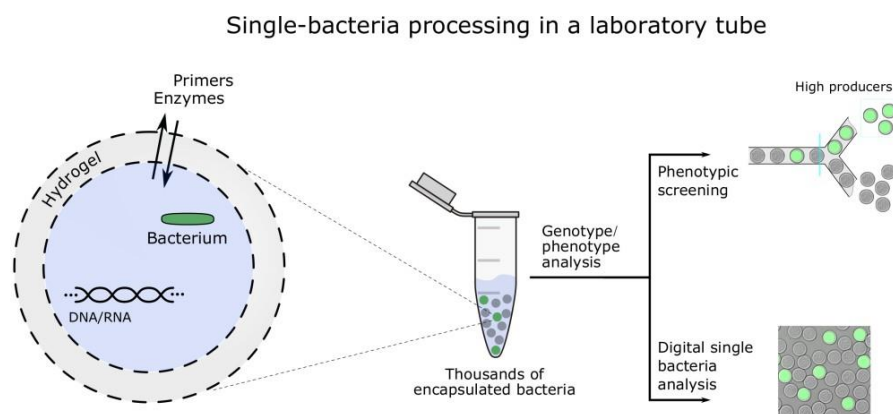


Figure 1. Semi-permeable capsules allow to analyse hundreds of thousands of individual bacteria using conventional molecular biology processing conditions.

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## 29. INVESTIGATION OF *KLUYVEROMYCES MARXIANUS* AS A HOST FOR THE EXPRESSION OF THE HETEROLOGOUS GENES FOR THE BIOCATALYST PRODUCTION

Aušra Kondrataitė<sup>1</sup>, Rimantas Šiekštelė<sup>1</sup>, Inga Matijošytė<sup>1</sup>

<sup>1</sup>Sector of Applied Biocatalysis, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania  
[ausra.kondratyte@gmc.stud.vu.lt](mailto:ausra.kondratyte@gmc.stud.vu.lt)

Biocatalysis is considered to be an effective and environmentally friendly alternative to traditional catalysis in chemical synthesis. It is extremely relevant in industry due to the properties, such as stereo-, chemo-, regioselectivity as well as high catalytic activity and mild operational conditions [1]. Biocatalytic processes have been applied in a number of fields including bioremediation, pharmaceutical, food, textile industries and polymer synthesis, *etc.*

The development of an efficient protein expression system is the key step in terms of biocatalyst production. Since the expression of genes in their native organisms is usually too low, scientists seek to design protein expression systems that would be capable of producing high yields of the target proteins. A typical expression vector consists of several crucial parts – origin of replication, promoter, terminator, and selection marker. To facilitate the secretion and purification of a protein, additional tags may be used. Each of these parts has to be selected wisely according to the gene of interest. The choice of expression host is another important aspect to be considered and, to this date, the most widely used organisms are bacteria and yeast [2].

In this study, we aim to develop a yeast expression system in order to synthesize several industrially important bacterial enzymes – laccase,  $\beta$ -carbonic anhydrase, and lipoxygenase – using *Kluyveromyces marxianus* as a host. *K. marxianus* serves as a highly applicable host regarding the GRAS (generally recognized as safe) status, thermotolerance, fast growth, ability to metabolize wide variety of carbon sources, *etc.* [3] Therefore, this microorganism has a great potential to be exploited in the production of the mentioned biocatalysts.

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## 30. HETEROLOGOUS EXPRESSION OF NOVEL BACTERIOCIN FROM THERMOPHILIC BACTERIA

Akvilė Vilpišauskaitė<sup>1</sup>, Saulė Ulinauskaitė<sup>1</sup>, Algirdas Kaupinis<sup>2</sup>, Mindaugas Valius<sup>2</sup>, Arnoldas Kaunietis<sup>1</sup>

<sup>1</sup>Department of Microbiology and Biotechnology, Institute of Biosciences, Life Sciences Center, Vilnius University, Saulėtekio ave.7, Vilnius, Lithuania

<sup>2</sup>Proteomics Centre, Institute of Biochemistry, Life Sciences Center, Vilnius University, Saulėtekio ave.7, Vilnius, Lithuania

[akvile.vilpisauskaite@gmc.stud.vu.lt](mailto:akvile.vilpisauskaite@gmc.stud.vu.lt)

Bacteriocins are a heterogeneous group of ribosomally synthesised antimicrobial peptides with the ability to kill closely related (narrow spectrum), or a diverse range of (broad spectrum), microorganisms [1]. They are classified into three major classes based on their structural and physicochemical properties: class I bacteriocins, class II bacteriocins, and class III bacteriocins. These antibacterials inhibit the growth of target organisms by functioning primarily on the cell envelope and by affecting gene expression and protein production within cells. These molecules have antimicrobial activity against pathogenic and deteriorating bacteria, which justifies their biotechnological potential. The use of bacteriocins has been reported for the following: food preservation, diverse therapeutic purposes such as treatment of peptic ulcer, spermicidal agent, and woman care, anticancerous agent, veterinary use, skincare, and oral care, and also for plant growth promotion in agriculture among others [2].

The genus *Geobacillus* comprises bacteria that are Gram-positive, thermophilic spore-formers, which are found in a variety of environments from hot-springs, cool soils, to food manufacturing plants, including dairy manufacturing plants [3]. *Geobacillus* has historically been associated with spoilage of canned food. However, in recent years it has become the subject of much attention due its biotechnological potential. One aspect of this genus that has not been fully explored or realized is its use as a source of novel forms of the ribosomally synthesized antimicrobial peptides known as bacteriocins [4]. Bacteriocins from thermophilic bacteria have the potential to be used in various industries that maintain higher temperatures, since thermophilic bacteria proteins are usually thermostable.

*Geobacillus stearothermophilus* 15, isolated from oilfield located in Lithuania, produces two bacteriocins geobacillin 26 (Geo26) and geobacillin 19 (Geo19). Since Geo26 have already been purified, heterologously expressed in *Escherichia coli* and characterized, the aim of our study is to clone, perform heterologous expression of recombinant novel Geo19 bacteriocin in *E.coli* and to characterize it. This bacteriocin can be assigned to class II, it has similarity to already characterized bacteriocin SDP. Native Geo19 is active against thermophilic bacteria, which often survive high temperatures during heat treatment of food and could therefore be widely applied in the food industry.

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# 31. IMMOBILIZATION OF ENZYMES VIA APPLICATION OF TRANSGLUTAMINASE

Ieva Ožiūnaitė, Jokūbas Krutkevičius, Inga Matijošytė

Sector of Applied Biocatalysis, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania  
E-mail: [ievaoziunaite@gmc.stud.vu.lt](mailto:ievaoziunaite@gmc.stud.vu.lt)

During the past decades, the application of enzymes in industrial processes has increased remarkably. Enzymes are versatile catalysts of biological origin that can catalyze reactions with great specificity and stereoselectivity under the benign environmental conditions (low temperature and pressure, aqueous medium, etc.)<sup>1</sup>. Enzymes could be applied almost everywhere, from the manufacturing of high-added value products to the degradation of plastic waste. The use of enzymes in industrial sectors underpins the transition of our society toward a more ecological and cost-effective economy. Despite all these advantages, industrial application of enzymes is often hampered by a lack of long-term operational stability and difficult recovery and re-use of the enzyme. These limitations can generally be overcome by immobilization of enzymes. These techniques improve enzyme properties, such as stability, facilitates separation from the product, enhance the efficient recovery and re-use of the enzyme. Basically, methods of enzyme immobilization can be divided into two categories – binding to a support and without support material (cross-linked enzymes), the latter is more practical and cost-effective<sup>2</sup>. However, the main struggle of the current technique is not sufficient mechanical stability and weak resistance to proteolytic degradation.

Transglutaminases (EC2.3.1.13) are a family of enzymes that catalyze an acyl transfer between peptide bound glutamine and  $\epsilon$ -amino group of the peptide bound lysine, resulting in most instances in the cross-linking of proteins *via* intra or inter  $\epsilon$ -( $\gamma$ -glutamyl)lysine isopeptide bonds<sup>3</sup>. Bonds formed by transglutaminase exhibit a high resistance to proteolytic degradation. This enzyme could be an alternative way to get more effective and stable enzyme immobilization method.

By this study we have investigated transglutaminase capability to cross-link lipase enzyme. The first goal was to purify enzyme from commercial transglutaminase mixture which contain additional substances. The second goal was to apply purified transglutaminase for immobilization of lipase. The obtained results in more detail will be presented during the poster session.

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## 32. ANTIMICROBIAL EFFECT OF GEOBACILLIN 26 BY ATOMIC FORCE MICROSCOPY

Mantė Rakauskaitė<sup>1,2\*</sup>, Arnoldas Kaunietis<sup>1</sup>, Marija Jankunec<sup>2</sup>

<sup>1</sup>Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania

<sup>2</sup>Institute of Biochemistry, Life Sciences Center, Vilnius University, Vilnius, Lithuania

[\\*mante.rakauskaite@gmc.stud.vu.lt](mailto:mante.rakauskaite@gmc.stud.vu.lt)

In the worldwide fight against bacterial resistance, there is a need for a novel family of antimicrobial drugs. Antimicrobial peptides (AMPs) are promising candidates to replace antibiotics. Still, the majority of them have an unclear mechanism of action. Atomic force microscopy (AFM) is a surface-sensitive technique and aims to study the physical properties of living biological cells under physiologically relevant conditions without the destruction of the sample. However, the studies involving live bacteria limits the necessity to attach a specimen to the substrate. Therefore, the existing protocol needs to be adapted. Here we present a protocol to immobilize Gram-positive bacterial cell membranes.

This study focuses on the antimicrobial effect of heat-labile Geobacillin 26 peptide against Gram-positive bacteria. The bacteria are attached to the substrate via crosslinking with glutaraldehyde. Time-resolved AFM images revealed no changes in the topography of the affected cell wall. This result is in line with recent studies, Geobacillin 26 was named as not a cell wall degrading enzyme [1]. However, the differences in cell volume of affected bacteria from a control group showed the leakage of inside fluids. That indicates the loss of membrane integrity. But the mode of action of Geobacillin 26 remains unclear. For this purpose, before and after the impact of Geobacillin 26, bacteria were evaluated by force spectroscopy (Fig.1). The technique where subtle changes in cell wall elasticity are detected.

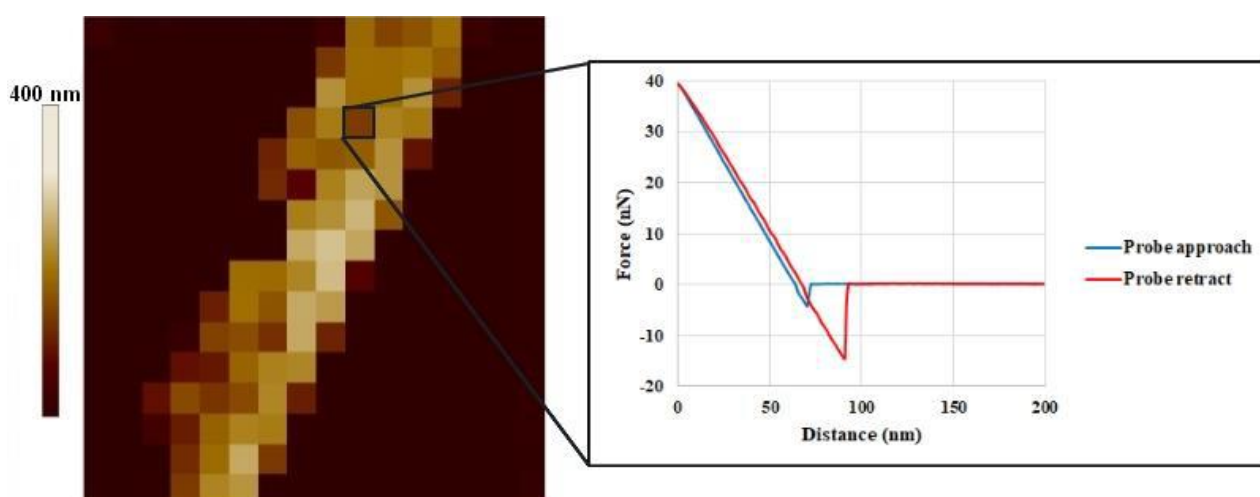


Fig. 1. Force spectroscopy analysis of bacteria control group.

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### 33. DESIGNING AN IMMUNO-PCR ASSAY FOR PNEUMOLYSIN DETECTION

Rugilė Jurevičiūtė<sup>1</sup>, Indrė Kučinskaitė-Kodzė<sup>1</sup>

<sup>1</sup>Life Sciences Center of Vilnius University, Lithuania  
[rugile.jureviciute@gmc.stud.vu.lt](mailto:rugile.jureviciute@gmc.stud.vu.lt)

According to the World Health Organization pneumonia is the leading cause of deaths from infectious diseases in children. Various pathogens can cause pneumonia but the most prevalent is *Streptococcus pneumoniae* (pneumococcus). This bacteria is found in the human respiratory tract's mucus and can result in pneumonia or more complicated cases such as otitis, sinusitis, even meningitis and sepsis. Pneumococcus is an opportunistic pathogen and has various mechanisms for evading the host immune system, making it tricky to detect and treat [1].

In current clinical practice, *S. pneumoniae* can be detected by microbiological and molecular biology methods; however, most of these methods lack sensitivity, and sample preparation is time-consuming [2]. To counter these throwbacks, we are adopting an immuno-PCR assay to detect *S. pneumoniae* toxin pneumolysin. Immuno-PCR is a chimera of two already widely used methods - enzyme-linked immunosorbent assay (ELISA) and quantitative PCR (qPCR). ELISA's main advantage is the specificity of monoclonal antibodies but requires a considerable amount of analyte to produce the signal. qPCR solves this problem with its ability to amplify the signal, although the sample takes longer to prepare. Immuno-PCR utilizes both of these methods and allows to detect of trace amounts of proteins in the samples.

Here, we are developing a system that consists of a pair of monoclonal antibodies (MAb) specific to pneumolysin - one immobilized on the plate surface and other conjugated with a nucleotide used for qPCR (Figure 1). When a sample is added PLY attaches to immobilized I MAb. Then, II MAb is added and binds to the I MAb and PLY complex. The oligonucleotide is separated from the complex with a restriction endonuclease, collected and amplified by qPCR. The qualities of immuno-PCR should highly enhance sensitivity and accuracy in detecting *S. pneumoniae* infection compared to currently used commercial tests. Here, we are developing a system that consists of a pair of monoclonal antibodies (MAb) specific to pneumolysin - one immobilized on the plate surface and other conjugated with a nucleotide used for qPCR (Figure1). When a sample is added PLY attaches to immobilized I MAb. Then, II MAb is added and binds to the I MAb and PLY complex. The oligonucleotide is separated from the complex with a restriction endonuclease, collected and amplified by qPCR. The qualities of immuno-PCR should highly enhance sensitivity and accuracy in detecting *S. pneumoniae* infection compared to currently used commercial tests.

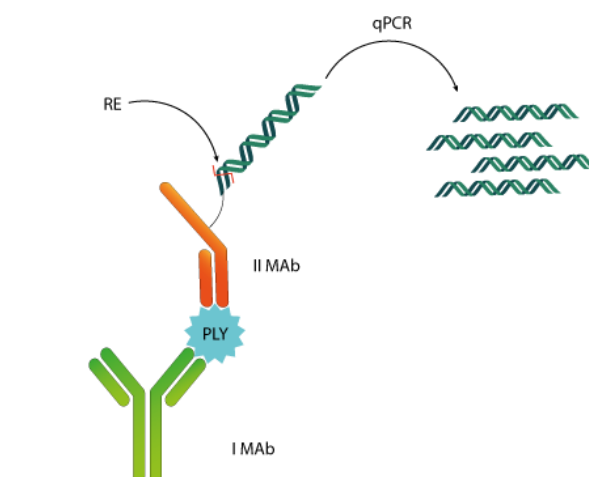


Figure1. Schematic model of immuno-PCR assay. PLY - pneumolysin, I MAb and II MAb - monoclonal antibodies, RE - restriction endonuclease, green spiral - oligonucleotide.

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## 34. SEARCH OF POLYOMAVIRUSES IN WILD BOAR BLOOD SERUM SAMPLES

Lina Krikščikaitė, Emilija Vasiliūnaitė, Alma Gedvilaitė

Vilnius University, Life Sciences Center, Institute of Biotechnology, Department of Eukaryote Genetic Engineering  
[lina.kriksikaite@gmail.com](mailto:lina.kriksikaite@gmail.com)

Viruses are infectious agents with RNA or DNA genomes that can replicate only inside the living cells of an organism. Viruses can infect all types of life forms and are important objects of scientific research. In this study, we investigated polyomaviruses (PyVs). PyVs are members of the Polyomaviridae family. They have small double-stranded circular DNA genomes of around 5000 base pairs. PyVs are very ubiquitous in human and animal populations, but symptoms of infection are usually detected in immunocompromised hosts. Up to now there are identified 13 PyVs species which could infect human, few of them are associated with cancerogenesis, other ones can affect respiratory and urinary tracts or other organ systems and cause diseases. PyVs of some animals can switch from one host to another host of related species. Wild boars (*Sus scrofa*) are a source of various pathogens including PyVs.

In this study, we tested 40 boar blood samples looking for PyVs DNA. Viral DNA was purified by using a commercial kit. Purified DNA was amplified with EquiPhi29 polymerase and used for further investigation which included two different types of polymerase chain reaction (PCR). We used nested PCR method with primers specific to a large T antigen (LTag) encoding gene and detected PyVs DNA in 2 samples of a total of 40 samples. Detected DNA sequences were most similar to the DNA sequences of human Merkel cell polyomavirus (MCPyV). Further analysis of samples by PCR with different sets of primers specific to LTag or capsid protein VP1 encoding genes proved that amplified DNA sequences were similar to LTag and VP1 gene sequences of MCPyV. New or already known PyVs of wild boars were not detected in tested samples.

In conclusion, it might be assumed that the presence of MCPyV DNR in tested samples might be a result of zoonosis or contamination.

## 35. ANTIOXIDANT ACTIVITY COMPARISON BETWEEN DIFFERENT CONCENTRATIONS OF NUTMEG SEED ESSENTIAL OIL

Inga Matulytė<sup>1,2</sup>, Giedrė Kasparavičienė<sup>1</sup>, Akvilė Mataraitė<sup>3</sup>, Jurga Bernatoniene<sup>1,2</sup>

<sup>1</sup>Department of Drug Technology and Social Pharmacy, Lithuanian University of Health Sciences, Lithuania

<sup>2</sup>Institute of Pharmaceutical Technologies, Lithuanian University of Health Sciences, Lithuania

<sup>3</sup>Faculty of Medicine, Lithuanian University of Health Sciences, Lithuania

[inga.matulyte@lsmu.lt](mailto:inga.matulyte@lsmu.lt)

Most plants, herbs, fruits, including nutmeg seeds, have an antioxidant effect [1]. It is necessary for the binding of free radicals and the protection of a live organism from negative influences, such as oxidative stress, which can cause central nervous system diseases, diabetes, age-related changes in appearance [2]. Not only flavonoids or phenols have an antioxidant effect, volatile compounds from essential oils (*Origanum vulgare*, *Salvia Rosmarinus*, *Syzygium aromaticum*, and other) can also bind free radicals and protect an organism from damage [3,4]. The aim of this study was to compare the dependence of antioxidant activity on different concentrations and different incubation time intervals in the dark of nutmeg essential oil.

Dried nutmeg seeds' (*Myristica fragrans*) country of origin is Grenada. Essential oil was prepared by using hydrodistillation – with a Clevenger-type apparatus. Hydrodistillation lasted 4 hours and the nutmeg powder was mixed with distilled water in a ratio for extraction of 1:20. After hydrodistillation, the hydrolat and essential oil were collected. Essential oil was separated from hydrolat and stored in an airtight bottle in refrigerator at 4 °C. Antioxidant activity was evaluated by using 2,2-diphenyl-1-picrylhydrazyl (DPPH, Sigma Aldrich, USA). A total of 1 mL of ethanolic DPPH solution (0.1 mM) was placed in a spectrophotometer cuvette and 100 µL of ethanolic essential oil solutions (from 0.2% to 20%) were added. All samples were incubated in the dark for 20 and 30 minutes, and absorbance was taken at 515 nm (UV Spectrophotometer UV-1800 (Shimadzu, Kyoto, Japan)). The results are presented as mean ± SD.

Antioxidant activity of nutmeg essential oil varied from 12.63±0.53% to 84.01±0.14% and from 13.82±0.19% to 85.00±0.19% (after 20 minutes and 30 minutes, respectively; fig. 1). A linear correlation between antioxidant activity and nutmeg essential oil's concentration was determined. Statistically significant changes ( $p < 0.05$ ) of antioxidant activity were determined in four out of seven samples (when comparing the dependence on the sample's time in the dark). After 30 minutes, samples with 1%, 2%, 5%, and 10% of nutmeg essential oil had a significantly higher antioxidant activity than the samples after 20 minutes (the average of differences of antioxidant activity is about 7.19%). In samples where the essential oil concentration was 0.2%, 0.5%, and 20% – significant variation was not found. Antioxidant activity of nutmeg essential oil varied from 12.63±0.53% to 84.01±0.14% and from 13.82±0.19% to 85.00±0.19% (after 20 minutes and 30 minutes, respectively; fig.1). A linear correlation between antioxidant activity and nutmeg essential oil's concentration was determined. Statistically significant changes ( $p < 0.05$ ) of antioxidant activity were determined in four out of seven samples (when comparing the dependence on the sample's time in the dark). After 30 minutes, samples with 1%, 2%, 5%, and 10% of nutmeg essential oil had a significantly higher antioxidant activity than the samples after 20 minutes (the average of differences of antioxidant activity is about 7.19%). In samples where the essential oil concentration was 0.2%, 0.5%, and 20% – significant variation was not found.

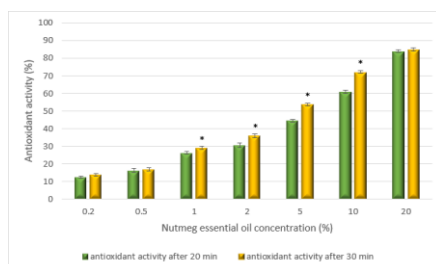


Figure 1. Antioxidant activity of nutmeg essential oil. \*  $p < 0.05$  versus antioxidant activity of samples after 20 min in the dark.

Absorption equilibrium is reached as early as 20 min in nutmeg essential oil samples at concentrations of 0.2%, 0.5%, and 20%, this is shown by statistically insignificant results

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## 36. SYNTHESIS AND BIOLOGICAL PROPERTIES INVESTIGATIONS OF SUBSTITUTED HYDRAZONES CONTAINING 1-PHENYLPYRAZOLE MOIETY

Ineta Meldaikytė<sup>1</sup>, Julija Mickevičiūtė<sup>1</sup>, Vlada Ganytė Oginskytė<sup>1</sup>, Indrė Styraitė<sup>1</sup>, Vilija Kederienė<sup>1</sup>

<sup>1</sup>Department of Organic Chemistry, Kaunas University of Technology  
[ineta.meldaikyte@ktu.edu](mailto:ineta.meldaikyte@ktu.edu)

1-Phenylpyrazole derivatives has many pharmacological, agrochemical, biological and chemical properties. There are drugs which excel anti-inflammatory effect, act as a cannabinoid receptor, suppress alcohol dehydrogenase or phosphodiesterase [1]. Also, 1-phenylpyrazole compounds act as antimicrobial, anticancer, antitubercular pharmaceuticals [2]. Pyrazole derivatives have been extensively studied from a biological point of view, but there are still a number of unexplored areas of pyrazole chemistry. One of the less studied areas is the synthesis of phenylpyrazole-based hydrazones and the study of biological activity. The application of hydroxypyrazoles is attractive because it is inexpensive starting material for the synthesis of pyrazole-hydrazone derivatives. Hydrazone and its derivatives contain biological activities as antioxidant, analgesic, antiparasitic, antiviral and antitumor [3, 4].

The aim of this work is to synthesize and investigate biological activity of new hydrazone analogues, containing 1-phenyl-1H-pyrazole scaffold.

Hydrazone derivatives containing 1-phenyl-1H-pyrazole moiety were obtained using several synthesis ways. Initially, 1-phenyl-3-hydroxypyrazole was obtained from phenylpyrazolidone using iron trichloride. Then alkylation reactions of hydroxypyrazole were performed by using benzyl bromide as alkylating agent. Finally, O-benzylated 1H-pyrazole-4-carbaldehyde was formed via Vilsmeier-Haack complex and functionalized by using different hydrazine reagents. The yields of the synthesized hydrazone compounds ranged between 46–91 %.

Biological studies of pyrazole-hydrazones have been extensively fulfilled. Antimicrobial study, reduction properties, DPPH inhibition, ABTS inhibition, antioxidant effect according to FRAP method and AOA were evaluated. Also, there were assessed studies of the germination of *Festuca arundinacea* and *Festuca rubra*. The synthesized compounds were tested for their in vitro antimicrobial activity using the disk diffusion method against Gram-negative bacteria *Rhizobium radiobacter*, *Escherichia coli* and *Xanthomonas campestris*.

3-(Benzyloxy)-4-[(E)-[2-(2-methylphenyl)hydrazinylidene]methyl]-1-phenyl-1H-pyrazole had the best antimicrobial properties, even at the lowest concentration. 3-(Benzyloxy)-4-[(E)-[2-(2-methylphenyl)hydrazinylidene]methyl]-1-phenyl-1H-pyrazole neutralized 100 % free DPPH radicals. The 3-(benzyloxy)-1-phenyl-4-[(E)-[2-(4-trifluoromethyl)phenyl]hydrazinylidene]methyl]-1H-pyrazole had the greatest influence on *Festuca rubra* and *Festuca arundinacea* germinated best with 3-(benzyloxy)-4-[(E)-(diphenylhydrazinylidene)methyl]-1-phenyl-1H-pyrazole.

Hydrazones containing 1H-phenylpyrazole scaffold showed the beneficial biological effect and might have potential application in various fields of medicine and advanced technologies.

Acknowledgements. Part of this work has received funding from the Research Council of Lithuania (LMTLT), agreement No [09.3.3-LMT-K-712-22-0082].

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## 37. SYNTHESIS AND BIOLOGICAL PROPERTIES OF N-ALKYLATED BENZO[B ]THIOPHENE-2-CARBOXYLATE DERIVATIVES

Paulina Voznikaitė<sup>2</sup>, Indrė Jaglinskaitė<sup>1</sup>, Gabrielė Armonaitė<sup>1</sup>, Vilija Kederienė<sup>1</sup>, Algirdas Šačkus<sup>1,2</sup>

<sup>1</sup>Department of Organic Chemistry, Faculty of Chemical Technology, Kaunas University of Technology, Radvilėnų pl. 19, LT-50254 Kaunas, Lithuania

<sup>2</sup>Institute of Synthetic Chemistry, Kaunas University of Technology, K. Baršausko g. 59, LT-51423 Kaunas, Lithuania  
[paulina.voznikaite@ktu.lt](mailto:paulina.voznikaite@ktu.lt)

Benzo[b]thiophene ring containing compounds are considered a pharmacologically important active scaffold that demonstrates almost all types of biological properties such as antioxidant, antibacterial, anticancer, anti-inflammatory and significantly more [1,2]. Previous studies have shown that benzo[b]thiophene derivatives demonstrated impressive results and high selectivity which greatly extends the limits of these compounds in new drug design or biotechnology. The presence of sulfur atom in different structures and molecules leads to varied applications in different areas such as technology, medicine and agriculture [3].

In order to produce new chemical substances that could potentially be developed into a new pharmacy preparations by optimizing its beneficial effects and minimizing its side effects, in this work the N-alkylated heterocyclic amines have been synthesized and biological properties of novel compounds were investigated.

The N-arylation of 3-aminobenzo[b]thiophene-2-carboxylates with a variety of functional group substituted aryl iodides was found to be greatly simplified by inexpensive, air-stable, catalyst systems, combining catalytic copper iodide salt with a set of structurally simple L-proline ligand. Mild, efficient, copper-catalyzed C-N coupling procedures for mentioned heterocyclic compounds were described in this work.

DPPH, ABTS and FRAP free radical scavenging methods are usually applied for measuring the antioxidant capacity of bioactive compounds. The purple color of DPPH is reduced to the corresponding pale yellow hydrazine as the free radical receives a hydrogen atom from antioxidant. The absorption band of resulting decolorization is registered at 517 nm. The free radical scavenging activity of a molecule can be evaluated by its effect on stable green colored solution of radical ABTS. The reaction is monitoring spectrophotometrically at 734 nm. The FRAP assay is used, and the antioxidant activity of examined compounds is characterized by their capacity to directly reduce Fe (III) to Fe (II), measuring the reduction reaction around 593 nm [4].

The synthesized N-substituted compounds were tested for their in vitro antimicrobial activity using the disk diffusion method against *R. radiobacter*, *X. campestris*, *E. coli* and *B. subtilis* bacteria.

The strongest antioxidant activity was observed in those benzo[b]thiophene ring containing compounds which had phenyl ring substituted in the para- position. Meanwhile, the strongest antibacterial properties showed derivatives with the ortho- position substituted on the phenyl ring.

N-alkylated benzo[b]thiophene-2-carboxylate derivatives exhibited the beneficial biological effects and might be promising for the monitoring of diseases, particularly those associated with free radical scavenging or therapeutic treatment against bacteria.

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## 38. PROTEOLYTIC STABILITY OF NISIN-LOADED ULVAN PARTICLES

Edita Kodytė<sup>1</sup>, Rūta Gruškienė<sup>1</sup>, Jolanta Sereikaitė<sup>1</sup>

<sup>1</sup>Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Lithuania  
[edita.kody@gmail.com](mailto:edita.kody@gmail.com)

One of the most popular bacteriocins in the food industry is nisin. It is recognized in more than 50 countries as a safe food preservative. Nisin does not harm human health, does not change the organoleptic properties of food, and has a broad antimicrobial spectrum [1]. However, the antimicrobial activity and stability of nisin may be reduced due to interactions with food compounds or due to environmental factors. Various encapsulation systems are being developed to solve this problem [2]. In this work, for particles preparation ulvan was used. Ulvan is a water-soluble anionic sulphated polysaccharide, which is derived from green algae, Ulvales. Ulvan is non-toxic, biocompatible and biodegradable. It also has a wide range of biological activity: antibacterial, immunostimulatory, anticancer, antioxidant, antiviral, anticoagulant effect. Due to its special properties, ulvan can be used to encapsulate nisin, thus protecting the nisin and providing additional beneficial properties [3].

Our study aimed to compare the proteolytic stability of nisin-ulvan loaded particles with free nisin.

Two types of proteases with different pH optimum and cleavage specificity were used, i.e. the protease Type XIII from *Aspergillus saitoi* and trypsin from porcine pancreas. The first protease was dissolved in acetic buffer solution at pH 5 and trypsin was dissolved in ammonium bicarbonate solution at pH 7. Nisin-loaded ulvan and free nisin samples at pH 5 and 7 were prepared, and were affected by protease Type XIII and trypsin, respectively. The concentration of ulvan in samples was 0.4 mg/mL, and the nisin concentration was in the range of 0.2–0.5 mg/mL. Proteolysis was performed for 24 h at 37 °C. Finally, all samples were centrifuged and the content of the proteolytic degradation products was analysed by the capillary zone electrophoresis method using 7100 Capillary Electrophoresis unit (Agilent Technologies).

Four peaks of protease from *Aspergillus saitoi* degradation products with different mass to charge ratios were observed in nisin samples. In the electrophoregram of nisin samples hydrolysed by trypsin, three major and three lower intensity peaks were obtained, the area of which also depends on the nisin content. If nisin-loaded ulvan particles undergo proteolytic degradation, the profile of the electrophoregram is the same as the sample containing only free nisin. However, the area of the respective peaks is different, i.e. the area of almost all peaks of nisin-loaded ulvan particles is smaller. Based on the obtained results, it can be stated that encapsulated nisin is more resistant to proteolytic degradation.

**Acknowledgements:**

We thank Professor Vassilios Roussis from the National and Kapodistrian University of Athens for the gift of the ulvan sample used in this study.

This research was funded by the European Social Fund under the No 09.3.3-LMT-K-712 “Development of Competences of Scientists, Other Researchers and Students through Practical Research Activities” measure. Grant No 09.3.3.-LMT-K-712-22-0040.

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# 39. THE INVESTIGATION OF TOTAL PHENOLIC CONTENT AND ANTIOXIDANT ACTIVITY OF THE TRIFOLIUM PRATENSE L. BLOSSOMS EXTRACTS

Jurga Andrėja Kazlauskaitė<sup>1,2</sup>, Jurga Bernatoniienė<sup>1,2</sup>

<sup>1</sup>Department of Drug Technology and Social Pharmacy, Lithuanian University of Health Sciences, Lithuania

<sup>2</sup>Institute of Pharmaceutical Technologies, Medical Academy, Lithuanian University of Health Sciences, Lithuania.

[jurga.andreja.kazlauskaite@stud.lsmu.lt](mailto:jurga.andreja.kazlauskaite@stud.lsmu.lt)

Red clover (*Trifolium pratense* L.) is an important forage plant that have been studied lately due to their health benefits [1]. *Trifolium* species have been reported to contain a wealth of biologically active secondary metabolites, of which phenolic compounds are one of the main classes [2]. These compounds have the scavenger capacity for oxidative free radicals, such as those derived from lipids and nucleic acids that underlie their utility in reducing the risk of certain age-related degeneration's [3] Isoflavones, the major phenolics present in the red clover, are categorized as phytoestrogens. Due to their structural similarity to  $\beta$ -estradiol, isoflavones may have a role in cancer prevention and the moderation of menopausal symptoms [4]. The aim of this study was to evaluate the total phenolic content and antioxidant activity of the *Trifolium pratense* L. flower heads extracts.

Plant aerial parts were harvested and dried, and then hydroalcoholic extracts were prepared. Ultrasound assisted extraction was performed using  $0.3 \pm 0.001$  g of dried and milled flower heads and 10 mL of 50 % ethanol. Temperature during extraction 40 and 60 °C. Sonication time 10 and 30 min. Part of the samples were heated for 1 hour after the sonication. Heat reflux extraction was done using  $0.3 \pm 0.001$  g of dried and milled flower that was mixed with 10 mL of 50% ethanol in a 250 mL round bottom flask and refluxed at 100 °C for 1 hour. Maceration results were used as control. Total phenolic content was determined based on Folin–Ciocalteu [5] method. Furthermore, has been evaluated using 2,2'-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid) (ABTS) [6] were used to measure the antioxidant capacity of T. Pratense hydroalcoholic extract. All experiments were performed in triplicate.

The results demonstrated that the red clover is a rich source of phenolic compounds. The highest yields of phenolic compounds were determined in the samples UH1 (46.57 mg GAE/g) and UH4 (49.95 mg GAE/g). Best antioxidant capacity showed the samples UH1 (67.06%), UH3 (67.77%), UH4 (88.23%) and HR (71.48%).

A significant correlation observed in the antioxidant activity of the extracts and their total phenolic content. An increase in antioxidant levels was observed with increasing levels of phenolic compounds. Increasing sonication time and temperature resulted in an increase in phenolic compounds. By prolonging the sonication time and increasing the temperature from 40 to 60 °C, the amounts of phenolic compounds in the extracts increased.

The authors would like to thank Open Access Centre for the Advanced Pharmaceutical and Health Technologies (Lithuanian university of Health Sciences) and for the opportunity to use modern infrastructure and perform this research. The authors declare no conflicts of interest.

This work was supported by the Research council of Lithuania grant no. 09.3.3 ESFA V 711 01 0001

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## 40. A CONVENIENT SYNTHETIC APPROACH FOR THE PREPARATION OF INDOLE-3-ACETIC ACID ANALOGUES

Selvina Bezaraite<sup>1</sup>, Monika Iškauskienė<sup>1</sup>, Asta Žukauskaitė<sup>2</sup>, Vida Malinauskienė<sup>1</sup>

<sup>1</sup>Department of Organic Chemistry, Kaunas University of Technology, Lithuania

<sup>2</sup>Department of Chemical Biology, Palacký University, Czech Republic

[selvina.bezaraite@ktu.edu](mailto:selvina.bezaraite@ktu.edu)

Plant hormones auxins, represented by indole-3-acetic acid (IAA) as the most important naturally occurring member of the family, influence numerous aspects of plant growth and development. Nowadays, phytohormone research largely depends on development of new phytohormone mimics which can be used to study and control phytohormone action and signalling. Ever since discovery of IAA as a plant growth regulating substance more than 80 years ago, plethora of synthetic auxin analogues have been developed. A number of them have found application as herbicides in agronomy and/or are commonly used in various biological applications. For instance, PEO-IAA antagonizes various auxin effects by competitively inhibiting the action of auxins by blocking the interaction between auxin receptor TIR1 and Aux/IAA proteins, while 5-F-IAA can be used to discriminate between transcriptional and non-transcriptional pathways of auxin signalling. Therefore, it is of a great importance to find ways to efficient produce IAA derivatives which could be of potential interest in plant research.

In this work, we employed Fischer indole synthesis as a convenient approach to access IAA starting from cheap starting material  $\gamma$ -butyrolactone. The structures of the obtained compounds were elucidated and confirmed by NMR, IR and mass spectrometry.

Acknowledgements:

This work was funded by the European Social Fund under the "Development of Competences of Scientists, other Researchers and Students through Practical research Activities" measure (No 09.3.3-LMT-K-712-22-0115).

# 41. DETECTION OF INTRACELLULAR BIOMARKERS IN MAMMALIAN CELLS USING PULSED ELECTRIC FIELDS

Justina Kavaliauskaitė<sup>1</sup>, Vytautas Kašėta<sup>2</sup>, Aida Kaušylė<sup>2</sup>, Arūnas Stirke<sup>1</sup>

<sup>1</sup>Department of Functional Materials and Electronics, Center for Physical Sciences and Technology, Lithuania

<sup>2</sup>Department of Stem Cell Biology, State Research Institute Centre for Innovative Medicine, Lithuania  
[justina.kavaliauskaite@ftmc.lt](mailto:justina.kavaliauskaite@ftmc.lt)

Reversible electroporation is the temporary permeabilization of cell membrane through the formation of transient pores created by short high voltage pulsed electric fields. This method has numerous applications in biology and biotechnology and has become an important technique in molecular medicine. Reversible electroporation is usually used to transfer macromolecules into the cells. However, the delivery of large molecules such as proteins into cells without loss of cell viability remains a challenge. In our study, we investigated whether pulsed electric fields parameters can be optimized for this purpose. The study was performed with Jurkat cell line and the primary mouse splenocytes.

We used several different pulsed electric fields parameters (with electric pulses of millisecond duration, msPEF) and an ELPORA devise designed at a Center for Physical Sciences and Technology [1] to investigate optimal conditions to introduce large molecules into cells that would, at the same time, allow retaining high viability rate. We used fluorescein-conjugated dextran as a size control and a labelled antibody against one of the most abundant proteins – actin – to determine if parameters under question allow transfer of intact functional protein in a well-established cell line environment. The electroporation efficacy and cell viability were evaluated by flow cytometry. After optimal conditions were determined, we further analyzed the efficiency of transfer of more specific antibody against a disease marker – interleukin-6 (IL-6) – in a primary mouse splenocytes. Splenocytes were specifically stimulated to produce IL-6.

We determined that msPEF parameters can be optimized for efficient transfer of intact large molecules such as antibodies/proteins into live cells without a significant loss of cell viability. We conclude that msPEF parameters can be successfully adjusted to detect intracellular biomarkers in viable cells. This is a new approach on how pulsed electric fields could be used in medicine and biological research to detect rare subpopulations of cells that produce specific markers and keep cells viable for further purposes. This would allow the use of these rare subpopulations of isolated cells for further research and personalized medicine [2].

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## 42. SYNTHESIS OF 2H-DIPYRAZOLO[1,5-a:4'3'-c]PYRIDINES

Inesa Zagorskytė, Eglė Arbačiauskienė and Algirdas Šačkus

Department of Organic Chemistry, Faculty of Chemical Technology, Kaunas University of Technology,  
Lithuania

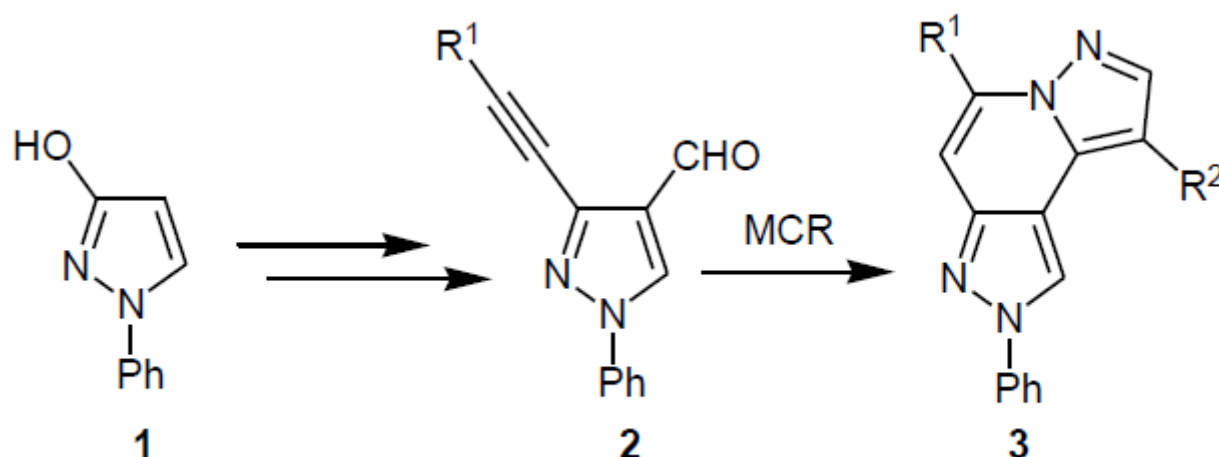
[inesa.zagorskyte@ktu.edu](mailto:inesa.zagorskyte@ktu.edu)

Condensed pyrazole derivatives possess a wide variety of pharmacological and biological activities such as anti-microbial, anti-fungal, anti-tubercular, anti-inflammatory, anti-convulsant, anticancer, anti-viral, angiotensin converting enzyme (ACE) inhibitory, neuroprotective and many others [1]. Betazole, celecoxib, antipirine, axitinib and other well-know drugs have a pyrazole nucleus in their structure.

Multicomponent reactions (MCR) has become a powerfull tool for the preparation of highly functionalized organic compounds in modern synthetic organic chemistry. [2]. MCR allows to build quick libraries of compounds of biological interest to identify new leads as potential therapeutic agents.

In continuation of our interest in synthesis and investigation of biological activity of pyrazole containing condensed heterocyclic compounds [2], herein we present an access to yet unknown 2H-dipyrzolo[1,5-a:4'3'- c]pyridine system employing a MCR approach.

A starting compound 1-phenyl-pyrazol-3-one (1) was transformed to 3-alkynyl-1-phenyl-1H-pyrazoles following a few step procedure we have previously reported [3a]. Compounds 2 were further reacted in a multicomponent reaction affording new polycyclic compounds with a 2H-dipyrzolo[1,5-a:4'3'-c]pyridine core (Scheme 1).



Scheme 1. Synthesis of 2H-dipyrzolo[1,5-a:4'3'-c]pyridines

The structures of the resulting compounds were confirmed by meas of LC/MS and NMR spectroscopy.

Acknowledgements:

This work was supported by the Research Council of Lithuania (Project No. S-MIP-20-60).

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### 43. ARABIDOPSIS THALIANA (L.) HEYNH. SEEDLING RESPONSE TO COLD PLASMA TREATMENT OF SEEDS IS MEDIATED BY REDOX ENZYMES.

Edgaras Tamelis<sup>2</sup>, Lauryna Ragauskaitė<sup>1</sup>, Dalia Gelvonauskiene<sup>1</sup>, Danas Baniulis<sup>1</sup>

<sup>1</sup>Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry,  
Babtai, Kaunas reg., Lithuania,

<sup>2</sup>Faculty of Natural Sciences, Vytautas Magnus University, Kaunas, Lithuania  
[e.tamelis@gmail.com](mailto:e.tamelis@gmail.com)

Successful seed germination and seedling establishment are critical steps in agricultural production and in the maintenance of natural ecosystems. Seed treatment with cold plasma (CP), is an emerging eco-agricultural technology that has potential application for enhancement of plant growth and adaptation. In addition to sterilization effect, treatment of seeds with CP has been shown to have effect on a broad spectrum of plant development and physiological processes. Although the body of information on the biological effects of the CP is growing, the complex biological mechanisms involved in the plant response remains vague. The objective of this study was to assess role of reactive oxygen species (ROS) producing and redox balance regulating enzymes in response of the model plant *Arabidopsis thaliana* (L.) Heynh. to the seed treatment with atmospheric pressure dielectric barrier discharge plasma source. Seeds of *A. thaliana* Columbia ecotype wild-type (Col-0) and gene knockout mutant lines of ROS producing enzymes NADPH oxidase AtRbohB and AtRbohF and enzymes involved in maintenance of the cellular redox balance, AtAPX1 and AtCAT2, were used in the study. Seeds were treated for 1.5 or 3.0 min at 3 mm distance from the CP source at 60–80% air humidity and stored for 7 days at 25 °C after the treatment. The significant changes in root length was observed for the 2 day old seedlings of Col-0, AtRbohB, AtRbohF and AtCAT2 germinated from the seeds treated for 3 min. AtRbohF showed an enhanced (~6 % compared to control) root growth response similar to that of the wild-type genotype, meanwhile the length of roots was reduced for the AtRbohB and AtCAT2 mutants (20 % and 10 %, respectively). In contrast to the effect observed for the roots, 3 min CP treatment had stimulating effect on hypocotyl development of the AtAPX1 mutant (~7 %). Meanwhile hypocotyls of the AtRbohF and AtCAT2 were shorter compared to control (9 % and 23 %, respectively). Hypocotyls of the Col-0 and AtRbohB mutant showed no response to CP treatment. The analysis confirmed that the redox enzymes play essential role in *A. thaliana* seedling phenotype formation in response to CP treatment and the effect could be linked to the role of ROS in seed germination and seedling organ development.

## 44. OCCURANCE OF KILLER YEASTS IN ACIDIC ENVIRONMENT

Zasčiurinskas, P.<sup>1,2</sup>, Strazdaitė-Žielienė, Ž.<sup>2</sup>, Dobrovolskis, L.<sup>1,2</sup>, Servienė, E.<sup>1,2</sup>

<sup>1</sup>Nature Research Centre, Vilnius, Lithuania;

<sup>2</sup>Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania;  
[paulius.zaschiurinskas@stud.vgtu.lt](mailto:paulius.zaschiurinskas@stud.vgtu.lt)

Killer yeasts are unicellular eukaryotic microorganisms secreting toxins lethal to susceptible cells. The biocidal activity is species- and strain-dependent. Yeast toxins are active at acidic pH, ensuring the high probability of finding new killer yeast strains in low-pH environment.

The objective of the present work was identification of killer yeasts in acidic environmental samples and evaluation of their biocidal properties. Samples were collected from swamp water, silage, and berries, such as cranberries (*Vaccinium oxycoccos*), lingonberries (*Vaccinium vitis-idaea*) and black chokeberries (*Aronia melanocarpa*). The pH of swamp water ranged from 5 to 7, the silage samples - from 4 to 5, and mashed berries - from 2.5 to 4. Ninety strains of yeast-like morphology, representing each of mentioned above habitat, were used for killer assay and molecular species identification. Nine biocidal strains were isolated from swamp water, ten - from berries and fourteen - from silage. Most of identified killer strains were inhabiting silage, however their diversity was the lowest - predominated yeasts were representatives of the genera *Wicherchamomyces*, *Candida*, and *Debaryomyces*. Biocidal yeast strains from the wetland ecosystem belonged to the genera *Candida*, *Cryptococcus*, *Metchnikowia*, *Aureobasidium*, and *Microdochium*. The highest diversity of killer yeasts was in the samples collected from the berries: they belonged to the genera *Candida*, *Metchnikowia*, *Cryptococcus*, *Curvibasidium*, *Hanseniaspora*, *Dothiora*, and *Krasilnikovozyma*. The killer assay was performed on MB media at pH 4.0, 4.8, and 5.6. The strongest biocidal properties of identified killer yeasts were observed at pH 4 and 4.8.

This study, focusing on the diversity of yeast in acidic environmental habitats and prevalence of killer yeasts, is the first such investigation in Lithuania. The obtained data offer new insights on killer or other biocidal systems existing in acidic environments. Identified biocidal yeast and their toxins may have high potential in food industry and human therapy. Research networking was funded by the COST Action CA18113 EuroMicroPH.

## 45. SYNTHESIS AND OPTIMIZATION OF PRUSSIAN BLUE BASED BIOCATHODE FOR ENZYMATIC GLUCOSE BIOFUEL CELL

Algimantas Kaminskas<sup>1</sup>, Asta Kausaite-Minkstimiene<sup>1</sup>

<sup>1</sup>NanoTechnas – Centre of Nanotechnology and Materials Science, Faculty of Chemistry and Geosciences,  
Vilnius University, Lithuanian

[algimantas.kaminskas@chgf.stud.vu.lt](mailto:algimantas.kaminskas@chgf.stud.vu.lt)

Prussian Blue (PB) is a hexacyanoferrate coordination compound with interesting electrochromic, electrochemical and magnetic properties. Due to high activity and selectivity towards the reduction of hydrogen peroxide, PB can be defined as an “artificial peroxidase” [1]. Hydrogen peroxide detection has its vital role in biochemical, chemical and other fields, so PB has been used in the construction of working electrodes for electrochemical biosensors or as cathodes in biofuel cells. Enzymatic biofuel cells (EBFCs) are devices capable of directly transforming chemical energy from redox enzymes to electrical energy via electrochemical reactions [2]. Glucose and oxygen are continuously replenished in body fluids and are accessible in organic tissues. Because of these properties, these materials are very attractive to researchers in developing glucose EBFCs that could utilize the fuelling properties of glucose and oxidizing properties of oxygen by supplying energy at the anode from blood glucose oxidation, and at the cathode using reduction of oxygen or hydrogen peroxide, which is produced during the enzymatic oxidation of glucose [3]. It is possible that, in time, energy will be supplied to implantable medical devices by miniature membrane-less EBFCs [4]. Although, over the last decade, researchers have also studied and published EBFCs that use enzymatic reactions but, powerful enough and long-term energy generating cells have not yet been discovered so, by modifying electrodes we can increase EBFCs properties.

The aim of this work was to develop PB based biocathode for enzymatic glucose biofuel cell. To achieve this, firstly, a graphite rod (GR) electrode was immersed in an electrochemical cell filled with a mixture of FeCl<sub>3</sub>, K<sub>3</sub>[Fe(CN)<sub>6</sub>], KCl, HCl and pyrrole-2-carboxylic acid (PCA). Stable and durable electrochemically coated PB and poly(pyrrole-2-carboxylic acid) (PPCA) nanocomposite film (PB-PPCA) was formed by cyclic voltammetry (CV). A second PPCA layer was then deposited on top of the PB-PPCA coated GR electrode also by CV. Finally, the glucose oxidase (GOx) enzyme was covalently linked to the carboxyl groups of the second PPCA layer. Immobilized GOx catalysed the reaction between glucose and dissolved oxygen and produced hydrogen peroxide, which was electrocatalytically reduced on the biocathode surface and caused an increase in the reduction current. The optimal conditions for the biocathode preparation were elaborated experimentally. CV was employed to investigate the electrochemical behaviour of the biocathode, while constant potential amperometry was used to estimate the ability to determine glucose. The obtained results showed that this simple biocathode design can be applied to improve the performance of EBFCs.

### Acknowledgment

This research was funded by a grant (No. S-LU-20-11) from the Research Council of Lithuania.

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## 46. WHY BOTHER WITH CARRIER? ENZYME IMMOBILIZATION VIA SIMPLE CARRIER-FREE METHOD

Laurynas Vaitkus, Justinas Babinskas, Inga Matijošytė

Sector of Applied Biocatalysis, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania  
[laurynas.vaitkus@chgf.stud.vu.lt](mailto:laurynas.vaitkus@chgf.stud.vu.lt)

In comparison to synthetic catalysts, biocatalysts are more friendly to an environment, have higher specificity, lower energy requirement; however, poor stability and recovery limits the performance of their soluble free state [1]. Researchers have been working for several decades on improving the stability of enzymes by immobilization. Notwithstanding, the majority of immobilization techniques require additional carriers what leads to a dilution and possible loss of enzyme catalytic activity. To overcome these problems, several carrier-free enzyme immobilization techniques have emerged such as CLEA (cross-linked enzyme aggregates), CLEC (cross-linked enzyme crystals), CLE (cross-linked enzymes) and others. Among them, CLEA seems to be the most effective, because it offers major advantages: low production price, excellent stability in various organic solvents and in a wide range of temperatures and pH [2]. The immobilization of enzymes by CLEA method consists of two steps: precipitation of an enzyme from an aqueous solution and its cross-linking with a bi-functional reagent. Many parameters such as pH, temperature, the amount of both the precipitate and the cross-linker, incubation time of both steps, affect the efficiency of immobilization, which leads to differences in the activity and stability of the formed CLEAs [3]. Therefore, the precipitation and cross-linking processes must be optimized in order to obtain the highest immobilized enzyme catalytic activity and stability.

During this study we have optimized the preparation of CLEA from the commercially available lipase Vilzim LPS from *Aspergillus niger*. Precipitation was optimized using different solvents (acetone, ethanol, 2-propanol, dimethoxyethane, 2-(2-methoxyethoxy)ethanol), exploring their various concentrations and precipitation time. Further, at optimal conditions precipitated lipase aggregates were used to investigate the cross-linking process parameters. More detailed results of this study will be presented during the poster session.

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### 47. COMPARISON OF NEW SUNITINIB DERIVATIVES ACTIVITY ON COLON CANCER CELL LINES IN NORMOXIA AND HYPOXIA CONDITIONS

Ugnė Endriulaitė<sup>1</sup>, Elias Maccioni<sup>2</sup>, Vilma Petrikatė<sup>1,3</sup>

<sup>1</sup>Department of Drug Targets Histopathology, Institute of Cardiology, Lithuanian University of Health Sciences, Lithuania

<sup>2</sup>Department of Life and Environmental Sciences, Cagliari University, Italy

<sup>3</sup>Institute of Physiology and Pharmacology, Lithuanian University of Health Sciences, Lithuania  
[ugne.endriulaityte@lsmu.lt](mailto:ugne.endriulaityte@lsmu.lt)

In 2018, there were 18.1 million new cancer cases and 9.6 million deaths related to it (Bray et al., 2018). Despite the progress in cancer treatment, there is still a need for advanced and more effective anticancer agents. Sunitinib is one of the kinase inhibitors which is used to treat kidney and gastrointestinal cancers after failure to the first choice treatments. (Arora & Scholar, 2005). The aim of our research was to evaluate and compare the activity of new sunitinib analogs synthesized at Cagliari University (Italy) in normoxia and hypoxia conditions.

In our study we used two human colon cancer cell lines HT-29 and HCT116. MTT assay was used to evaluate the metabolic activity of cells after treatments with analogs in normoxia and hypoxia conditions. Half maximal effective concentration (EC50) has been established that shows the concentration of compounds causing 50% reduction of cell metabolic activity. Wound healing assay was used to evaluate antimetastatic activity of compounds. Compounds were used at 10, 50 and 90% of their established EC50 values. Results were compared to the clinically used drug sunitinib.

In normoxia and hypoxia conditions, tested compounds 1, 6 and 7 statistically significantly stronger reduced the viability of both cancer cell lines compared to sunitinib. The most active one was compound 1. It was from 2.7 (against HT-29) to 57.5 times (against HCT116 cell line) more active compared to sunitinib. In normoxia condition compound 1 statistically significantly inhibited the migration of both cell lines. The difference was determined after 72 h of incubation with 90% of EC50 concentration of compound. In hypoxia, HCT116 line cells did not show any statistically significant differences compared to the control. HT-29 line cells migration was statistically significantly inhibited after 24 h of incubation with compound 1 at 50% and 90% of its EC50 concentration compared to the control. Compound 6 statistically significantly inhibited cell migration at 10% and 90% of its EC50 concentration compared to the control. Sunitinib did not have such an impact.

In conclusion, sunitinib derivatives 1, 6 and 7 are more effective than sunitinib in vitro in normoxia and hypoxia conditions and could be developed further as anticancer agents against colon cancer.

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## 48. INVESTIGATION OF CYTOTOXIC RESPONSES OF HEALTHY AND PATHOLOGICAL HUMAN MYOCARDIUM-DERIVED MESENCHYMAL STEM CELLS

Andre Aleksandraviciute<sup>1</sup>, Rokas Miksiunas<sup>1</sup>, Kestutis Rucinskas<sup>2</sup>, Vilius Janusauskas<sup>2</sup>, Siegfried Labeit<sup>3</sup>, Daiva Bironaite<sup>1</sup>

<sup>1</sup>Department of Regenerative Medicine, State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania.

<sup>2</sup>Vilnius University, Faculty of Medicine, Centre of Cardiac Surgery, Clinic of Cardiac and Vascular Diseases, Vilnius, Lithuania.

<sup>3</sup>Department of Integrative Pathophysiology, Universitätsmedizin Mannheim, Mannheim, Germany.  
[an.aleksandraviciute@gmail.com](mailto:an.aleksandraviciute@gmail.com)

Cardiomyopathies are a heterogeneous group of myocardial diseases associated with mechanical or electrical disorders of cells causing inappropriate functioning of the left ventricle [1]. Dilated cardiomyopathy (DCM) is agreed to be most common form of cardiomyopathy responsible for 40–50% of cases of heart failure leading to requirement of heart transplantation [2]. Many toxic causes, including drugs, environmental agents, substances of abuse or natural toxins and other are involved in pathophysiological mechanisms of DCM [3]. Therefore, in this study the molecular mechanisms explaining a response of human healthy and dilated myocardium-derived mesenchymal stem cells (hmMSC) to chemical and mechanical exposures have been investigated. hmMSC have been isolated from human healthy and dilated myocardium biopsies, cultivated in IMDM growth media with 10 percent of FBS and subjected to the different concentrations of toxic environment mimicking naphoquinone or to an extra mechanical overload using Flexcell equipment. The response of healthy and pathological hmMSC to the toxic exposures have been investigated by cell viability kit CCK-8, detection of apoptosis and various pro- and anti-cell surviving mechanisms by western blotting.

Data of this study show that healthy and pathological hmMSC differently responded to the toxic exposures with more prominent effect on healthy compared to the pathological cells. The obtained data also suggest what types of mechanisms regulating surviving of myocardium cells can participate in the heart regeneration processes. Moreover, the targeted regulation of heart cell death/survival mechanisms could help to search for new therapeutic or DCM preventing means.

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## 49. DEPENDENCE OF JNK SIGNALING PATHWAY MOLECULES ON INTERCELLULAR CONTACTS

Aurimas Stulpinas, Natalija Krestnikova, Aušra Imbrasaitė, Audronė V. Kalvelytė

Life Sciences Center, Vilnius University

**Background.** Intercellular contacts regulate the fate of cells during biotechnological and biomedical processes. They influence results of bioproduction, regenerative medicine, anticancer therapy. Cell-cell contacts affect many functional properties such as cell survival, growth, metabolism, protein synthesis, gene expression, etc. Cells in a monolayer or within 3D structures, cells in damaged tissue as well as circulating cancer cells, either single or in groups, wherever they are used, – differently interact with each other, and these interactions lead to different changes in their functioning. Elucidation of the influence of cell-cell contacts on the activity of cell fate-regulating signaling is essential in predicting cell response to the changing conditions. Basal activity of signaling molecules is an important factor here.

c-Jun N-terminal kinase (JNK) is a subfamily of mitogen-activated protein kinases (MAPKs), which is activated by diverse stress signals. JNK regulates important cellular activities and plays roles in cancer cell response to both targeted- and chemo-therapies. JNK has dual functions and can mediate both cell transformation and apoptosis through a variety of cell-context dependent mechanisms.

A member of activating protein-1 (AP-1) transcription factor family, c-Jun may be the effector through which JNK exerts its biological function, although opposing functions of components of JNK pathway have been reported. Therefore, the role and context-dependence of each component needs to be understood. Here, we focus on dependency of JNK signaling pathway molecules on cell-cell contacts.

**Methods.** Human non-small cell lung cancer adenocarcinoma A549 and muscle-derived Myo stem cell lines were grown under standard conditions. Cells incubated under agitation were fractionated in order to obtain non-aggregated (suspension) and aggregated cell fractions. Protein expression and phosphorylation was analyzed by Western blotting using antibodies against total and phosphorylated JNK and c-Jun. Cell viability was determined by using the resazurin reagent, mode of cell death was assessed by staining with a mixture of AO/EB fluorescent dyes. Specific inhibitor of JNK (SP600125) and proteasomal inhibitor (MG132) were selected for the role of JNK and c-Jun expression studies.

**Results.** JNK was shown to be required for proliferation of the cells studied, and their protection against anoikis. Modeling different cellular states – confluent and subconfluent, single cells in suspension or aggregated, – expression and phosphorylation profile of JNK and c-Jun was studied.

Inhibitory role of intercellular contacts on the c-Jun protein level was determined in A549 and Myo cells when grown in confluency or in aggregates, whereas expression of JNK did not change. The studies indicated a possible proteasomal degradation of c-Jun. Moreover, studies revealed the opposite dependence of molecules of the same signaling pathway – phospho-c-Jun and phospho-JNK – on cell culture density: JNK phosphorylation increased with increasing cell density, whereas phosphorylation of downstream JNK target c-Jun decreased.

**Conclusions.** Given the importance of c-Jun in regulating genes involved in the main cellular processes, changes in its amount may be important for its biological contribution. The findings suggest that the outcome of c-Jun N-terminal kinase activation in cells may be dependent on the amount of c-Jun, which itself is determined by intercellular contacts.

**Acknowledgment.** The work was supported by grant No. 01.2.2-LMT-K-718-01-0072 (LMTLT).

## 50. THE INFLUENCE OF [PSI<sup>+</sup>] PRION PROTEIN ON YEAST *SACCHAROMYCES CEREVISIAE* CELL GROWTH AND INTRACELLULAR PROTEIN ACCUMULATION

Neda Jonutyte<sup>1</sup>, Skaistė Jančauskytė<sup>1</sup>, Justina Jurgelevičiūtė<sup>1</sup>, Eglė Lastauskienė<sup>1</sup>

<sup>1</sup>Department of Microbiology and Biotechnology, Institute of Biosciences, Life Sciences Center, Vilnius University, Sauletekio avenue 7, LT-10257 Vilnius, Lithuania.

[neda.jonutyte@gmc.stud.vu.lt](mailto:neda.jonutyte@gmc.stud.vu.lt)

Yeast models of amyloid-related neurodegenerative diseases have become powerful tool for high-throughput chemical and drug screening to reduce amyloid aggregation and toxicity [1]. Yeast and fungal prions are self-propagating, amyloid structures of native soluble proteins. [PSI<sup>+</sup>]-is the prion form of the translation termination factor Sup35 protein that results in increased nonsense suppression in yeast *Saccharomyces cerevisiae* cells. The prion protein can fold into structurally different infectious states leading to different phenotypes [2]. The yeast prion [PSI<sup>+</sup>] variant was first isolated as strong or weak, indicating the strength of the prion phenotype, reflecting the degree of lack of the normal protein [3]. Individual [PSI<sup>+</sup>] variants have different mitotic stability ([PSI<sup>+</sup>] loss rate), translation termination activity [4]. The aim of this study was to determine the effect of *S. cerevisiae* 74D-694 [PSI<sup>+</sup>] prion variants on yeast cell growth and intracellular protein accumulation. Methods of cultivation, prionization, electroporation, protein isolation and imaging were used in the study. The study of growth dynamics found that the prionized [PSI<sup>+</sup>] S, M, L, XL variants produced the highest amount of biomass compared to the variants of the respective sizes. Statistically significant differences of doubling time ( $p < 0,05$ ) were found. Analysis of intracellular insoluble proteins showed: [PSI<sup>+</sup>] S, M, L, XL variants have more proteins greater than 70 kDa and less than or equal to 30 kDa compared to the corresponding cured using GuHCl variants ([psi<sup>-</sup>] S, M, L, XL). Analysis of intracellular soluble proteins showed that the prionized [PSI<sup>+</sup>] S, M, L, XL variants have a lower concentration of the hypothetical Sup35 protein compared to the corresponding [psi<sup>-</sup>] S, M, L, XL cured GuHCl variants. In [PSI<sup>+</sup>] variants Sup35 protein is prionized, therefore insoluble and less visible in the soluble protein fraction compared to [psi<sup>-</sup>] variants with high levels of soluble Sup35 protein.

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# 51. NEURAL DIFFERENTIATION AND NEURON-TYPE SPECIFIC MARKER EXPRESSION OF HUMAN AMNIOTIC FLUID-DERIVED STEM CELLS GROWN IN 3D CULTURES

Elizabet Beržanskytė, Aistė Zentelytė, Giedrė Valiulienė, Rūta Navakauskienė

Department of Molecular Cell Biology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Vilnius, Lithuania

[elizabet.berzanskyte@gmc.stud.vu.lt](mailto:elizabet.berzanskyte@gmc.stud.vu.lt)

Neurodegenerative disorders lead to changes in the number, structure and function of cells in the central nervous system or even cellular death. Thus, amniotic fluid stem cells (AFSCs) could be a valuable tool in the reconstruction of neural tissue. AFSCs are advantageous because they are easy to isolate from amniocentesis samples, display high proliferation potential as well as the ability to differentiate towards lineages from all three germ layers.

However, in regenerative medicine the method of cell culture is equally important to the type of the stem cells itself. Most of the time *in vitro* stem cells are grown in monolayers, however, these cells usually fail to mimic cells naturally occurring in live organisms. A solution to this problem could be 3D cultures where cells tend to display more similar morphology, differentiation, proliferation, polarization potentials, cell-cell interaction, and microenvironment to the *in vivo* cells.

Our research was aimed to evaluate the potential of AFSCs grown in 3D cultures to differentiate towards neural cell lineage. Stem cells were obtained using a two-stage isolation protocol. First, they were expanded in monolayer culture and then transferred to the 96-well Nunclon™ Sphera™ plates and grown as spheroids in 3D cultures. Cells from 2D cultures were characterized and were positive for pluripotent stem cell markers (*NANOG*, *SOX2*, *OCT4*, *REX1*) and mesenchymal stem cell markers (CD44, CD73, CD90, CD105, CD146, CD166). The neural differentiation of the spheroids was induced using a protocol that comprised commercial supplement NeuroCult™ and biomolecules such as NGF, BDNF, retinoic acid, and KCl. Later the expression of neural genes (*NES*, *NSE*, *NGF*, *BDNF*, etc.) and microRNAs (miR-16-5p, miR-135a-5p, miR-1202, miR-124-3p, miR-103a-3p, miR-155-5p, miR-146a, miR-93-5p, miR10a, miR-210, miR-199a-3p, miR-223) was examined using RT-qPCR. It was discovered that there are some changes in the expression of specific genes and microRNAs in the differentiated cells grown in 3D compared to those grown in 2D cultures.

The results of this study bring new insights about the ability of AFSCs to differentiate into neural-like cells, especially when grown in 3D cultures. Yet, additional research is needed to apply them in stem cell therapy.

## 52. ESTABLISHMENT AND CHARACTERIZATION OF A NEW PANCREATIC DUCTAL ADENOCARCINOMA CELL LINE CAPAN-26

Eglė Žalytė<sup>1</sup>, Veronika Dedonytė<sup>2</sup>, Benediktas Kurlinkus<sup>3</sup>, Audrius Šileikis<sup>3</sup>, Peter Schemmer<sup>3,4</sup>, Mindaugas Valius<sup>1</sup>

<sup>1</sup>Proteomics Centre, Institute of Biochemistry, Vilnius University Life Sciences Centre, Vilnius, Lithuania

<sup>2</sup>Department of Botany and Genetics, Institute of Biosciences, Vilnius University Life Sciences Centre, Vilnius, Lithuania

<sup>3</sup>Institute of Clinical Medicine, Clinic of Gastroenterology, Nephrology, and Surgery, Faculty of Medicine, Vilnius University, Vilnius, Lithuania

<sup>4</sup>General, Visceral and Transplant Surgery, Department of Surgery, Medical University of Graz, Graz, Austria  
[egle.zalyte@gf.vu.lt](mailto:egle.zalyte@gf.vu.lt)

Pancreatic ductal adenocarcinoma is one of the deadliest forms of human cancer. Disease variability makes pancreatic cancer difficult to combat using mainstream treatment modalities such as chemotherapy and radiotherapy. For many years, cell lines have proved to be a useful in vitro model for preclinical cancer research and American Type Culture Collection (ATCC) provides a panel of them for different cancer types, including pancreatic. However, the strength of cell lines lies in their diversity: each cell line represents a unique cancer case. Thus, we are constantly in need of new cell lines that can recapitulate primary tumors.

Here we present a novel pancreatic ductal adenocarcinoma cell line Capan-26. This cell line was derived from a primary tumor of a 65 years-old female Lithuanian patient with pancreatic ductal adenocarcinoma stage II. To our knowledge, it is the first pancreatic cancer cell line derived from a Lithuanian patient. We characterized the cells functionally and genetically: determined their growth rate, detected expression of pancreatic cancer and stem cell markers, confirmed that cells form colonies in soft agar, analyzed karyotype and found mutations of several cancer related genes. Also, we found out that Capan-26 cell line is sensitive to drugs that are used for pancreatic cancer treatment. In conclusion, we believe that Capan-26 will be a valuable tool for cancer studies in the future.

This work was funded by “Healthy Ageing” programme of the Research Council of Lithuania (LMTLT); agreement No. S-SEN-20-16.



## 53. MOLECULAR ANALYSIS OF MICRO RNAS THAT ARE POTENTIALLY RELATED TO CANCER CELL METASTASIS

Kamilė Normantaitė, Rimvilė Prokarenkaitė, Eglė Balčiūnaitė, Kęstutis Sužiedėlis

Laboratory of Molecular Oncology, National Cancer Institute  
[kamile.normantaite@gmc.stud.vu.lt](mailto:kamile.normantaite@gmc.stud.vu.lt)

Metastasis is the leading cause of death in cancer patients. Early diagnosis of the progression of tumour before the formation of metastasis would make cancer treatment more efficient. However, that requires identification of molecular biomarkers that are specific to different stages of tumour metastasis. Accumulating evidence indicates a crucial role of micro RNAs (miRNAs) in the initiation and progression of cancer, acting either as oncogenes or as tumour suppressors. miRNAs are small ~22 bp long non-coding RNAs that play key roles in the regulation of gene expression at the posttranscriptional level. miRNA expression profiling has been shown to be associated with tumour development, progression and response to therapy, suggesting their possible use as diagnostic and predictive biomarkers [1].

The previous study at VU Institute of Biosciences and National Cancer Institute revealed a substantial number of miRNAs that are potentially related to non-small cell lung cancer metastasis. Microarray data demonstrated a signature of significantly altered expression of 77 miRNAs in mouse lung cancer cells grown under 2D and ECM based 3D cell culture conditions [2].

The aim of this study was to investigate the implication of identified miRNAs in the metastatic process. Bioinformatical target analysis and pathway enrichment analysis of miRNAs revealed nineteen miRNAs that are potentially involved in cancer metastasis as their target genes are related to processes such as cell migration, adhesion, invasion, metastasis, actin dynamics, cytoskeleton organization and ECM-receptor interaction. Three of them – mmu-miR-195a-5p, mmu-miR-207, mmu-miR-500-3p – were selected for further molecular analysis. We used CRISPR/Cas9 genome editing tool to knock down selected miRNAs in mouse Lewis lung carcinoma (LLC-1) cells. Cas9 nuclease was targeted to genomic DNA by a single-guide RNA consisting of a 20-nt guide sequence and a scaffold [3]. Deletion of miRNA gene was generated using two different sgRNAs.

Results show that monoallelic deletion of the sequence encoding miR-195a significantly reduced the expression of miR-195a. Furthermore, cell proliferation and cell cycle analyses revealed substantial decrease in mutant cell proliferation along with the accumulation of the cells in S-phase. In addition, wound healing assay demonstrated that miR-195a knock-down notably inhibited migration of LLC-1 cells. Taken together, the present study suggested that miR-195a may be potentially implicated in metastasis of lung cancer.

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# 54. INVESTIGATION OF GARDNERELLA SPP. CNA PROTEIN USING IMMUNOLOGICAL METHODS

Raminta Reškevičiūtė, Indrė Dalgėdienė, Aurelija Žvirblienė, Milda Plečkaitytė

Vilnius University, Life Sciences Center, Institute of Biotechnology, Department of Immunology and Cell Biology

[raminta.reskeviciute@gmc.vu.lt](mailto:raminta.reskeviciute@gmc.vu.lt)

Bacterial vaginosis (BV) is one of the most common vaginal infections associated with impaired vaginal microflora that affects women of childbearing age. BV is associated with various adverse outcomes not only relating to women's fertility, reproductive health, or pregnancy, but also women's mental health [1]. *Gardnerella* spp. is the most associated bacterium with BV and has been detected in almost all vaginal samples from women suffering from BV [2].

Adhesins are cell-surface components that are responsible for the recognition of host cells and adhering to them. Studies on other bacteria show that various adhesins are proven virulence factors that are significant in bacteria's pathogenesis [3, 4]. However, the adhesins of bacterium *Gardnerella* spp. are very little studied and described.

After the amino acid sequence of *Gardnerella* spp. collagen adhesin (CNA) protein determination, a hypothesis was proposed that CNA allows *Gardnerella* spp. to bind to host extracellular matrix (EM) proteins.

The interaction between recombinant *Gardnerella* spp. CNA and selected EM proteins was investigated by various ELISA method variations and other immunochemical methods. Monoclonal antibodies (MAbs) against recombinant CNA protein were created using hybridoma technology. Anti-CNA MAbs were used to investigate the interaction between the CNA and EM proteins: type I, III, IV collagens, fibronectin, and fibrinogen. Using indirect ELISA method we detected that CNA interacts with all EM proteins used in the experiment (Fig. 1).

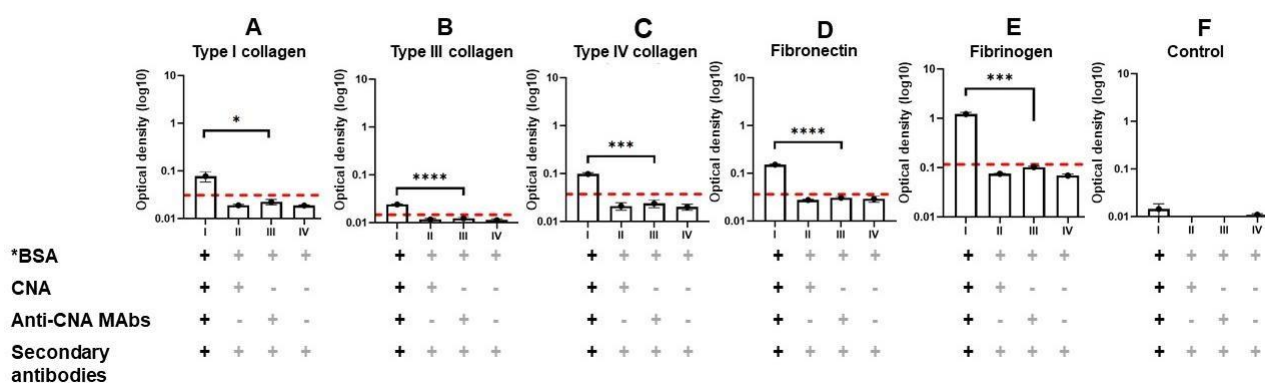


Fig. 1. The investigation of interaction between *Gardnerella* spp. CNA protein and selected EM proteins: A – type I collagen; B – type III collagen; C – type IV collagen; D – fibronectin; E – fibrinogen; F – control, no EM proteins were adsorbed. The  $t$ -test was applied, where \* $p < 0.05$ , \*\*\* $p < 0.001$ , \*\*\*\* $p < 0.0001$  ( $n = 4$ ). The red line indicates the mean of an optical density plus three standard deviations. \*BSA – bovine serum albumin.

Results and created molecular tools, anti-CNA MAbs, raise new questions and hypotheses. In the future, we seek to use more complex model systems to investigate the interactions between native CNA and epithelial cells in vitro. This would allow to investigate and understand molecular *Gardnerella* spp. pathogenesis mechanisms.

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## 55. GENERATION OF MONOCLONAL ANTIBODIES AGAINST ATLANTIC COD PARVALBUMIN AND THEIR REACTIVITY WITH RECOMBINANT FISH ALLERGENS

Aiste Sliziene, Vytautas Rudokas, Gintautas Zvirblis and Aurelija Zvirbliene

Vilnius University Life Sciences Center Institute of Biotechnology, Sauletekio al. 7, Vilnius, Lithuania  
[aiste.imbrasaite@gmc.vu.lt](mailto:aiste.imbrasaite@gmc.vu.lt)

### Background

Atlantic cod is one of the commonly consumed fish species, that can be found in Northern Europe and North America's eastern coast. Even though fish is an excellent source of nutrients, ingestion or inhalation of fish allergens can trigger severe allergic reactions. Gad m 1 is Atlantic cod parvalbumin, an allergen, found in fish muscle. It is a small (about 10-12 kDa), highly stable calcium-binding protein, that shares high sequence identity among different fish species (carp, salmon). Monoclonal antibodies (MAbs) against allergens of different fish species may provide useful reagents for fish allergy studies and diagnostics.

### Methods

MAbs against natural Atlantic cod parvalbumin (Gad m 1) were generated by hybridoma technology and characterized using enzyme-linked immunosorbent assay (ELISA). Recombinant salmon parvalbumin (Sal s 1), Baltic cod parvalbumin (Gad c 1), Atlantic cod parvalbumin (Gad m 1) and Atlantic herring parvalbumin (Clu h 1), fused with maltose-binding protein (MBP), were expressed in *E.coli* and purified. The pattern of cross-reactivity of the MAbs with different fish allergens were analysed by ELISA and Western blot.

### Results

In total, four MAbs (7B2, 2C1, 18H3 and 16B3) against Gad m 1 were generated, that were of IgG1 isotype and showed high affinity to the antigen. The reactivity of MAbs with recombinant Sal s 1-MBP, Gad c 1-MBP, Gad m 1-MBP and Clu h 1-MBP was investigated. MAbs 2C1 and 7B2 reacted with Gad c 1-MBP and Gad m 1-MBP, while MAbs 18H3 and 16B3 were reactive with Gad m 1-MBP and Clu h 1-MBP. None of the MAbs reacted with Sal s 1-MBP. All MAbs were shown to recognise linear epitopes of certain allergens by Western blot.

### Conclusions

The newly developed MAbs against Atlantic cod parvalbumin (Gad m 1) may serve as a promising tool for antigenic characterization of different fish allergens. This study indicates that all four fish parvalbumins share common epitopes that are recognised by MAbs raised against Gad m 1.

## 56. ABLATION OF MURINE TUMORS USING NEW HIGH-FREQUENCY NANOSECOND ELECTROPORATION AND ITS INFLUENCE ON CHANGES IN IMMUNE CELL SUBPOPULATIONS

Augustinas Želvys<sup>1</sup>, Austėja Balevičiūtė<sup>1</sup>, Eivina Radzevičiūtė<sup>1</sup>,  
Vitalij Novickij<sup>2</sup>, Irutė Girkontaitė<sup>1</sup>

<sup>1</sup>State Research Institute Centre for Innovative Medicine, Department of Immunology, Vilnius, Lithuania

<sup>2</sup>Institute of High Magnetic Fields and Department of Electrical Engineering, Vilnius Gediminas Technical University, Vilnius, Lithuania  
[augustinas.zelvys@gmc.stud.vu.lt](mailto:augustinas.zelvys@gmc.stud.vu.lt)

Chemotherapy is used as an anticancer therapy, but high drug doses have adverse effects on patients. Thus, to decrease the doses of chemotherapeutics, electroporation (EP) is employed. Pulsed electric fields (PEFs) facilitate intratumoral drug delivery used in electrochemotherapy (ECT). Microsecond ECT is already used in clinical trials for the elimination of tumors. However, standard microsecond treatment procedure (ESOPE [1]) lacks the efficiency, induces necrotic thermal effects, or severe unintended muscle contractions.

Nanosecond electrical pulses are not used in anticancer therapies as they are still in research. In our previous works [2], we have shown that nanosecond PEFs (nsPEF) can cause lower tumor cell viability, better permeabilization for drug delivery, and less tumor-surrounding tissue necrosis. Now, we present a new modality of high-frequency nanosecond electroporation for successful ECT in vivo (Fig. 1) and its influence on changes in immune cell subpopulations..

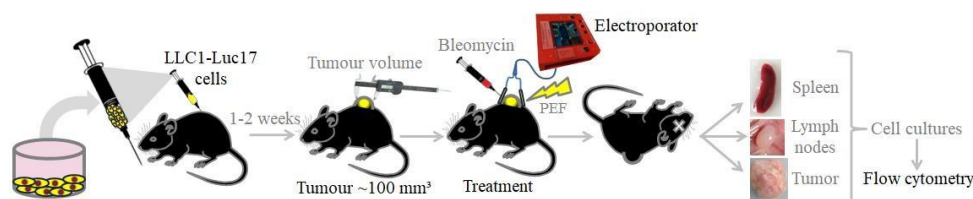


Figure 1. Investigation of ECT effect on changes of mice tumor volume and organ cell populations.

Tumors were induced by injecting C57BL/6J mice with LLC1 cells subcutaneously, grown to reach 100 mm<sup>3</sup>. Afterwards, mice were treated with standard microsecond (ESOPE) and different modalities of nanosecond EP, in combination with bleomycin (ECT). ESOPE was compared to the nanosecond ECT (3.5 kV/cm, 200 pulses) with different nsPEF: nsPEF1 (200 ns, 1 kHz), nsPEF2 (200 ns, 1 MHz), nsPEF3 (700 ns, 1 kHz), nsPEF4 (700 ns, 1 MHz).

C57BL/6 mice were divided into untreated, ESOPE and four nsPEF-treated groups. Treatment efficiency was evaluated by comparing mice survival and tumor volumes. Compared to untreated mice, the survival of nsPEF2, nsPEF3 and nsPEF4-treated mice was significantly longer (Mantel-Cox test,  $p < 0.05$ ). Compared to the untreated mice, ECT with nsPEF3 and nsPEF4 has resulted in significantly reduced or eliminated tumors, relating to prolonged mice lifespan.

Flow cytometry was performed to evaluate the changes in B220<sup>+</sup>, CD4<sup>+</sup>, CD8<sup>+</sup> immune cell subpopulations. The percentage of B220<sup>+</sup> was higher in the tumor and lymph nodes of nsPEF3 and ESOPE-treated mice. PEF treated mice had enlarged spleens. nsPEF3 and ESOPE treatment resulted in an increased number of myeloid cells and lymphocytes in the spleen. T and B cell percentage was significantly lower in the spleens of nsPEF3 and ESOPE-treated mice, compared with the untreated mice. However, a significantly higher percentage of B220<sup>+</sup>CD4<sup>+</sup>CD8<sup>+</sup> lymphocytes was in PEF-treated mice. Therefore, further studies are necessary to find out this negative lymphocyte population.

Our study has shown that nanosecond ECT is as efficient as ESOPE procedures for carcinoma tumor ablation. High-frequency nanosecond ECT causes less muscle contractions and necrosis due to decreased thermal effects. nsPEFs may induce a better immune cell response, prolonging mice lifespan. These advantages could be promising in the future anticancer therapies, gradually replacing ESOPE.

*Acknowledgement: The project was funded by the Research Council of Lithuania (No. S-MIP-19-22).*

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## 57. *CORRYLUS AVELLANA* ALLERGEN COR A 2 AND COR A 9: EXPRESSION, PURIFICATION AND ANALYSIS IN *ESCHERICHIA COLI*

leiva Lingytė<sup>1</sup>, Jūta Rainytė<sup>1</sup>, Rasa Petraitytė-Burneikienė<sup>1</sup>, Gintautas Žvirblis<sup>1</sup>

<sup>1</sup>Vilnius University Life Sciences Center, Institute of Biotechnology, Department of Eukaryote Gene Engineering

Nowadays allergies are being recognized as a substantial public health burden in developed countries. From a wide range of different allergens, food products cause allergic diseases in more than 3–4 % of the adult population in Westernized countries. One group of well-defined allergens are the different types of nuts which can lead to severe systemic and even fatal reactions in the human body.

Allergen extracts are often used in allergy diagnostics. However, these heterogenic mixtures are composed of many components which can cause cross-reactivity in allergy tests. An alternative for these extracts could be the use of recombinant allergens, which increases diagnostic assay sensitivity as well as its analytical specificity. To obtain high-amounts of soluble recombinant allergens, *Escherichia coli* protein synthesis systems are a great option due to their easy manipulation and scale-up process.

In this work, the hazelnut, *Corylus avellana* Cor a 2 and Cor a 9 allergens were expressed in *E. coli* BL21 (DE3) and Rosetta (DE3) pLysS strains. The recombinant protein was also fused with a highly soluble partner – maltose-binding protein (MBP), which promotes recombinant protein solubility and proper folding. Recombinant allergens were purified with Ni-NTA affinity chromatography. To test the antigenic properties of rCor a 2 and rCor a 9 – an enzyme-linked immunosorbent assay was prepared with blood serum samples from patients positive for nut allergy.

This study aimed to purify and characterize recombinant allergens rCor a 2 and rCor a 9.

## 58. DEVELOPMENT OF MONOCLONAL ANTIBODIES AGAINST HOUSE DUST MITE ALLERGEN DER P 21 FOR THE QUANTIFICATION OF ALLERGEN COMPONENT IN ALLERGEN EXTRACTS

Vytautas Rudokas, Gintautas Žvirblis, Aurelija Žvirblienė

Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania

[vytautas.rudokas@gmc.vu.lt](mailto:vytautas.rudokas@gmc.vu.lt)

Allergy is a type I hypersensitivity reaction to the environmental antigens that usually cause little or no problem to most people. The house dust mite (HDM) is one of the most important and widely spread allergen sources causing allergy and its related complications, such as bronchial asthma. Recombinant allergens are being used in molecular allergy diagnostics systems and can also be employed in the development of monoclonal antibodies (MAbs). Since the allergen extracts from different manufacturers lack proper standardization regarding to its composition [1], MAbs against specific allergen components can be used for the quantification of allergen components in allergen extracts.

This study aimed to purify recombinant HDM allergens and then develop, characterize and apply MAbs against one of the purified allergens. Here, we studied 9 allergens from HDM *Dermatophagoides pteronyssinus* and *Dermatophagoides farinae*, which were previously fused with maltose binding protein (MBP), expressed in *E. coli* and were purified using affinity liquid chromatography of MBP-passenger proteins. The allergens were Der f 1, Der f 2, Der p 10, Der p 21, Der p 23, Der p 24, Der p 26, Der p 30 and Der p 36. A TEV protease hydrolysis sequence was inserted in between each allergen and its MBP sequence. MBP and TEV also had 6 histidine sequence inserted. Hydrolysis reaction was optimized to detach MBP from purified allergens and one selected allergen – rDer p 21 was purified using second step affinity liquid Ni-NTA chromatography. Blood serum from HDM allergic patients was analyzed and complex formation, between rDer p 21 and IgE (antibodies against native allergens), was observed. These results led to the development of MAbs against purified rDer p 21. Five hybridoma cell lines producing high affinity MAbs of IgG isotype were generated using hybridoma technology [2]. The MAbs were grouped into two categories according to their recognized epitopes and a sandwich enzyme-linked immunosorbent assay (Fig. 1) system for the quantification of Der p 21 was developed and optimized.

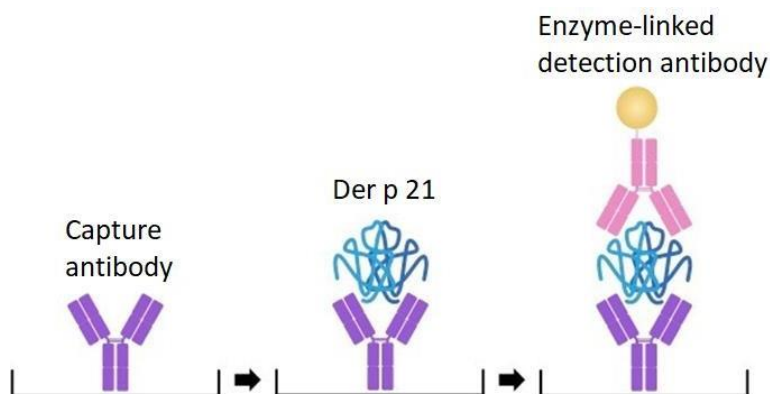


Figure 1. Scheme of sandwich enzyme-linked immunosorbent assay.

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## 59. BIOSYNTHESIS OF RECOMBINANT ALLERGENS USING MAMMALIAN CELLS EXPRESSION SYSTEM

Agne Rimkute<sup>1</sup>, Dovile Stravinskiene<sup>1</sup>, Gintautas Zvirblis<sup>2</sup>

<sup>1</sup>Department of Immunology and Cell Biology, Life Sciences Center, Vilnius University, Vilnius, Lithuania

<sup>2</sup>Department of Eukaryote Gene Engineering, Life Sciences Center, Vilnius University, Vilnius, Lithuania

[agne.rimkute@gmc.vu.lt](mailto:agne.rimkute@gmc.vu.lt)

IgE-mediated allergic reactions are the most common hypersensitivity disorders affecting up to 30% of the population worldwide and still rising. Due to the high prevalence of these diseases, accurate diagnosis is critical to ensure the most effective treatment. Immunological test systems for allergy diagnostics currently mostly involve allergen extracts, which are heterogeneous mixture of various allergic and non-allergic components. Levels of the main allergen and the additional substances could vary among different batches of the product. In contrast, recombinant allergens can be produced as defined molecules with known immunologic and biological features [1]. The usage of purified recombinant allergens instead of allergen extracts could increase the accuracy of allergic reaction detection, as well as reduce cross-reactions. Recombinant proteins could be produced using various expression systems, including mammalian cells. The ability to perform post-translational modifications that are not present in proteins produced in prokaryotes makes mammalian cells expression system an advantageous production platform [2].

This study aimed to produce recombinant secreted maltose-binding protein (MBP) fused wasp's allergen Ves v 2 (rVes v 2-MBP) and cow's milk allergen Bos d 4 (rBos d 4-MBP) in mammalian cells. Synthetic DNA fragments coding these allergens were cloned to mammalian expression vector and transiently transfected into Chinese hamster ovary (CHO) cells. Secreted rBos d 4-MBP was purified using MBP affinity tag and confirmed using immunoblotting and indirect enzyme-linked immunosorbent assay using monoclonal antibodies against MBP. However, recombinant Ves v 2 allergen was not secreted into cells growth media but was detected in intracellular compartment of the cell. To determine if synthesized recombinant protein antigenicity is similar to natural allergen, blood serum samples from patients allergic to cow's milk were tested with rBos d 4-MBP. Also, blood serum samples were tested using the same recombinant allergen produced in *E. coli*.

Results show that serum samples with allergen-specific IgE recognizes rBos d 4-MBP antigen produced in CHO cells, suggesting that recombinant protein contains IgE-binding epitopes. Nevertheless, rBos d 4-MBP protein produced in *E. coli* was not recognized by specific IgE.

In conclusion, recombinant Bos d 4 fused with MBP expressed in mammalian cells is most likely to be similar to native protein as compared to the same recombinant allergen produced in *E. coli*. These results indicate that mammalian cells expression system is suitable for eukaryotic protein production and synthesized recombinant proteins could be used for improving molecular allergology tests in vitro.

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## 60. FEEDING WITH BOVINE COLOSTRUM ENHANCES INTESTINAL MICROBIOTA DEVELOPMENT IN NEONATAL CALVES

Ramunė Grigalevičiūtė<sup>1</sup>, Paulius Matusevičius<sup>2</sup>, Rita Plančiūnienė<sup>3</sup>, Vilma Zigmantaitė<sup>1</sup>, Audrius Kučinskas<sup>1</sup> and Povilas Kavaliauskas<sup>1,4,5,6</sup>

<sup>1</sup>Biological research center, Lithuanian University of Health Sciences . Kaunas, Lithuania.

<sup>2</sup>Department of Animal Nutrition, Lithuanian University of Health Sciences. Kaunas, Lithuania.

<sup>3</sup>Institute of Microbiology and Virology, Lithuanian University of Health Sciences. Kaunas, Lithuania.,

<sup>4</sup>Institute of Infectious Diseases and Pathogenic Microbiology.

<sup>5</sup>Joan Sanford I. Weill Cornell Medical College, New York, United States.

<sup>6</sup>Institute for Genome Sciences, University of Maryland School of Medicine, Baltimore, United States.

[ramune.grigaleviciute@lsmuni.lt](mailto:ramune.grigaleviciute@lsmuni.lt)

**Introduction.** Early and adequate intake of colostrum is critically important for the health and survival of neonatal calves. Colostrum is rich in immunoglobulins, growth factors, vitamins, and bioactive molecules that are highly secreted for the first 4 hours post-partum. However, the role of colostrum in the development of gut microbiota in ruminants remains poorly understood. This study is aimed to characterize the role of colostrum in the development of bovine gut microbiome and assessment with blood parameters at different time points.

**Material and methods.** A ten (n=10) Holstein Friesian cattle were fed to colostrum (COL) (n=5) and artificial colostrum replacement (control) (RD) (n=5) for 6 hours after birth. The animals were evaluated on day 1, 7, and 30 after feeding. The fecal samples were collected at different time points and were used for 16S rRNA metagenomic analysis. Blood samples were collected from v. jugularis and used for the morphological and biochemical analyses.

**Results.** The feeding with COL resulted in significantly higher body mass of 38.54 kg ( $\pm 0.5$  kg) at day 7 in comparison to RD 34.00 kg ( $\pm 0.5$  kg) ( $p < 0.05$ ). Feeding with COL and RD resulted in time-dependent increase in blood monocytes (MON) and neutrophil (NEU) counts. Colostrum diet resulted in significantly higher ( $p < 0.05$ ) numbers of MON at day 1 ( $1.9 \times 10^2$  cells/ $\mu$ L) and day 7 ( $3.25 \times 10^3$  cells/ $\mu$ L) in comparison to the control group ( $1.88 \times 10^2$  and  $3.27 \times 10^2$  cells/ $\mu$ L respectively). Moreover, the colostrum diet induced greater numbers of NEU at day 1 and 7 ( $1.76 \times 10^2$  and  $4.64 \times 10^2$  cells/ $\mu$ L) in comparison to RD ( $2.21 \times 10^2$  and  $4.63 \times 10^2$  cells/ $\mu$ L). 16S rRNA analysis demonstrated higher numbers of Operational Taxonomic Unit (OTU) among animals fed with colostrum in comparison to RD. Feeding with COL resulted in significantly higher OTU numbers of Enterobacteriaceae, Veillonellaceae in comparison to RD. Among animals that received RD, Enterobacteriaceae, Streptococcaceae were dominant species at day 1. The prevalence of probiotic species (Lactobacillaceae, Lachnospiraceae) was greater in animals with COL diet.

**Conclusion.** This study has characterized the influence of bovine colostrum on the development of calf intestinal microbiota. Bovine colostrum demonstrated strong immunomodulatory activity. Further studies are needed to better understand the molecular mechanisms leading to microbiota modulation and immunomodulation.

# 61. DENDRITIC CELL VACCINE MODULATION IN VITRO WITH CHARACTERISED EPITHELIAL OVARIAN CANCER CELL LINE LYSATE

Eglė Žymantaitė<sup>1</sup>, Jan Aleksander Kraško<sup>2</sup>, Agata Mlynska<sup>2</sup>, Emilija Paberalė<sup>2</sup>

<sup>1</sup>Faculty of Medicine, Vilnius University, Lithuania

<sup>2</sup>Immunology Laboratory, National Cancer Institute, Vilnius, Lithuania

[egle.zymantaite@mf.stud.vu.lt](mailto:egle.zymantaite@mf.stud.vu.lt)

Epithelial ovarian carcinoma (EOC) is a highly heterogeneous and aggressive disease that remains the most lethal gynaecological malignancy. EOC is the most frequent ovarian tumour, distinguished by high mortality in late stages when conventional therapies are no longer effective. The role of the immune system is pivotal for efficient control and destruction of cancer cells. Dendritic cells (DCs) serve as orchestrators of the cytotoxic response of the adaptive arm of the immune system and as such is an attractive target or component of cutting edge cell-based immunotherapies against cancer. DC vaccines have shown promising results in ovarian and several other types of cancers such as melanoma, prostate, lung cancer [1]. Some of the most commonly used are DC-based vaccines loaded with allogeneic cancer cell line lysate, which mimics the tumour present in the patient. Lysates developed from different cancer cell line models of the same tumour might, however, have a dissimilar effect on DC maturation and surface protein expression. That is because different cell lines represent distinct variances of the same disease and, therefore, have different protein constitutions. [2,3]. In our study, we undertook the task of creation of a DC-based vaccine with different ovarian cancer cell line lysates and their mixture to find out whether unique EOC cell lysates have an impact on dendritic cell maturation. We characterised four different EOC cell lines: OV7, SKOV3, COV362, A2780; under standardised culture conditions, by performing flow cytometry (FACS) (Fig. 1.) and analysing 27 immune-related genes expression by quantitative real-time PCR (qPCR). Cytokine array was used to evaluate 105 cytokines concentration in produced cancer cell line lysates later used for dendritic cell maturation. We noted that the four ovarian cancer cell lines differ both in stemness- related CD274, CD44, CD105, CD73 marker expression (FACS), and in immune-related gene expression (qPCR). In agreement, the cytokine array revealed that ovarian cancer cell line lysate profiles are highly diverse.

After cancer cell line characterisation, we successfully produced DCs loaded with different ovarian cancer cell line lysates and their mixture. DC characterisation was performed using FACS, maturation markers: CD11c, CD80, CD83, MHCII, CCRX7 were examined. We strongly believe that our findings will lead to development of more potent cell-based immunotherapies for patients suffering from EOC and, possibly, other tumors.

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## 62. NLRP3 INFLAMMASOME ACTIVATION BY VIRAL-LIKE PARTICLES IN MACROPHAGES

Kristina Mašalaitė<sup>1</sup>, Asta Lučiūnaitė<sup>1</sup>, Aurelija Žvirblienė<sup>1</sup>

<sup>1</sup>Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania  
[kristina.masalaite@gmc.vu.lt](mailto:kristina.masalaite@gmc.vu.lt)

Inflammasomes are intracellular protein complexes and important components of the innate immune system. The best described inflammasome is NLRP3, which contains three major components – nucleotide-binding and oligomerization domain-like receptor, adapter protein apoptosis-associated speck-like protein (ASC) and procaspase-1 [1]. NLRP3 inflammasome activation results in cleavage and activation of inflammatory cytokines, like IL-1 $\beta$ , and inducing inflammatory cell death – pyroptosis [2]. Activation of NLRP3 inflammasome is associated with various diseases, including asbestosis, gout, Alzheimer's disease and auto-inflammatory diseases. In our previous research we showed that NLRP3 inflammasome is activated by soluble amyloid-beta protein oligomers and protofibrils [3]. The aim of this study was to extend the latter research and determine whether oligomeric proteins of different structure activate NLRP3 inflammasome in macrophages.

Human monocytic cell line THP-1, differentiated to macrophages were selected as cell culture model for this study. Cells were treated with various viral oligomeric proteins: filaments forming measles and mumps viral proteins [4, 5]; spherical viral-like particles (VLPs) of these polyomaviruses – Karolinska Institutet polyomavirus (PyV), Merkel cell PyV [6], John Cunningham PyV [7]. MCC950 was used to inhibit NLRP3 inflammasome activation. NLRP3 activation was studied by evaluating cell viability by LDH detection assay, IL-1 $\beta$  and TNF- $\alpha$  cytokine release by ELISA, active caspase-1 detection using fluorescent probe FLICA, which binds to the activated caspase-1, and ASC speck detection using THP-1 macrophages expressing ASC protein fused to green fluorescent protein.

It was found that filament-like nucleocapsid proteins of measles and mumps viruses did not cause any inflammatory response in macrophages. In contrast spherical VLPs of polyomaviruses triggered cell death, induced IL-1 $\beta$  secretion and ASC speck formation in human cell model indicating NLRP3 inflammasome activation. In addition, it was demonstrated that cathepsins are involved in NLRP3 activation induced by polyomavirus VLPs. To conclude, our results demonstrate that viral proteins can activate inflammasome depending on their structural properties.

### Funding:

The study was funded by Research Council of Lithuania (LMTLT), project no. S-SEN-20-11.

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### 63. RESEARCH ON MICROPLASTICS POLLUTION OF LAKE GINEITIŠKĖS BOTTOM SEDIMENTS

Asta Kovrigina<sup>1</sup>, Vaidotas Valskys<sup>1</sup>

<sup>1</sup>Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania

[asta.kovrigina@gmc.stud.vu.lt](mailto:asta.kovrigina@gmc.stud.vu.lt)

**Introduction:** Over the last decade, pollution of the environment by microplastics (< 5 mm) has received an increasing attention and is now an emerging research topic. While many studies have focused on quantifying the amount of microplastics in the marine environment, there are relatively few studies on microplastics in inland water bodies [1]. Recent studies have reported high concentrations of microplastics in lakes and rivers, although the understanding of several factors affecting source, transport and destiny is still limited [2].

**Aim:** To investigate and evaluate microplastics pollution of surface and subsurface sediments of Lake Gineitiškės.

**Materials and methods:** Samples were taken at pre-selected points according to a prepared grid using ArcMap software. 32 bottom sediment samples were taken in Lake Gineitiškės: 16 surface (0–10 cm) and 16 subsurface (10–20 cm) bottom sediment samples.

The samples are rich in organic matter, thus 50 mL of 30 % hydrogen peroxide was added. The sample was mixed well and left for 1 week. The reaction was considered complete when no more air bubbles formed. The samples were mixed with distilled water in a polypropylene cylinder (300 mL) with a plunger to expel the sample. Sample homogenized and further 20 mL of olive oil was added. The samples were homogenized and left to stand for 30 minutes. The samples were frozen at -26°C (5 hours). The ice with solidified oil is pushed out on the filter paper (90 mm diameter, RED bar). After the oil got warmed, the samples were extracted with hexane to remove the oil traces; the filter was air-dried and microplastics were identified with a stereomicroscope.

**Results:** In surface sediments the fiber length varied from 0.1 mm to 5 mm. The amount of microplastics in 1 kg of sediments ranged from 50 particles kg<sup>-1</sup> to 400 particles kg<sup>-1</sup>; the size of fragments ranged from 0.1 mm to 0.4 mm; the amount of microplastics in 1 kg of sediment ranged from 50 particles kg<sup>-1</sup> to 200 particles kg<sup>-1</sup>. In subsurface bottom sediments, fiber length varied from 0.1 mm to 4.2 mm; microplastics concentration in 1 kg of sediment ranged from 50 particles kg<sup>-1</sup> to 300 particles kg<sup>-1</sup>, fragment size ranged from 0.1 mm to 0.2 mm, microplastics concentration in 1 kg of sediment ranged from 50 particles kg<sup>-1</sup> to 150 particles kg<sup>-1</sup>. The results show that the abundance, type, and size of microplastics in surface and subsurface sediments of different functional areas are different.

**Conclusions:** Lake Gineitiškės is a very shallow lake, which is characterized by intensive sedimentation [3, 4], therefore microplastics were found in both surface and subsurface bottom sediments. The total concentration of microplastics found in the surface sediment layers was 3800 particles kg<sup>-1</sup> while in the subsurface sediments it was 3000 particles kg<sup>-1</sup>. Preliminary sources of microplastics could be the urbanized area [5], intensive construction activities, and transport around the lake. Microplastics can also settle from the air [6], and they can be carried by wind and water currents [3].

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## 64. PREVALENCE OF SARCOCYSTIS PARASITES INFECTING FARMED ANIMALS IN WATER SAMPLES FROM LITHUANIA

Baranauskaitė, A.<sup>1,2</sup>, Strazdaitė-Žielienė, Ž.<sup>2</sup>, Prakas, P.<sup>2</sup>, Servienė, E.<sup>2</sup>

<sup>1</sup>Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania;

<sup>2</sup>Nature Research Centre, Vilnius, Lithuania;

[agne.terese@gmail.com](mailto:agne.terese@gmail.com)

Parasites of the *Sarcocystis* genus are protozoans that infect mammals, birds, and reptiles. They are characterized by obligatory two-host prey-predator life cycle. Intermediate host (including humans) may become infected after consuming food or water contaminated with sporocyst of *Sarcocystis*. Some *Sarcocystis* spp. are pathogenic to domestic and wildlife animals. These parasites were most comprehensively investigated in farmed animals. Until now, *Sarcocystis* infection has been mainly studied by analysing animal carcasses. The objective of the present study was to optimize the molecular-based method for the identification of *Sarcocystis* species from farmed animals (cattle, pig, sheep and goat) in water samples.

During this work, primer pairs that were specific and suitable for the identification of *S. bovifelis*, *S. cruzi*, *S. hirsuta*, *S. tenella*, *S. capracanis* and *S. miescheriana* parasites were selected and optimal conditions for PCR targeting *cox1* were determined. Using selected primer pairs, 72 water samples collected from lakes, rivers, reservoirs and the Baltic sea were analysed. Based on molecular methods, *Sarcocystis* parasites infecting farmed animals were identified in 79.2 % of water samples (57/72). The highest infection rate was detected in reservoirs (87 %): 57 % *S. capracanis*, 48 % *S. bovifelis*, 30 % *S. tenella*, 22 % *S. cruzi*, 13 % *S. miescheriana* and 4 % *S. hirsuta*. The prevalence of *Sarcocystis* infection in rivers (76 %) was similar to the one detected in lakes (74 %). Usually one of the species (35%) less often two species (19 %), very rarely three (9 %) or four species (6 %) were identified in individual sample. Three of the species selected for research were identified in the sample from the Baltic sea: *S. bovifelis*, *S. tenella* and *S. capracanis*. This study provides the first insight into contamination of water bodies in Lithuania with sporocysts of *Sarcocystis* species infecting farmed animals.

## 65. NEW TO SCIENCE SPECIES OF THE GENUS ANTOCHA OSTEN SACKEN, 1860 (DIPTERA: LIMONIIDAE)

Radvile Markeviciute

Nature Research Centre, Akademijos str. 2, LT-08412, Vilnius, Lithuania

[radvile.mark@gmail.com](mailto:radvile.mark@gmail.com)

Genus *Antocha* Osten Sacken, 1860 with three subgenera *Proantocha* Alexander, 1919, *Antocha* (s. str.) Osten Sacken, 1860 and *Orimargula* Mik, 1883 includes 158 species, four subspecies and, according to Catalogue of the Craneflies of the World, this genus is recorded from all zoogeographic regions [2]. Adults of this genus are small (3 mm) to medium-sized (11 mm) crane flies, which are found near streams or rivers, where larval stage develops [1]. The wings of these insects are wide with large, nearly right-angled anal angle. The subgenus *Antocha* (s. str.) is the most species-rich subgenus of the genus *Antocha*, with 112 valid species and two subspecies, including 30 species and two subspecies recorded from China[1].

The taxonomy of the genus remains unclear; several species are known only from the unique female holotypes and no phylogenetic analysis of *Antocha* has been undertaken. Due to lack of information, it is difficult to identify species of this genus, thus this group of insects is not well known. Moreover, insects of the genus *Antocha* form a large part of the river's benthos and could be used for biomonitoring of rapidly flowing rivers, but it is still impossible to identify many species of this genus larval stage.

The aim of the study is to present a newly discovered species of the genus *Antocha* and to provide information about habitat, illustrations of distinctive features and DNA sequences for scientific community.

This research was carried out in Sichuan province (China). Insects of the newly discovered species were caught with a light trap in 2016. The most distinctive features of this species are sclerotized and twisted into spirals genital structures – parameres, which are different in other species.

This type of taxonomic work provides a foundation for other scientific works. If the scientific name and systematic of any life form is unclear, it will be impossible to repeat an experiment, so the results will be incomparable, and hypotheses will be untested.

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## 66. MOLECULAR IDENTIFICATION OF SARCOCYSTIS SPECIES AND THEIR PREVALENCE IN ENVIRONMENTAL SAMPLES

Šikšniūtė, E.<sup>1,2</sup>, Baranauskaitė, A.<sup>1,2</sup>, Prakas, P.<sup>2</sup>, Servienė, E.<sup>1,2</sup>, Strazdaitė-Žielienė, Ž.<sup>2</sup>

<sup>1</sup>Nature Research Centre, Vilnius, Lithuania;

<sup>2</sup>Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania;  
[emilija.siksniute@stud.vgtu.lt](mailto:emilija.siksniute@stud.vgtu.lt)

Parasites of the *Sarcocystis* genus are protozoans that infects mammals (including humans), birds and reptiles, and are characterized by having obligatory two-host life cycle. Sarcocysts of these parasites are formed in muscles or CNS of the intermediate host, whereas oocysts/sporocysts develop in the definitive host. Some of the *Sarcocystis* species are harmful to humans, domestic and wild animals. Animals may become infected by consuming water or food containing sporocysts.

The detection of *Sarcocystis* parasites in the environmental samples is complicated by their low concentration and the lack of an appropriate research methods. The objective of the present work was to create and optimize molecular-based method for the identification of pathogenic *Sarcocystis* species and those that use economically important animals as their hosts from environmental samples. During this work, the optimal amount of sample required to detect oocysts/sporocysts and conditions for parasite gDNR purification and PCR were determined. The genomic DNA was extracted from soil, fodder and water sediment samples, and species-specific PCR targeting *cox1* was performed. Based on molecular methods, *Sarcocystis* were identified in 84.62 % of water sediment samples (22/26), 38.10 % of soil samples (8/21) and 100 % in fodder samples (11/11). The greatest diversity of *Sarcocystis* spp. were detected in soil samples, taken from pasture: 33.33 % *S. capracanis*, 9.52 % *S. cruzi*, 9.52 % *S. hirsuta*, and 9.52 % *S. tenella*, 4.76 % *S. bovifelis* and 4.76 % *S. miescheriana*. Five *Sarcocystis* species were found in fodder (*S. capracanis* 100 %, *S. hirsuta* 72.73 %, *S. tenella* 54.55 %, *S. miescheriana* 9.09 % and *S. bovifelis* 9.09 %) and two in water sediment samples (*S. hirsuta* 30.77 % and *S. capracanis* 73.08 %). This study provides the first insight into detection of *Sarcocystis* species prevalence in the environmental samples in Lithuania. The development and optimization of molecular-based method for the identification of *Sarcocystis* will allow the rapid and accurate detection of sporocysts in the natural environment and may be used to prevent the outbreak of the disease.



## 67. A MODIFICATION OF THE METAL-BINDING GROUP OF CARBONIC ANHYDRASE INHIBITORS LEADS TO IMMENSE BINDING AFFINITY LOSS

Denis Baronas<sup>1</sup>, Virginija Dudutienė<sup>1</sup>, Vaida Paketurytė<sup>1</sup>, Visvaldas Kairys<sup>2</sup>, Aivaras Vaškevičius<sup>1</sup>, Asta Zubrienė<sup>1</sup>, Daumantas Matulis<sup>1</sup>

<sup>1</sup>Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Life Sciences Center, Vilnius University, Saulėtekio 7, Vilnius LT-10257, Lithuania

<sup>2</sup>Department of Bioinformatics, Institute of Biotechnology, Life Sciences Center, Vilnius University, Saulėtekio 7, Vilnius LT-10257, Lithuania  
[denis.baronas@gmc.vu.lt](mailto:denis.baronas@gmc.vu.lt)

The aim of this work is to determine the factors leading to high selectivity and affinity of metalloenzyme inhibitors toward their counterpart protein. In this research, the 12 human isoforms of carbonic anhydrases (CA) were chosen as a model metalloenzyme protein to study protein-ligand recognition. Primary fluorinated benzenesulfonamides were selected as ligands of CAs that bind with picomolar affinity. A modification with a single methyl of their headgroup yielded secondary sulfonamides. Such an approach of introducing minor modifications of the sulfonamide group gives insight why primary benzenesulfonamides are such good inhibitors.

Interaction of secondary sulfonamides with CAs was determined by biophysical techniques such as fluorescent thermal shift assay, stopped-flow assay of inhibition of enzymatic activity and isothermal titration calorimetry.

In this study we showed that secondary sulfonamides exhibit the U-shape dependence of dissociation constants as a function of pH like primary sulfonamides (Fig. 1 A). Such dependence indicated the protein binding-linked protonation reactions that have to be subtracted from observed binding reaction in order to obtain the intrinsic binding constants that are necessary to perform structure – thermodynamics analysis of protein-ligand interaction. Intrinsic thermodynamic parameters of binding were calculated for secondary benzenesulfonamides by subtracting protonation reactions that are linked to the binding reactions. The obtained values were compared to intrinsic parameters of analogous primary benzenesulfonamides. Secondary sulfonamides bind to CA much weaker, by up to 30 kJ/mol, than analogous primary sulfonamides.

Crystal structures showed that secondary and primary benzenesulfonamides bound to the active site of CAs in different orientations (Fig. 1 B) despite the fact that secondary sulfonamide binding mechanism remains the same as primary sulfonamides.

Computational modeling showed the atomic contributions to the binding energetics and molecular docking was in agreement with crystal structures giving the insight of the mechanism of secondary sulfonamide binding to CA.

The reduction of binding affinity of secondary benzenesulfonamides compared with analogous primary sulfonamides is most likely due to different and unfavorable orientation of secondary sulfonamide in the active site of CA.

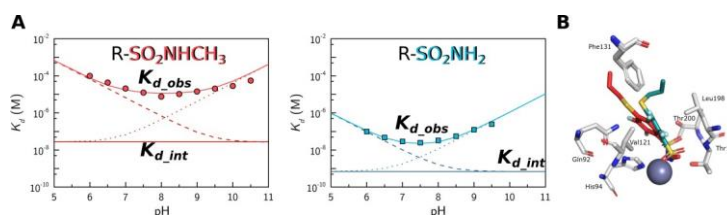


Figure 1. (A) The U-shape dependence of dissociation constants as a function of pH (red circles – secondary sulfonamide, blue squares – primary sulfonamide). (B) Superimposed X-ray crystallographic structures of CAII with bound secondary sulfonamide (blue) and primary sulfonamide (red).

## 68. THE THERMODYNAMIC STUDIES OF CARBONIC ANHYDRASE II AND ACETAZOLAMIDE INTERACTION

Eglė Vitkūnaitė and Lina Baranauskienė

Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Life Sciences Center,  
Vilnius University, Vilnius, Lithuania

[egle.vitkunaite@gmc.stud.vu.lt](mailto:egle.vitkunaite@gmc.stud.vu.lt)

Carbonic anhydrases are proteins of lyase family that catalyzes the reversible reaction of carbon dioxide hydration to bicarbonate ion and proton in vivo [1]. There are twelve catalytically active  $\alpha$ -CA isoforms in humans. Some of them are associated with human diseases such as glaucoma, oedema, epilepsy, cancer.  $\alpha$ -CA isoforms inhibition with small molecule drugs, containing unsubstituted sulfonamide group, is used to control some of these diseases. Nonetheless, there are some concerns using currently created drugs: side effects, non-specific binding, or inhibition of other proteins. Therefore, there is a need for developing CA isoform specific drugs.

Human CA II is a cytosolic isoform which has the highest enzymatic activity of all human CAs and is widely distributed in different organs and tissues. hCA II is a small, monomeric, non-glycosylated protein, which is readily available and has a well-studied 3D structure. All these properties make hCA II a perfect model protein for investigation of biomolecules interactions [2]. However, analysis of CA binding to its ligands is not straightforward. The hCA II binding to ligand that has sulfonamide group is linked to several concomitant reactions that includes protonation and deprotonation. Therefore, the observed binding affinity and enthalpy depends on reaction conditions [4]. However, only intrinsic thermodynamic parameters, i. e. independent on reaction conditions, can be directly linked to crystallographic information, and then such results of structure-thermodynamics relationships analysis can be used for rational drug design [3].

In this study we focus on intrinsic thermodynamic parameters of hCA II – acetazolamide interaction. We used two biophysical methods: isothermal calorimetry titration and fluorescence thermal shift assay. We analysed this reaction by performing experiments in various pH, buffers and temperature. According to dependence of observed binding parameters on reaction conditions we calculated the intrinsic binding parameters ( $K_d$ ,  $\Delta H$ ,  $C_p$ ).

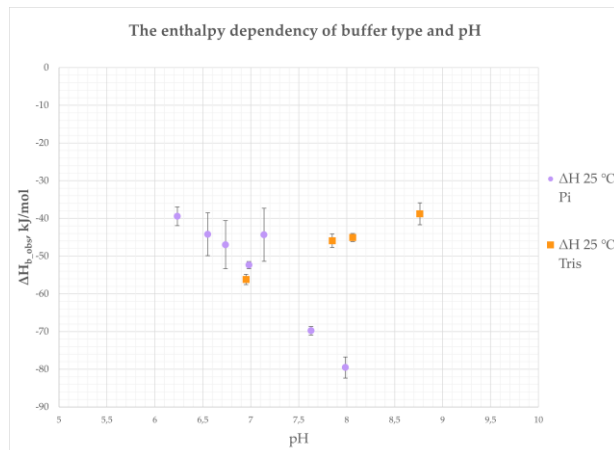


Figure 1. The dependence of observed enthalpy of hCA II and acetazolamide interaction on buffer type and pH. The experiments were performed at 25 °C temperature in 0.05 M phosphate buffer with 0.1 M NaCl (purple circles) or 0.05 M Tris buffer (orange squares), with 0.1 M NaCl.

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## 69. PH DEPENDENT SELF-ASSEMBLING OF H2TPPS4 IN AQUEOUS SOLUTIONS

Kornelija Buivydaite<sup>1,2</sup>, Marijus Plečkaitis<sup>1,2,3</sup>, Ričardas Rotomskis<sup>1,3</sup>

<sup>1</sup>Biomedical Physics Laboratory, National Cancer Institute, P. Baublio str. 3b, LT-08406 Vilnius, Lithuania

<sup>2</sup>Life Sciences Center, Vilnius University, Saulėtekio av. 7, LT-10257 Vilnius, Lithuania

<sup>3</sup>Biophotonics group of Laser Research Centre, Vilnius University, Saulėtekio av. 9, LT-10222 Vilnius, Lithuania

[kornelija.buivydaite@gmc.stud.vu.lt](mailto:kornelija.buivydaite@gmc.stud.vu.lt)

Recently, supramolecular assemblies of porphyrins have been of growing interests because the aggregate packing structure can be easily tuned through ionic interactions among the cationic core, charged substituents and/or the nature of the inorganic anion. Controlling the packing structure of functional dye molecules by supramolecular methods is a challenging task for tailoring functional materials with desired properties.

The association in aqueous solutions tetrasodium 5,10,15,20-tetrakis(4-sulfonatophenyl) porphyrin (H2TPPS4), which was detected by visible spectra, has been not clarified. In acidic media it has been described that new species appear with absorptions at 490 and 706 nm. In terms of excitonic coupling, aggregated dyes with blue- and red-shifted absorption bands are referred to as H- and J-aggregates. Due to the distinct optical properties, control of the formation of H- and J-aggregated states of dyes has attracted much research interest.

In this study, we investigated how different pH affects the formation of ionic species of H2TPPS4 molecules, their absorption and fluorescence spectra. The solutions were made by diluting H2TPPS4 in deionized water and then changing pH accordingly, with aqueous NaOH (for the pH 7), HCl (for pH 4 to pH 1), or H2SO4 (for pH -1) solutions. At the pH 7 tetra-anion form of H2TPPS4 was observed with Soret band at 414 nm and Q bands at 516, 553, 594 and 634 nm, respectively. Under 414 nm excitation, fluorescence was observed at 644 nm and 702 nm. By lowering to the pH 4, the H2TPPS4 changed to diacid form as two additional protons attached to the free nitrogens at the center of H2TPPS4 molecules. Bathochromic shift of the Soret band (435 nm) was observed in the absorption spectrum and the Q bands were reduced to 645 nm with the shoulders around 550 nm, 594 nm. H2TPPS4 diacid form under 435 nm excitation had a fluorescence peak at 670 nm. By lowering the pH even more at pH 1 the H2TPPS4 formed the zwitterionic form with not only the center nitrogens blocked with protons, but two substitute sulfo groups as well. In this form the H2TPPS4 molecules start to actively self-aggregate forming J-aggregates with a distinguished absorption spectrum and Soret band at 490 nm, and Q band at 708 nm. Under 490 nm excitation, the fluorescence peak of the J-aggregates measured at 714 nm. Then, using H2SO4 the H2TPPS4 solution of pH -1 was reached and at this pH no self-aggregation was observed, as not only all the center nitrogens, but even all the substitute sulfo groups of H2TPPS4 were blocked by protons. This led to an absorption spectrum with the Soret band at 438 nm and Q band at 649 nm (bathochromic shift compared to the diacid form of the H2TPPS4) and fluorescence (under 434 nm excitation) peak at 682 nm.

The results showed that at certain acidic conditions H2TPPS4 can form J-aggregates due to positive electrostatic interactions, however if pH gets too low, all SO<sub>3</sub><sup>-</sup> groups are blocked and the aggregates can no longer form..

## 70. OPTICAL MAPPING OF THE HEART IN SWINE MODEL

Vilma Zigmantaitė<sup>1</sup>, Ramunė Grigalevičiūtė<sup>1</sup>, Audrius Kučinskas<sup>1</sup>, Regina Mačianskienė<sup>2</sup>, Jonas Jurevičius<sup>2</sup>

<sup>1</sup>Biological research center, Lithuania University of Health Sciences, Kaunas, Lithuania.

<sup>2</sup>Institute of Cardiology, Lithuanian University of Health Sciences, Kaunas, Lithuania.

[vilma.zigmantaite@gmail.com](mailto:vilma.zigmantaite@gmail.com)

The emergence of optical imaging has unquestionably revolutionized the investigation of cardiac electrical activity as a major tool for elucidating the mechanisms of various cardiac pathological conditions, including arrhythmia [1]. Optical mapping (OM) is recognized as a promising tool to register the electrical activity of the heart. Most cardiac OM experiments are performed in ex vivo isolated heart models [2]. The study of the explanted heart is an excellent model, but the evaluation of the results is complicated by the fact that the autonomic nervous system and neurohumoral regulation are vital in cardiac function; therefore, in vivo studies are a closer step to bring the research to practical use [3] [4].

Our aim was to develop an OM system fitted for large animals and suitable to record cardiac electrical activity under the same conditions which are widely used in cardiac surgery.

All experiments involving animals were performed according to the European Community guiding principles and approved by the State Food and Veterinary Service of the Republic of Lithuania. We used a swine model, open-chest OM study. In the study we tried to record the electrical activity of the pig heart using different methods of cardiac immobilization in order to reduce heart movement artefact. Several different heart fixation methods were used in the experiments: a hand-made frame, an Octopus Evolution tissue stabilizer and chemical cardiac arrest during artificial blood circulation mode. Using the fluorescent voltage-sensitive dyes we succeed to record the electrical activity of the heart in vivo.

Using handmade frame or tissue stabilizer in experiments shows that the optical registration of cardiac electrical activity in vivo was successful because the obtained signals were sufficient to register electrical behavior throughout mapping area. However, the optical signal was not ideal, since residual motion and contraction artefacts still occurred and the field of view is quite limited using mechanical fixation (Fig. 1). The study results show that standard artificial blood circulation guarantees controlled conditions under which OM can be applied to record electrical activity on the whole heart in large animals.

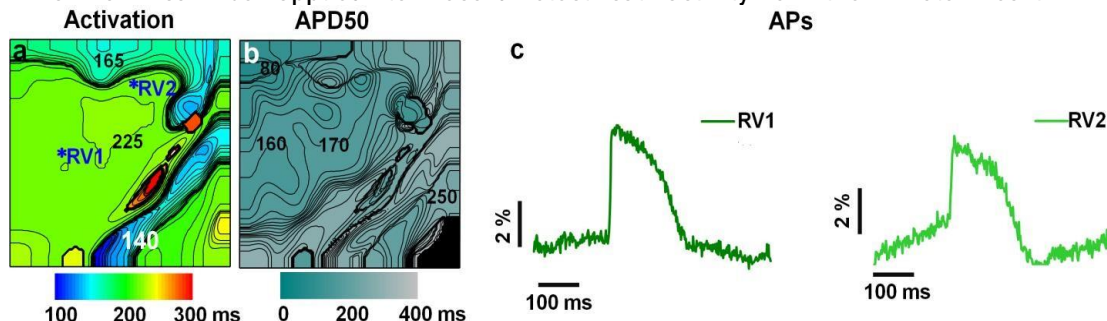


Figure 1. Electrical activity in the pig heart immobilized by the frame *in vivo* and recorded by optical mapping using dyes under physiological blood circulation. The obtained activation time and APD50 maps show that the action potential can be recorded this way, but the quality of the action potentials is limited because the OAP is scattered at the edges of the map area due to contraction (C, RV1 and RV2).

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# 71. CORRELATION OF INVASIVE AND NON-INVASIVE ARTERIAL BLOOD PRESSURE MEASUREMENTS IN EXPERIMENTAL SWINE MODEL

Dovilė Kiudelytė<sup>1,2</sup>, Vilma Zigmantaitė<sup>1</sup>, Ramunė Grigalevičiūtė<sup>1</sup>, Audrius Kučinskas<sup>1</sup>

<sup>1</sup>Biological research center, Lithuania University of Health Sciences, Kaunas, Lithuania.

<sup>2</sup>Faculty of Veterinary Medicine, Lithuania University of Health Sciences, Kaunas, Lithuania.

[dovikiud@gmail.com](mailto:dovikiud@gmail.com)

Blood pressure measurement is one of the most important patient monitoring parameters in both veterinary and human medicine and is still one of the most inaccurately performed. Accurate measurement of blood pressure is necessary to classify individuals, to ascertain blood pressure-related risks, and to guide management. [1] Invasive arterial blood pressure (IBP) measurement is a 'golden standard' for measuring blood pressure, but the complexity of the procedure and risk of side effects outweigh its accuracy in veterinary medicine. [2] An alternative to the IBP measurement is a non-invasive arterial blood pressure (NIBP) measuring method, which allows fast data acquisition, and doesn't require an invasive procedure, therefore it eliminates all the risks that IBP measurement carries and can be used in long-term clinical care or experiments. [3] The ideal goal is to combine the accuracy of IBP measurement with the simplicity and safety of NIBP measurement.

The aim of this research was to evaluate correlation of IBP and NIBP measurements in experimental swine model during anesthesia.

All experiments were performed according to the European Community guiding principles and approved by the State food and veterinary Service of the Republic of Lithuania. Our study included 20 Lithuanian local breed pigs weighing 30 – 40 kg. All animals were premedicated and put under general anesthesia, then monitored for eight hours. There were two main methods used to assess blood pressure values. IBP was monitored via catheter in the right common carotid artery. NIBP was measured either on the right front leg, between fetlock and carpal joints, or on the right hind leg between fetlock and tarsal joints. We calculated correlations between IBP and NIBP mean values using Statistica program for Windows and using geometrical interpretation obtained accurate formulas to determine IBP mean values from NIBP.

The results show that NIBP of both front and hind legs significantly correlates with IBP (Pearson correlation coefficient 0,85256). However, we observed generally higher IBP mean values compared to NIBP. Therefore, for a more precise evaluation of blood pressure in experimental setting, we suggest using the formula: mean IBP =  $8,8448 + 0,96148 \cdot \text{mean NIBP}$ , to calculate IBP when measuring NIBP on front leg or mean IBP =  $30,587 + 0,80860 \cdot \text{mean NIBP}$  when measuring NIBP on hind leg.

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## 72. RELATIONSHIP BETWEEN SUBJECTIVE EXPERIENCES AND BRAIN ELECTRIC MICROSTATES: INVESTIGATION USING OPTIMAL NUMBER OF CONFIGURATIONS

Povilas Tarailis<sup>1</sup>, Thomas Koenig<sup>2</sup>, Inga Griškova-Bulanova<sup>1</sup>

<sup>1</sup>Institute of Biosciences, Life Science Center, Vilnius University, Lithuania <sup>2</sup>Translational Research Center, University Hospital of Psychiatry, Bern, Switzerland  
[povilas.tarailis@gmc.vu.lt](mailto:povilas.tarailis@gmc.vu.lt)

One of the options to evaluate resting-state electroencephalogram (EEG) is to apply microstates approach. The recorded oscillations are defined as „states“ characterized by a unique, fixed spatial distribution of electrically active neurons with time varying amplitude [1]. These brain electrical microstates qualify for basic building blocks of mental and emotional processes [2], and their temporal properties can be modulated by various conditions, tasks and subjective experiences which can be quantified by resting state questionnaires.

In the previous study [3], using 4 canonical microstates (A, B, C, D), Pipinis et al. showed correlation between contribution of microstate C and somatic awareness. However, based on methodological literature [4], when only 4 microstates are used, spatially similar but functionally different microstates are merged into a single microstate class C. In this study, we aimed to determine the optimal number of microstates based on optimization criteria, relate them to different subjective experiences during resting state and localize their cortical sources.

5 min. eyes-closed resting state EEG data from 197 participants were collected using 64 channels EEG. After the recording, participants filled in Amsterdam Resting-State Questionnaire (ARSQ) [5]. EEG was subjected to microstates analysis where topographies at Global Field Power (GFP) peaks were submitted into k-means algorithm. Based on two measures of fit – Cross-Validation (CV), which predicts residual variance, and Krzanowski–Lai index (KL), which evaluates ratio of the relative difference of the within-clusters dispersion [6] – the optimal number of clusters was determined as 7. Duration, occurrence, contribution and transition probabilities were calculated for each of 7 microstates. Spearman's correlation coefficient between microstate parameters and scores on ARSQ categories were calculated. Cortical sources were estimated using low resolution electromagnetic tomographic analysis (LORETA).

Extracting more than canonical 4 microstate classes and using data-driven methods to determine the optimal number of clusters we showed that spatially similar but functionally different topographies – microstate C and microstate F – display distinct correlation patterns with different subjective experiences during the resting state and have distinct cortical sources.

Our result confirms that:

1. The optimal number of clusters should be estimated for each dataset using optimization criteria, rather than choosing a fixed number based on literature;
2. Use of resting state questionnaire which quantifies participants' cognitive state during resting state condition can potentially help to improve the interpretation or increase the sensitivity and specificity of neuroimaging biomarkers in clinical and pharmacological studies.

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## 73. REVIEW OF NEUROCHEMISTRY BEHIND CHANGES OF AUDITORY STEADY-STATE RESPONSE (ASSR) IN SCHIZOPHRENIA

Urtė Jašinskytė, Rasa Girijotaitė, Inga Griškova-Bulanova, Robertas Guzulaitis

Department of Neurobiology and Biophysics, Life Sciences Center, Vilnius University, Lithuania  
[urte.jasinskyte@gmc.stud.vu.lt](mailto:urte.jasinskyte@gmc.stud.vu.lt)

Schizophrenia is a multifaceted disorder that affects socialization, cognitive processes, mood and behavior, and has long-term negative health consequences. The disease affects about 1% of the population, and the first symptoms appear at a very young age. Despite the prevalence of the disease and the burden on society, the causes are not entirely clear. An Auditory Steady-State Response (ASSR) can be used to assess changes in brain activity that are caused by the disease. ASSR is based on the idea that neural networks in the central nervous system have a constantly changing activity - otherwise known as oscillations - and these oscillations can be triggered by a rhythmically presented sensory stimulus. ASSR as a biomarker of schizophrenia is gaining prominence not only due to consistent changes in the disease, but also observed changes and high-risk individuals, which would predict the disease at very early stages [1], [2].

ASSR is mainly used in human studies, therefore method is limited to non-invasive assessment. Investigation of mechanisms (molecular, cellular, network) requires use of invasive methods, therefore animal models allow studies on mechanisms behind ASSR generation and its alterations in disease. Research in animal models not only allows implementation of more precise recording techniques (local field potential, single cell, etc.), but also permits manipulations (pharmacology, electrical stimulation, optogenetics, etc.) to establish causality [3].

Here, we reviewed the literature exploring neurochemical mechanisms underlying changes of ASSR during schizophrenia (SZ). Most prominent ASSR is being induced as a response to 40 Hz stimulation in humans [4], therefore this frequency is most commonly used in animal studies. ASSR is quantified through time-frequency decomposition of EEG/LFP data and as a phase-locking index (PLI) where the degree of phase consistency across trials is measured [5], [6]. These parameters were taken into account when reviewing the appropriate articles. Different neurochemical manipulations, where agonists and/or antagonists were used, had different effects on ASSR power and PLI parameters. This demonstrates the complexity of schizophrenia and suggests that multiple neurotransmission systems are altered during disease. This review demonstrates how animal models can be helpful in fundamental understanding of brain function and malfunction during disease.

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## 74. PHOTOMECHANICAL EFFECT INDUCED ON INDRADERMAL TATTOO PIGMENTS WITH PICOSECOND Nd: YAG LASER

Justinas Baleišis<sup>1</sup>, dr. Romualdas Rudys<sup>1</sup>

<sup>1</sup>Department of Biomodels, State Research Institute Centre for Innovative Medicine, Lithuania

[Justinas.baleisis@imcentras.lt](mailto:Justinas.baleisis@imcentras.lt)

A Tattoo is a permanent mark or design made on the body by the introduction of pigment through ruptures in the skin. Currently the tattoos market is growing at a phenomenal rate. This has led in growth of tattoo removal procedure as well. Methods of tattoo removal include dermabrasion, skin grafts or plastic surgery, and laser surgery. The most appealing form is the Q-switched nanosecond domain lasers, that up until recently were the gold standard in the field. Complex and multicolor pigment induced tattoos are a challenge to remove and promising results are seen in the published data regarding the use of shorter – picosecond domain lasers [1]. The reason behind that being that for picosecond domain pulses a lower fluence is needed during the treatment sessions due to the higher peak power and greater photoacoustic effect, which can offer less side-effects and a better pigment clearance [2].

When stating the term “tattoo removal” it is necessary to explain, that the “removal” is the fragmentation of the targeted particle into smaller fragments. Thermal and stress confined laser-energy deposition in the targeted particle is needed, to generate an internal pressure wave or acoustic wave. Fracture can occur when the tensile force of the acoustic wave exceeds the corresponding limit of the particle. That can be affected by the influence of the used laser pulse length, as with shorter length can be noted an increase of particle fracture. This relationship has its limits when the acoustic wave confinement time is longer than the pulse length [3].

Most commercially available lasers excel at 300–600 ps. In our study, we aimed to investigate the laser-tissue interactions of a picosecond domain Nd: YAG laser, that can generate 150 ps pulses and energies ranging up to 250 mJ and 120 mJ for 1064 and 532 nm wavelength, respectively. Thus, providing insight in shorter pulse efficacy and safety in the use for tattoo pigment fragmentation. The effects of 150 ps ultrashort pulses were not currently described, and the area was absent of in vivo animal studies that could offer great translational value, when interpreting study results from animal to human. For that reason, this study was performed on a in vivo porcine model.

As excessive energy delivered to the superficial skin can lead to blistering, prolonged healing, scurrying and change in pigmentation [4] it is important to optimize the fluence of the laser energy. Impact of different fluencies for side-effect development and pigment fragmentation efficacy were investigated on different color tattoos of known pigment composition. This has been carried out by changing the wavelength, spot size and pulse energy of the laser. The tattoo removal was analyzed using clinical macroscopic examination and histochemistry.

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### 75. LONG-TERM GENOTOXIC AND CYTOTOXIC EFFECTS OF MICROPLASTICS ON LARVAL-STAGE SALMO TRUTTA

Agnė Bučaitė<sup>1</sup>, Milda Stankevičiūtė<sup>1</sup>, Janina Pažusienė<sup>1</sup>

<sup>1</sup>Laboratory of Genotoxicology, Nature Research Centre, Lithuania  
[bucaite.agne@gmail.com](mailto:bucaite.agne@gmail.com)

Microplastics (MPs) are widespread in the marine environment and are becoming an emerging contaminant worldwide. MPs are able to cause a variety of adverse effects on organisms from oxidative stress and inflammation to developmental and growth disorders that can reduce population fitness. Unfortunately, the toxicity of MPs in the early life stages of fish is not yet fully understood or adequately studied, since most ecotoxicological studies so far have been conducted on adult or juvenile fish. The aim of this study was to evaluate the genotoxic and cytotoxic potential of long-term (113 days) exposure to microplastics using sea trout (*Salmo trutta*) larvae as experimental animals. It is important to notice that non-ingestion related toxicity of MPs was investigated, as yolk-sac larvae do not feed exogenously. *S. trutta* were exposed to polystyrene (PS), polyethylene terephthalate (PET) and polyethylene (PE) at environmentally realistic concentrations. Micronucleus and other erythrocytic nuclear abnormalities were used as biomarkers of cytogenetic damage. The formation of micronuclei (MN), nuclear buds (NB), nuclear buds on filament (NBf), blebbed nuclei cells were assessed as genotoxicity endpoints, and 8-shaped nuclei, fragmented apoptotic (FA) and bi-nucleated (BN) cells as cytotoxicity endpoints. Total genotoxicity levels were assessed as the sum of the frequencies of the detected genotoxicity endpoints. This study revealed significantly higher total genotoxicity values in all MPs exposure groups compared to the control. Increased occurrence of nuclear buds was observed in all MPs exposure groups, while elevated micronuclei frequencies were only noticed in PS and PET groups. Fragmented apoptotic and bi-nucleated cells were not detected, making 8-shaped nuclei erythrocytes the only observed cytotoxic aberration. There was no significant difference in frequencies of cytotoxicity endpoints in MPs exposure groups compared to the control. Different polymer types showed different toxicity (PS being the most genotoxic while PE was the least genotoxic). Considering that there is an extensive amount of polymer types, each of them designed with specific characteristics, which might have different effects on organisms, further research is required. Likewise, not only polymers by themselves but experiments with their additives also show increased toxicity and bioavailability of different pollutants or pathogens. Finally, cytogenetic potential of nanoplastics should also be investigated.

## 76. METHYLATION ANALYSIS OF VITAMIN D METABOLIC PATHWAY GENES IN RHEUMATOID ARTHRITIS PATIENTS

Aistė Gerulaitytė<sup>1</sup>, Justina Gaiževska<sup>1,2</sup>, Eglė Puncevičienė<sup>3,4,5</sup>, Lina Vencevičienė<sup>6,7</sup>, Rasa Sabaliauskaitė<sup>2</sup>, Irena Butrimienė<sup>3,4,5</sup>, Sonata Jarmalaitė<sup>1,2</sup>

<sup>1</sup>Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania

<sup>2</sup>National Cancer Institute, Vilnius, Lithuania

<sup>3</sup>Centre of Rheumatology, Vilnius University Hospital Santaros klinikos, Vilnius, Lithuania

<sup>4</sup>Institute of Clinical Medicine, Clinic of Rheumatology, Traumatology Orthopaedics and Reconstructive Surgery, Faculty of Medicine, Vilnius University, Vilnius, Lithuania

<sup>5</sup>State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania

<sup>6</sup>Clinic of Internal Medicine, Family Medicine and Oncology, Faculty of Medicine, Vilnius University, Vilnius, Lithuania

<sup>7</sup>Centre of Family Medicine, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania

[aiste.gerulaityte@gmc.stud.vu.lt](mailto:aiste.gerulaityte@gmc.stud.vu.lt)

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune multi-factorial disease that adversely affects the quality of life. Multiple environmental and genetic factors have been associated with increased risk for RA. Early diagnosis and therapeutic intervention or treatment can prevent severe disease manifestations in patients suffering from this autoimmune disease. Vitamin D (VitD) has important role as a natural immune modulator via regulating the expression of genes which have been implicated in the pathophysiology of autoimmune diseases. Reduced VitD intake has been linked to increased susceptibility to the development of rheumatoid arthritis (RA) and VitD levels has been found significantly lower in the RA patients, as compared to the controls. Moreover, recent studies demonstrate that epigenetics play important roles in the pathogenesis of RA, especially DNA methylation modification.

The aim of this study was to analyze the frequency and intensity of methylation of genes in the VitD metabolism pathway in RA.

In this study, we used bisulfite conversion and pyrosequencing as tools to determine DNA methylation of three genes (VDR, CYP24A1, CYP2R1) in 35 RA patients samples and 41 healthy controls. The results revealed that gene methylation frequency (MF) and methylation intensity (MI) showed no significant difference between RA patients and controls ( $p > 0.05$ ). Furthermore, the data demonstrated that gene MF or MI does not differ significantly with VitD concentration. However, overall CYP24A1 methylation level was significantly higher in comparison to VDR ( $p < 0.0001$ ) and CYP2R1 ( $p < 0.0001$ ) genes. Also, CYP24A1 gene MI showed a significant positive correlation with RA patients age ( $r = 0.5190$ ;  $p = 0.0017$ ). Moreover, the results revealed a significant difference between VDR methylation frequency and RA impact of disease (RAID) ( $p = 0.0182$ ).

In conclusion, although no significant differences in gene methylation were found between patients and controls, we can suppose from other results that methylation of DNA is significantly involved in the pathogenesis of RA and could be applied as a promising biomarker in the disease progression but more studies are needed.

**Acknowledgements:** This project has received funding from the Research Council of Lithuania (LMTLT), agreement No. S-MIP-17-12.

# 77. ANALYSIS OF NON-INVASIVE BIOMARKERS FOR INCREASED RISK ASSESSMENT OF CLEAR CELL RENAL CELL CARCINOMA

Augustė Vipartaitė<sup>1</sup>, Kristina Žukauskaitė<sup>2</sup>, Rasa Sabaliauskaitė<sup>1,2</sup>, Sonata Jarmalaitė<sup>1,2</sup>

<sup>1</sup>Institute of Biosciences, Life Science Center, Vilnius University, Vilnius, Lithuania

<sup>2</sup>National Cancer Institute, Vilnius, Lithuania

[auguste.vipartaite@gmc.stud.vu.lt](mailto:auguste.vipartaite@gmc.stud.vu.lt)

Renal cell carcinoma (RCC) is the most common type of kidney cancer. Most cases of RCC are discovered incidentally by non-invasive imaging techniques and survival is highly dependent upon the stage of disease at diagnosis, with the late-stage metastatic disease having only a 12% 5-year survival rate [1]. RCC is a highly heterogeneous disease that includes several histological subtypes. The most common and particularly challenging histology is clear cell renal cell carcinoma (ccRCC), which represents up to 75% of RCCs [2]. Sequencing of primary ccRCC tumors demonstrated that *VHL*, *PBRM1*, *BAP1*, and *SETD2* are the most commonly mutated genes [3]. Remarkably, ccRCC is associated with a loss of the short arm of chromosome 3p which represents the first genetic event that has a long latency period (more than 30 years) between this genetic change diagnosis and development of ccRCC (Figure 1). Early identification of the disease has high importance because it can impact clinical management. Considering the lack of sufficient non-invasive markers for ccRCC diagnosis, liquid biopsies such as blood plasma could provide an attractive and minimally invasive method to identify diagnostic biomarkers in ccRCC patients.

This study aimed to determine the changes in the gene expression of *VHL*, *PBRM1*, *SETD2*, and *BAP1* in the plasma of patients with ccRCC compared to samples from people with benign tumors to adapt them for initial risk assessment and early disease diagnosis.

In the present study, 9 plasma samples of ccRCC patients and 16 plasma samples from patients with non-malignant kidney tumors were analyzed. Clinical samples were collected during 2019–2021 at National Cancer Institute. Plasma mRNA levels were evaluated using reverse transcription polymerase chain reaction.

The results of our study revealed significant differences in *VHL*, *SETD2*, and *BAP1* mRNA expression levels in plasma samples of ccRCC patients compared to benign tumors (all  $P < 0.05$ ). Gene expression analysis revealed that *VHL*, *SETD2*, and *BAP1* plasma mRNA levels in ccRCC samples were significantly lower compared to noncancerous cases. Such differences may have occurred before the onset of the disease. Because of the lack of early-stage symptoms, precocious detection of chromosome 3p loss associated with decreased *VHL*, *PBRM1*, *SETD2*, and *BAP1* expression would help to identify an increased risk of renal cancer and to provide the most appropriate treatment on time.

In conclusion, detection of *VHL*, *PBRM1*, *SETD2*, and *BAP1* expression changes in blood plasma samples may serve as potential biomarkers and could help to define the occurring disease and thus guide to patient management.

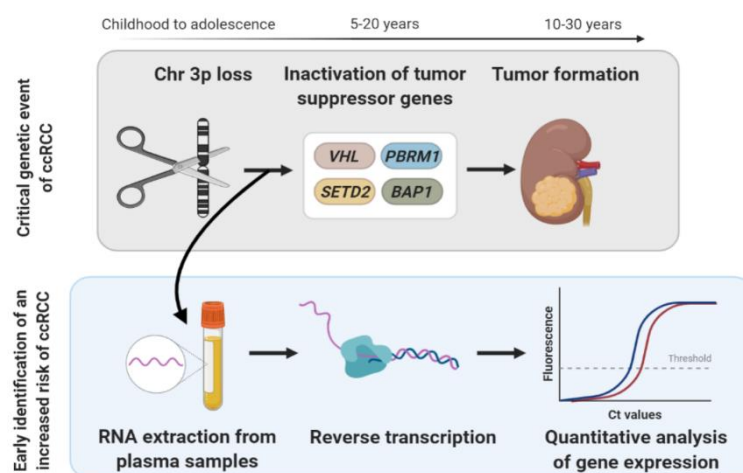


Figure 1. Schematic representation of ccRCC formation and potential possibility of early disease prediction.

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## 78. ANALYSIS OF PROSTATE CANCER ASSOCIATED GENES' SINGLE NUCLEOTIDE POLYMORPHISMS IN PROSTATE CANCER PATIENTS' COHORTS OF LITHUANIA

Mantautas Šimkus<sup>1</sup>, Justina Gaiževska<sup>1,2</sup>, Paulius Bosas<sup>2</sup>, Arnas Bakavičius<sup>2,3,4</sup>, Albertas Ulys<sup>2</sup>, Rasa Sabaliauskaitė<sup>2</sup>, Feliksas Jankevičius<sup>3,4</sup>, Sonata Jarmalaitė<sup>2</sup>

<sup>1</sup>Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania

<sup>2</sup>National Cancer Institute, Vilnius, Lithuania

<sup>3</sup>Urology Centre, Vilnius University Hospital Santaros klinikos, Vilnius, Lithuania

<sup>4</sup>Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania

[mantautas.sim@gmail.com](mailto:mantautas.sim@gmail.com)

**Introduction:** Prostate cancer (PC) is one of the most common types of cancer [1]. Despite the recent progress of diagnosis and research, it remains a significant medical problem [2]. The attention is paid to the identification of genetic variation which increases susceptibility because it could help to develop screening strategies and clinical management [3]. Numerous single nucleotide polymorphisms (SNPs) which might play an aggregate role in PC susceptibility have recently been identified. The goal of the study was to identify several SNPs found in genes associated with PC using quantitative polymerase chain reactions (qPCR) and explore correlations between SNPs and clinical characteristics of PC patients.

**Methods:** DNA was extracted from the leucocytes of PC patients whose cancer is in the early stages (n = 128) and castration-resistant prostate cancer (CRPC) patients (n = 47). qPCR with TaqMan assays were used to identify SNPs found in HOXB13, KLK3, CDKN1B, RFX6 and ANO7 genes. Associations between the form of cancer and clinical characteristics, genotypes and clinical characteristics were analyzed.

**Results:** The genotypes of early-stage PC and CRPC patients in reference to the SNPs of HOXB13, KLK3, CDKN1B, RFX6 and ANO7 genes were identified. There was no significant difference between early-stage PC and CRPC patients regarding the distribution of analyzed genotypes and alleles' frequencies ( $p > 0.05$ ). Significant associations between the form of cancer and patients' Gleason score, prostate-specific antigen (PSA) level and the existence of metastasis were identified ( $p < 0.001$ ). No significant associations between alleles and the form of cancer, genotypes and the form of cancer, age of diagnosis, PSA level, Gleason score, T category, the existence of metastasis or the localization of metastasis were found ( $p > 0.05$ ).

**Conclusions:** The genotypes of patients in both cohorts were determined. Significant associations between the form of cancer and clinical data were found, although there were no significant associations between identified genotypes and analyzed clinical characteristics. To identify a more realistic situation in the country, wider research involving more patients could be conducted.

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## 79. Polyomaviruses: a model for virus-host co-evolution

Emilija Vasiliūnaitė<sup>1</sup>, Alma Gedvilaitė<sup>1</sup>

<sup>1</sup>Department of Eukaryote Gene Engineering, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania

[emilija.vasiliunaite@gmc.vu.lt](mailto:emilija.vasiliunaite@gmc.vu.lt)

Polyomaviruses (PyVs) are widespread, small (~5 kb) double-stranded DNA viruses that infect mammals, birds, and fish. PyVs are associated with various human diseases, including cancer, progressive multifocal leukoencephalopathy, nephropathy, skin and respiratory tract diseases, however, pathogenesis normally occurs only in immunocompromised patients. Since their discovery in the 1950s, polyomaviruses have been a model for research of important cellular processes, such as oncogenesis and replication. Recently, pathogenesis, evolution and zoonotic abilities of PyVs are being extensively studied. SARS-CoV-2 pandemic emphasized the importance of virus evolution, emergence and host-change studies and lack of reliable viral models for such research. Despite few known zoonoses (simian virus 40 is known to infect humans; Sorex araneus polyomavirus 1, formerly known as Human polyomavirus 12, was first identified in human hosts, later on – in shrews), polyomaviruses are thought to co-evolve with, rather than change their hosts, however, host change and recombination events were an important part of PyV evolution. Rodents, the largest group of mammals, is one of the biggest reservoirs of zoonotic diseases. Rodents have been relatively little investigated for PyVs (rodent PyVs make up only 10% of all known mammalian PyVs, while rodents cover 40% of all mammalian diversity), making them a good target to search for new polyomaviruses. Furthermore, closely related rodent species and their PyVs are a suitable model for the analysis of virus-host coevolution.

In this study, we examined 288 vole (genus *Microtus*) kidney and 96 chest cavity fluid samples from several locations in Europe: Lithuania, Spain, Germany, Czech Republic and the Netherlands. Two polyomavirus species were identified in *Microtus arvalis* samples: previously identified Common vole polyomavirus (*Microtus arvalis* polyomavirus 1, MarPyV1) and a novel polyomavirus – *Microtus arvalis* polyomavirus 2 (MarPyV2). Whole-genome sequences of MarPyV2 from Germany, Spain and the Netherlands were obtained through PCR amplification and Sanger sequencing. Sequenced MarPyV2 genomes had a size of 4985–4999 bp and a whole-genome identity of ~74% to a mouse polyomavirus (*Mus musculus* polyomavirus 2) and ~62% to MarPyV1. We conducted a virus-host co-evolution analysis among populations and identified that virus-host co-evolution was the most important event for the inter-population diversity of this virus.

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## 80. CIRCULATING MIRNAS AS DIAGNOSTIC BIOMARKERS FOR PERIODONTITIS: NEW DIAGNOSTIC APPROACH?

**Benita Buragaite-Staponkiene<sup>1</sup>, Kristina Snipaitiene<sup>1</sup>, Adomas Rovas<sup>2</sup>, Ruta Matuleviciute<sup>1</sup>, Egle Punceviciene<sup>3</sup>, Irena Butrimiene<sup>3</sup>, Alina Purienne<sup>2</sup>, Sonata Jarmalaite<sup>1</sup>**

<sup>1</sup>Human Genome Research Group, Life Sciences Center, Vilnius University, Vilnius, Lithuania

<sup>2</sup>Vilnius University Hospital Zalgiris Clinic, Vilnius, Lithuania

<sup>3</sup>Vilnius University Hospital Santaros Clinics, Vilnius, Lithuania

Periodontitis (PD) is highly prevalent inflammatory disease, affecting tooth-supporting tissues, resulting in loss of the periodontal junction and destruction of alveolar bone. In addition to its adverse effects on quality of life, PD is also associated with patients' increased susceptibility to other systemic disorders, e.g., diabetes, obesity, rheumatoid arthritis, respiratory and cardiovascular diseases, and even cancer. Epigenetic regulation plays an important part in pathogenesis of PD and microRNAs (miRNAs) are one of the most critical modulators that impact periodontal homeostasis. The present study aimed to reveal miRNAs associated with PD in gingival tissue and bodily fluids, including gingival crevicular fluid (GCF), saliva and plasma, and to assess the utility of those miRNAs as minimally- and non-invasive diagnostic markers for PD.

MiRNA profiling of eight gingival tissue samples was performed and four significantly differently expressed miRNAs were selected for validation in extended cohort of gingival tissue samples (N=61) and for further analysis in corresponding liquid biopsy specimens (GCF, saliva and plasma, N=171) by means of quantitative reverse transcription PCR (RT-qPCR).

Microarray analysis revealed a number of miRNAs that were significantly overexpressed in PD-affected gingival tissues as compared to periodontally healthy tissues. After thorough validation, miR-199a-5p, miR-483-5p, miR-3198 and miR-4299 were analyzed in bodily fluids. The level of miR-3198 was significantly higher in GCF collected from patients with PD ( $P=0.019$ ). The level of salivary miR-199a-5p was identified to be increased in severe PD cases ( $P=0.019$ ), and decreased in PD-affected participants' plasma samples ( $P=0.008$ ), as compared to periodontally healthy individuals. Moreover, significant alterations in the abundance of GCF miR-3198 and plasma miR-199a-5p were strongly associated with main periodontal outcome parameters. The best significant diagnostic performance was demonstrated by combination of GCF miR-3198 and miR-4299 ( $AUC=0.86$ ,  $P<0.0001$ ) with 68% sensitivity and 96% specificity.

The current study revealed that altered expression of miR-199a-5p, miR-483-5p, miR-3198, miR-4299 in gingival tissues significantly correlated with the presence and severity of PD. Furthermore, the levels of GCF miR-3198, -4299 and plasma miR-199a-5p were associated with PD, suggesting that these miRNAs may serve as minimally- or non-invasive tool for PD diagnostics.



# 81. CYTOSINE MODIFICATIONS EXHIBIT CIRCADIAN OSCILLATIONS THAT ARE INVOLVED IN EPIGENETIC AGING AND COMPLEX DISEASE

Gabriel Oh<sup>1</sup>, Karolis Koncevičius<sup>2</sup>, Sasha Ebrahimi<sup>1</sup>, Matthew Carlucci<sup>1</sup>, Daniel Erik Groot<sup>1</sup>, Akhil Nair<sup>1</sup>, Aiping Zhang<sup>1</sup>, Algimantas Kriščiūnas<sup>2</sup>, Edward S. Oh<sup>1</sup>, Viviane Labrie<sup>1</sup>, Albert H. C. Wong<sup>1</sup>, Juozas Gordevičius<sup>2</sup>, Peixin Jia<sup>1</sup>, Miki Susic<sup>1</sup>, Art Petronis<sup>1,2</sup>

<sup>1</sup>The Krembil Family Epigenetics Laboratory, The Campbell Family Mental Health Research Institute, Centre for Addiction and Mental Health, Toronto, Canada

<sup>2</sup>Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania  
[karolis.koncevicius@gmc.vu.lt](mailto:karolis.koncevicius@gmc.vu.lt)

Epigenetic factors are found to be involved in the circadian machinery; however, despite extensive efforts, detection and characterization of circadian DNA modification at a nucleotide (cytosine) level has been elusive. In order to address this gap we performed a mapping of oscillating modified cytosines (osc-modC) in mouse liver and lung tissues [1] as well as purified human neutrophils [2] and were able to detect the presence of osc-modCs in all investigated tissues (8.2%, 35.6%, and 8.6% of variable cytosines were found to be oscillating in mouse liver, mouse lung, and human neutrophils respectively).

Interestingly, our detected osc-modCs belonged to the category of cytosines exhibiting a high degree of inter-individual variation, which has traditionally been treated as stochastic. In addition, osc-modCs showed several links to the circadian transcriptome and, importantly, were associated with the aging epigenome: oscmodCs tended to precede age-dependent DNA modification changes, and their amplitude strongly correlated with the magnitude of the aging effect. Such linking of the two temporal dimensions (dynamic circadian cycles and linear molecular aging) provides a possible new interpretation of aging and its proximal causes.

Since aging is closely related to human disease, we next asked if there were any associations between osc-modCs and human diseases. For this, we re-analyzed the data from epigenome-wide association studies of four human diseases: chronic lymphocytic leukemia [3], schizophrenia [4], obesity (BMI) [5], and type II diabetes [6]. We detected that cytosines showing differential modification in disease were statistically significantly associated with osc-modCs detected in our human neutrophil experiment (odds ratio ranged from 1.2 to 8.1; with p values ranging from  $3.2 \times 10^{-3}$  to  $2.8 \times 10^{-47}$ ), which suggests that circadian epigenomic dysregulation might be involved in the etiopathogenesis of disease.

In summary, the existence of cytosines with circadian DNA modification dynamics may provide a new perspective on several fundamental questions in biomedical research. In the broadest sense our findings hint towards a common mechanism underlying circadian rhythmicity, aging, and disease.

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## 82. DATA COLLECTION FOR THE STATISTICAL CLASSIFICATION OF BIOSIGNATURE INFORMATION

Aivaras Vilutis<sup>1,2</sup>, Diana Gentry<sup>2</sup>

<sup>1</sup>Vilnius University, NASA International Internships Program, *Lithuania / USA*

<sup>2</sup>NASA Ames Research Center, *USA*

[aivaras.vilutis@gmc.stud.vu.lt](mailto:aivaras.vilutis@gmc.stud.vu.lt)

In our work we present a novel method for life detection. We have started our work asking ourselves whether machine learning could help us classify various samples into life – potential and life non – potential. We address one of the main astrobiological problems mentioned in the Ladder of Life – there is no universal criteria for life yet and numerous resources highlight the need to identify measurable parameters for the evidence of life. [1] This work is the first stage of developing a universal biosignature information classifier and is based on crucial data collection and labeling. All of this is a beginning of a new way to answer a fundamental question of our Universe – are we alone?

Such a classifier must be trained on parameters to classify systems. Systems are observable things, such as a rock, polymer, or a tree, that can be represented by a set of measurements. Parameters are these measurements, corresponding to features like chemical complexity or physical structure. The biggest challenge here is to provide a good training data set that is diverse, reliable and has suitable parameters corresponding to mission instruments [2]. We introduce a data set that consists of over 20 different systems that are unambiguously alive (i.e. *Escherichia coli*, diatoms, etc.) or abiogenic (i.e. meteorites, lunar rocks, stardust, etc.). We also provide data and databases on selected parameters – main and trace/rare elemental abundance [3], elemental composition [3], mineral composition, isotopic fractionation [3], elemental ratios [3], enantiomeric excess [4], various spectroscopy, differences of sugars and amino acids [5].

Future perspectives are also highlighted – a lot of additional collected data was ambiguous but can still be used as an advanced approach in the upcoming stages. We will expand the training set to include non – extant biogenic systems, such as stromatolites, ichnofossils, phytoliths and other bacterial encrustation. We also identify possible biogenic systems that include sponges, other lichens, and species like *Mus musculus*, *Drosophila melanogaster*, *Danio rerio*. These model organisms are valuable because the literature already provides nearly every parameter of interest.

Later on, different classification algorithms will also have to be tested using the training and validation data collected here. Even if some of the data was not available for specific systems, there were similar systems that have had these parameters characterized. These systems still have to be included – that leads us to the conclusion that combining data is crucial if we want to distinguish alive systems from the abiogenic ones.

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### 83. ANALYSIS OF THE PREVALENCE OF SINGLE NUCLEOTIDE POLYMORPHISM IN THE CHRNA5 GENE AND ITS INFLUENCE ON NICOTINE DEPENDENCE

Viltė Juozokaite<sup>1</sup>, Titas Radzevicius<sup>1</sup>, Ugnė Meškauskaitė<sup>1</sup>

<sup>1</sup>Seventh Fort of Kaunas Fortress, Educational Biochemistry Laboratory, Archyvo St. 61, Kaunas, LT 50157, Lithuania

[vjuozokaite@gmail.com](mailto:vjuozokaite@gmail.com), [titas.radzevicius@gmail.com](mailto:titas.radzevicius@gmail.com)

**Introduction.** Nicotine addiction is known to be one of the toughest to cure. Research has shown that only 5% of smokers quit smoking successfully after their first try [1]. In addition, nicotine use is apparent among the youth – a conducted study has proven that around one in three high school students use nicotine products and often choose electronic cigarettes [2]. From a genetics perspective, scientists have proven that individuals, possessing a single nucleotide polymorphism (SNP) rs16969968 (78590583G>A) in the CHRNA5 gene, are more likely to become dependent on nicotine products [3]. Implementation of genetic testing related to CHRNA5 gene's SNP would make it possible to use personalised medicine methods to help the addicted overcome their dependence and warn non-smokers of the possible consequences of consuming nicotine-containing substances.

**Aim of the study.** Investigate the prevalence of CHRNA5 gene's SNP in smokers and non-smokers as well as determine their genetic likelihood of developing a nicotine addiction.

**Methods.** Individuals (n = 62) fill consent forms and questionnaires which contain information about their relationship with nicotine-containing products. DNA sample (saliva) is processed and genetic material is extracted by using Chelex resin. Specific DNA fragments are amplified during the polymerase chain reaction (PCR). The restriction fragment length polymorphism (RFLP) method is used to cut out the specific DNA fragments by the TaqI restriction enzyme. After running the agarose gel electrophoresis, fragments are evaluated in the ultraviolet (UV) light. The data were analyzed using statistical calculation methods (Chi-square, p-value).

**Results.** The most common genotype in the smokers and non-smokers groups was GA (associated with a slightly higher risk of dependence) with a frequency of 0.613 and 0.581, respectively. The frequency of AA genotype (associated with the highest risk to develop nicotine addiction) was 0.129 (group of smokers) and 0.065 (group of non-smokers). After the comparison of genetic results and questionnaire answers, it was determined that the polymorphism of CHRNA5 did not have a significant influence on nicotine dependence ( $p > 0.05$ ). It is likely that the statistical results were found to be unreliable because of the small sample size; therefore, it would be appropriate to repeat the study with an increased number of participants.

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## 84. ORANŽINIŲ TATUIRUOČIŲ DAŽŲ POVEIKIS ŽINDUOLIŲ LĄSTELĖMS IN VITRO

Agnė Žaromskytė<sup>1</sup>, Silvija Andruškevičiūtė<sup>1</sup>

<sup>1</sup>Kauno tvirtovės VII fortas, Edukacinė biochemijos laboratorija, Archyvo g. 61, LT 50157, Lietuva  
[agne.zaromskyte@gmail.com](mailto:agne.zaromskyte@gmail.com)

**Įvadas.** Tatuiruotės, šiomis dienomis, yra ypač populiari saviraiškos priemonė tarp jaunų žmonių. Apklausos rodo, kad Europos sąjungoje tatuiruotes turi 12% bendros populiacijos, o 18–20 amžiaus žmonių tarpe šis rodiklis didesnis ir siekia iki 25% [1]. Istoriskai spalvų pigmentams išgauti buvo naudojami įvairūs mineralai, tačiau šiais laikais virš 80 % dažų spalviklių yra organiniai anglies junginiai, iš kurių 60 % yra azo dažai. Tam tikri atskiri tatuiruočių dažų komponentai gali būti neutralūs, tačiau reaguodami tarpusavyje gali tapti toksiškais [2]. Dėl to yra svarbu įvertinti, ar tatuiruočių sudedamosios medžiagos nekeičia savo savybių sąveikaudamos tarpusavyje bei jas veikiant UV ar lazerio spinduliais. Jau yra įrodyta, kad azo dažai skyla UVB bei saulės spinduliuose, o titano oksidas yra skilimo reakcijų katalizatorius. Šių reakcijų produktai yra pavojingi, potencialiai gali būti toksiški bei kancerogeniški [3]. Azo pigmentai yra uždrausti naudoti kosmetinių priemonių gamybai, tačiau nėra jokių apribojimų jų naudojimui tatuiruočių rašalų gamyboje [4].

**Darbo tikslas.** Įvertinti, ar į radikalus suskilę azo dažai, esantys oranžiniuose tatuiruočių dažuose, gali toksiškai veikti žinduolių ląsteles in vitro.

**Metodai.** Oranžiniai tatuiruočių dažai (pigmentai: „Geltonas 83“ („Yellow 83“), „Oranžinis 16“ („Orange 16“)) ir 1:1 maišyti oranžiniai:balti tatuiruočių dažai (titano oksidas ir oranžinių dažų pigmentai), savaitę veikiami ultravioletinėmis B bangomis. UVB veikų ir neveiktų azo dažų poveikis CHO ląstelėms nustatomas žaizdos gijimo ir MTT jautrumo testais. Žaizdos gijimo testui ląstelių monoslouksnis buvo paveikiamas 0,5 % dažų koncentracija, žaizda daroma su 200 µl pipetės antgaliuku ir fotografuojama po 0 valandų, 2 valandų, 6 valandų ir 24 valandų, naudojant programą „ImageJ“ apskaičiuojamas žaizdos gijimo greitis. MTT testu tiriamas 0,5 % ir 0,1 % koncentracijos tatuiruočių dažų poveikis CHO ląstelėms. Po inkubacijos su dažais, ląstelės veikiamos MTT reagentu, kuris gyvybingose ląstelėse formuoja formazano kristalus, matuojama šviesos sugertis, įvertinama gyvybingų ląstelių koncentracija.

**Rezultatai.** MTT testas parodė, kad 0,5 % ir 0,1 % koncentracijos 1:1 maišyti oranžiniai:balti tatuiruočių dažai turėjo panašų poveikį CHO ląstelėms nepriklausomai nuo dažų veikimo UVB. Ląsteles veikiant UVB veiktais oranžiniais dažais (0,5 % koncentracija), jų gyvybingumas buvo mažesnis (63%) nei veikiant paprastais oranžiniais dažais (73 %), bet skirtumas nebuvo statistiškai reikšmingas. Žaizdos gijimo testas atskleidė, kad UVB veikti oranžiniai dažai nulėmė lėtesnę ląstelių migraciją ir proliferaciją po 2 val. (gijimo greitis 12 500 µm<sup>2</sup>/h) lyginant su UV neveiktais dažais paveiktomis ląstelėmis (16 750 µm<sup>2</sup>/h). Priešingi rezultatai gauti ląsteles veikiant oranžiniais:baltais dažais (žaizdos gijimas ląsteles paveikus UVB veiktais dažais – 46 000 µm<sup>2</sup>/h, UVB neveiktais dažais – 23 250 µm<sup>2</sup>/h), tačiau abiem atvejais skirtumai buvo statistiškai nereikšmingi.

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# 85. SKIRTINGŲ FITOHORMONŲ SANTYKIŲ MAITINAMOJOJE TERPĖJE POVEIKIS ŽIEDINIO KOPŪSTO (BRASSICA OLERACEA VAR. BOTRYTIS L.) EKSPLANTŲ AUGIMUI IR VYSTYMUISI MIKRODAUGINIMO METU

Augustė Adomaitytė<sup>1</sup>, Gabija Vaitkevičiūtė<sup>1</sup>, Aida Rudokaitė<sup>1</sup>

<sup>1</sup>Kauno tvirtovės VII fortas, biochemijos edukacinė laboratorija, Archyvo g. 61, Kaunas, LT50157 Lietuva  
[augustei.ad@gmail.com](mailto:augustei.ad@gmail.com)

Mikrodauginimo metodas – efektyvus būdas užauginti didelį kiekį augalų, turinčių tą patį genetinį kodą – t. y. klonų. Šis metodas gali būti naudojamas norint užauginti sveiką augalą, nepalietą virusų, bakterijų ar grybų, bandant išsaugoti nykstančias rūšis ar išgauti didelį kiekį genetiškai identiškų augalų. [1] Mikrodauginimas pasaulyje plačiai naudojamas ne tik komerciniu lygmeniu, bet ir atliekant įvairius mokslinius tyrimus, kuriems reikalingas didelis kiekis genetiškai identiškų augalų. Nors šis klonavimo metodas gali būti pritaikytas daugumai augalų, kiekvienas augalas išsiskiria tam tikrais aspektais. Vienas iš jų – skirtingas reikiamų hormonų balansas. [2] Tyrimu siekta išsiaiškinti tinkamiausią hormonų pusiausvyrą mitybinėje terpėje žiediniam kopūstui (*Brassica oleracea* var. *Botrytis* L.) mikrodauginti. Norint nustatyti optimaliausią hormonų santykį buvo pasirinkti trys skirtingi fitohormonai: N6-furfuriladeninas (kinetinas), indolo-3-actio rūgštis (IAA) bei 6 – Benzilaminopurinas (BAP), ir ištirtos šešios skirtingos jų santykių variacijos.

## **Darbo tikslas:**

Ištirti skirtingų fitohormonų (kinetino, indolo-3-actio rūgšties (IAA) ir 6-Benzilaminopurino (BAP)) santykių maitinamojoje terpėje poveikį žiedinio kopūsto (*Brassica oleracea* var. *Botrytis* L.) eksplantų augimui ir vystymuisi mikrodauginimo metu.

## **Darbo metodai:**

Tyrimas atliktas in vitro sąlygomis panaudojant žiedinį kopūstą (*Brassica oleracea* var. *Botrytis*) kaip modelinę sistemą. Žiedinio kopūsto eksplantai auginami Murashige ir Skoog (MS) terpėje su skirtingomis hormonų koncentracijomis ir deriniais 24 valandų šviesos fotoperiode. Renkami duomenys, reikalingi statistikai: eksplantų aukštis (mm), lapų formavimasis (naudojama skalė 0–3), šaknų formavimasis (naudojama skalė 0–3). Vykdyta statistinė analizė: dispersinė analizė (ANOVA), taip pat Tukey HSD testas. Nustatyta, kuris iš hormonų derinių yra efektyviausias *Brassica oleracea* var. *Botrytis* mikrodauginimui.

## **Rezultatai:**

Penkių savaičių augimo laikotarpyje buvo matuojamas eksplantų ūgis, jų šaknų bei lapų vystymasis. Atlikus gautų duomenų statistinę analizę nustatyta, jog 3-ią savaitę tarp žiedinio kopūsto eksplantų, augusių hormonų santykiais besiskiriančiose mitybinėse terpėse, statistiškai reikšmingo skirtumo nebuvo. Visgi, remiantis 5-os savaitės eksplantų ūgiais buvo matoma, jog eksplantai efektyviau auga mitybinėje terpėje, kurioje yra IAA ir 6-BAP mišinys nei terpėje be hormono, terpėje su IAA nei terpėje be hormono bei terpėje su kinetino ir IAA mišiniu nei terpėje be hormono. Norint paskatinti lapų formavimąsi, efektyviausiai veikia kinetino ir IAA mišinys, o šaknų formavimasis efektyviausias mitybinėje terpėje esant IAA hormonui arba terpėje be hormonų.

[1] George, E.F., Hall, M.A. ir Klerk, G.-J.D. (2008). Micropropagation: Uses and Methods. Plant Propagation by Tissue Culture, p.29–64.

[2] Kyte, L., Kleyn, J., Scoggins, H. ir Bridgen, M. (2013). Plants from Test Tubes – An Introduction to Micropropagation. 4-tas leidimas Timber Press, pp.28–34..

## 86. LAIMO BORELIOZĘ SUKELIANČIŲ BORRELIA BURGDORFERI SENSU LATO BAKTERIJŲ NUSTATYMAS JONAVOS MIESTE SURINKTOSE IXODES RICINUS RŪŠIES ERKĖSE

Beata Kolesnikovaitė<sup>1</sup>, Ugnė Meškauskaitė<sup>1</sup>

<sup>1</sup>Kauno tvirtovės VII fortas, Edukacinė biochemijos laboratorija, Archyvo g. 61, Kaunas, LT 50157, Lietuva  
[beata.kolesnik@gmail.com](mailto:beata.kolesnik@gmail.com)

**Įvadas.** Sergamumas erklių platinamomis ligomis (EPL) vis didėja. Žmonėms ir gyvūnams erkės gali perduoti įvairių ligų sukėlėjus: erkinio encefalito, Laimo ligos (LL), babeziozės, anaplazmozės, erlichiozės, tularemijos sukėlėjus [1]. Tai pavojingos ligos, kai kuriais atvejais galinčios sukelti neįgalumą ar net mirtį. Žmogui pavojingiausios EPL yra Laimo boreliozė ir erkinis encefalitas. Laimo liga yra viena iš dažniausių erklių sukeltų ligų [2]. Sergamumas LL pastaraisiais metais Jonavos mieste yra 5–6 kartus didesnis nei erkinio encefalitu [3]. LL ligos gydymo strategija remiasi antibiotikų terapija, kurios sėkmė priklauso nuo ankstyvos ligos diagnostikos [4,5]. Siekiant sumažinti sergamumą LL itin svarbi nuolatinė LL paplitimo stebėseną, tačiau trūksta tyrimų apie Laimo boreliozės patogenų paplitimą ir dinamiką erkėse [6]. Todėl žinant LL sukėlėjų paplitimą Jonavos mieste aptinkamose erkėse, galima nustatyti skirtingų vietų žaliųjų erdvių saugumą lankytojui, planuoti erklių naikinimo prevencines priemones. Ypač aktualu turėti greitas ir patikimas monitoringo priemones, paremtas molekulinės biologijos sprendimais. Taip pat svarbus ir visuomenės informavimas, apie patogenais užsikrėtusių erklių mastą.

**Tyrimo tikslas.** Nustatyti keturiose Jonavos miesto rekreacinėse vietose surinktų Ixodes ricinus rūšies erklių užsikrėtimą Borrelia burgdorferi sensu lato bakterijomis.

**Metodai.** Pasitelkiant baltą audinį erkės surinktos iš 4-ų Jonavos miesto gyventojų dažnai lankomų objektų: Jonavos Joninių slėnio, prie Laukagalių g. ir Gaidžiūnų g. esančios pievos ir Taurostos parko dviračių tako šalikėlio. Iš surinktų erklių išskirta DNR, kuri polimerazinės grandininės reakcijos (PGR) metu padauginama naudojant pagal genų seką sumodeliuotus pradmenis. Gauti PGR produktai vizualizuojami elektroforezės pagalba ir įvertinami ultravioletinėje šviesoje, nustatant, ar tirtoji erkė yra I. ricinus rūšies ir B. burgdorferi s. l. bakterijų nešiotija.

**Rezultatai.** Ištyrus 88 Ixodes ricinus erkes nustatyta, kad B. burgdorferi s. l. bakterijų nešiotijos yra 21 (23,86 %) I. ricinus erkė. Daugiausia LL bakterijomis infekuotų erklių yra prie Taurostos parko dviračių tako – iš 23-ų surinktų I. ricinus erklių B. burgdorferi s. l. bakterijų nešiotijos buvo 9-os erkės (39,13 %).

[1] Mead, P., Petersen, L., Kersh, G. (2019). Lyme and other tickborne diseases increasing. Centers for disease control and prevention. Interneto prieiga: <https://www.cdc.gov/media/dpk/diseases-and-conditions/lyme-disease/index.html>

[2] Murray, T. S., Shapiro, E. D. (2010). Lyme disease. Clinics in Laboratory Medicine, 30(1), 311–28.

[3] Čapkauskienė, V. (2020). Erklių platinamų ligų profilaktikos priemonių taikymo rekomendacijos Jonavos rajono savivaldybės administracijai ir ūkio subjektams. NVSC Kauno departamento Jonavos skyrius.

[4] Heinz, F. X., Stiansny, K., Holzmann, H., Grgic-Vitek, M., Kriz, B., Essl, A., Kundi, M. (2013). Vaccination and tick-borne encephalitis, Central Europe. Emerging Infectious Diseases, 19(1), 69–76.

[5] Rizzoli, A., Hauffe H., Carpi G., Gi Vourc, H., Neteler, M., Rosa, R. (2011). Lyme borreliosis in Europe. Euro Surveill, 16(27), 25–32.

[6] Arbočienė, J. (2019). Jonavos rajono sergamumo erklių platinamos ligos analizė. NVSC Kauno departamento Jonavos skyrius.

# 87. NILERED SINTEZĖ IR SOLVATOCHROMINIŲ SAVYBIŲ TYRIMAS

Meda Surdokaitė<sup>1</sup>, Marta Marčiulionytė<sup>1</sup>

<sup>1</sup>Kauno tvirtovės VII fortas, Edukacinė chemijos laboratorija, Archyvo g. 61, Kaunas, LT 50157, Lietuva  
[medasurd@gmail.com](mailto:medasurd@gmail.com)

**Įvadas.** Pramonėje didžiuliais kiekiais naudojami organiniai tirpikliai, kurių paklausa bei vartojimas vis didėja. Kiekvieną dieną milijonams darbuotojų tenka susidurti su organiniais tirpikliais ir neretam su jų poveikiu sveikatai: toksiškumu nervų sistemai, kvėpavimo takų, inkstų bei kepenų pažeidimais, neigiamu poveikiu reprodukcinei sistemai, vėžiu [1]. Organiniai tirpikliai naudojami neteisingai, patekę didelėmis koncentracijomis ir kiekiais į aplinką gali kelti riziką gamtai bei ekologijai. Tarptautinė darbo organizacija rekomendavo šiam klausimui skirti didelį prioritetą [2]. Tirpikliai naudojami plačiai; jų poveikis sveikatai yra rimtas; organinių tirpiklių kelių bei ribines vertes reikia kontroliuoti, todėl ypač svarbu turėti prieinamą, lengvai naudojamą metodiką saugumo prevencijai ir ribinių koncentracijų nustatymui. Šiandien organinių junginių aptikimui naudojami brangūs instrumentinės analizės metodai, kurių dėl tyrimui reikalingų kaštų ir laiko sąnaudų nepavyksta plačiai taikyti, todėl aktualūs greitos diagnostikos metodai. Tam galima taikyti solvatochromines savybes turinčius junginius. Nilo raudonasis (NileRed) gali būti pritaikytas naudojimui kaip indikatorius atpažinti skirtingus organinius tirpiklius, kadangi gali būti fiksuojamas ir regimojoje, ir UV šviesoje.

**Tyrimo tikslas.** Susintetinti NileRed dažą bei ištirti solvatochromines savybes.

**Metodai.** Sintezės metodas: nitrozavimo reakcijos metu iš 3-dietilaminofenolio gaunamas 5-(dietilamino)-2-nitrosfenolis – tarpinis reakcijos produktas, kartu su 1-naftoliu ištirpdomi organiniame tirpiklyje [3]. Reakcijai vykti reikalinga aukšta temperatūra – tirpiklio virimo temperatūra. Pasibaigus reakcijai, Nilo raudonojo dažas nuo tirpiklio atskiriamas rotacinio garinimo metodu. Su paimtu dažo mėginiu ruošiamas koncentruoto acetono tirpalas ir plonasluoksnės chromatografijos metodu tikrinama, ar susidarė dažas ir kokios priemaišos mišinyje po reakcijos liko. Kizelgūro ir dažo mišinys yra įnešamas į sausos vakuuminės chromatografijos kolonėlę ir gryninamas [4]. Chromatografijai naudojami heksanoizopropanolio arba heksano-acetono tirpiklių mišiniai su poliškumo didėjimu. Solvatochrominių savybių tyrimo metodas: sintezės metu gautas Nilo raudonasis tirpinamas organiniuose tirpikliuose. Nustatyti naudojama 0,01g dažo su kizelgūru ir 5ml organinio tirpiklio. Gauti spalviniai tirpalų pokyčiai įvertinami regimojoje ir UV šviesoje.

**Rezultatai.** Išgryninus tarpinį produktą apskaičiuota išeiga, kuri siekia 87,89%. Susintetintas Nilo raudonasis dažas pasižymi solvatochrominėmis savybėmis, geriausiai tirpsta alkoholiuose ir acetone (ryškiausia spalva regimojoje spinduliuotėje, UV šviesoje sunkiai švyti dėl didelės koncentracijos), sunkiausiai tirpsta alkanuose (heksane), cikloalkanuose (cikloheksane), netirpsta vandenyje. Nuo tirpiklio poliškumo priklauso Nilo raudonojo savybė tirpti.

[1] Interneto prieiga: <https://osha.europa.eu/lt/publications/summary-state-art-report-reproductive-toxicants>

[2] Interneto prieiga: [https://www.vdi.lt/Forms/Tekstas1.aspx?Tekstai\\_ID=2433](https://www.vdi.lt/Forms/Tekstas1.aspx?Tekstai_ID=2433)

[3] Markus Borgardts, Kathrin Verlinden, Manuel Neidhardt, Tobias Wöhrle, Annika Herbst, Sabine Laschat, Christoph Janiak and Thomas J. J. Müller, SYNTHESIS AND OPTICAL PROPERTIES OF COVALENTLY BOUND NILE RED IN MESOPOROUS SILICA HYBRIDS – COMPARISON OF DYE DISTRIBUTION OF MATERIALS PREPARED BY FACILE GRAFTING AND BY CO-CONDENSATION ROUTES, December 2015

[4] Daniel Sejer Pedersen,\* Christoph Rosenbohm, DRY COLUMN VACUUM CHROMATOGRAPHY, September 2001, Denmark.



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