THE COINS

International Conference of Life Sciences

Abstract book
2020

Vilnius University
Students' Representation
in Life Sciences Center

Foreword

Dear participants of The COINS 2020,

It is my great pleasure to welcome all of you to the 15th annual International Conference of Life sciences – The COINS 2020. Born from the initiative of students, this conference aims to spread news and science to a wider audience every year.

The main desire of the project is to create the kind of atmosphere where everyone - keynote speaker as well as attendee - will feel like a part of the warm and welcoming scientific community. Therefore, in the following three days, you will be able to get acquainted with various people of the world of science and the impact they have had on it. The conference hosts scientists introducing their famous discoveries, companies presenting cutting edge innovations, students talking about their research, and guests who are willing to share their valuable insight during the panel discussion.

I am pleased to say that due to the passion and curiosity of all the people who are gathering here in Vilnius, The COINS 2020 has become increasingly relevant, unified and accessible to all.

So let's go deeper, seek wider and reach higher during today's already 15 years old conference.

Sincerely,
Coordinator of the COINS 2020
Elizabet Beržanskytė

Content

Programme

February 25th

08:30-09:30	Registration
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10:00-11:00	**Panel disscussion: Networking in science**
Session 1: Molecular Biology	
11:00-12:00	**Keynote speaker: NOBEL PRIZE WINNER Aaron Ciechanover – The Revolution of Personalized Medicine: Are We Going to Cure all Diseases and at What Price?**
12:00-12:30	Coffee break
12:30-13:15	**Keynote speaker: Joan Taylor –Novel Insulin Technologies**
13:15-14:15	Lunch
14:15-15:00	**Keynote speaker: Matthias W. Hentze – A New Continent of the RNA World**
15:00-15:10	Break
15:10-15:25	Student presentation: **Aistė Zentelytė** - Amniotic fluid - the untapped source of stem cells

15:30-16:15	**Keynote speaker: Augustas Pivoriūnas – Extracellular Vesicles As a New Mode of Intercellular Communication and Potent Novel Theapeutic Tools Against Neurodegenerative Diseases**
16:20-16:35	Student presentation: **Karolina Kriaučiūnatė** - In vitro modeling of Alzheimer's disease: potential role of astrocyte-derived extracellular vesicles
19:00	Evening Ceremony

February 26th

08:30-09:30	Registration

Session 2: Genetics

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10:20-11:05	**Keynote speaker: Alina Urnikytė – Inferring Micro evolutionary Processes in Local Human Populations**
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11:35-11:50	Student presentation: **Milda Narmontė** - New approach for genome-wide single-base resolution profiling of 5-hydroxymethylcytosine

11:55-12:40	**Keynote speaker: Peter Hegemann – Multicomponent Optogenetics: Sensing is not Understanding**
12:45-13:00	Student presentation: **Austėja Balevičiūtė** - Transfection of CHO-K1 Cells Using Nanosecond Electroporation
13:00-14:00	Lunch
13:30-14:45	**COMPANY FAIR**

Session 3: Neuroscience

14:45-15:30	**Keynote speaker: Marius Bauža – There and Back Again: How Do Animals Navigate**
15:35-16:20	**Keynote speaker: Tomaš Paleniček – The Neuropsychological Effects of Psilocybin: Focus on Cognitive Processing and Brain Activity, Implications for Treatment**

February 27th

08:30-09:30	Registration

Session 4: Accesing The Complexity Of Living Systems

09:30-10:15	**Keynote speaker: Jonas Cicėnas – Kinases, Cancer and OMICS**

0:20-11:20	**Live video lecture: NOBEL PRIZE WINNER Jean-Marie Lehn – A Journey from Molecular towards Adaptive Chemistry**
11:20-11:50	Coffee break
11:50-12:35	**Keynote speaker: Luca Mazzitelli – Biology at High Resolution with 10X Genomics: from single cell applications to spatial transcriptomics**
12:40-12:55	Student presentation: **Vilius Malūnavičius** - Protein engineering of Geobacillus lipolytic enzymes - from enzyme fusions to directed evolution
13:00-14:00	Lunch
14:00-15:00	**POSTER SESSION**
15:00-15:30	**“Vilnius-Lithuania iGEM” team presentation**
15:30-16:00	Closing ceremony and awards

Ambassadors

Aušrinė Armonaitė

Lithuanian liberal politician

Member of the Seimas in Lithuania

Was an appointed Member of the Parliament in 2016

Since 2019, she is the chairwoman of the Freedo**m Party**

Gintaras Valinčius

Biochemist, director of the Center for Life Sciences at Vilnius University

He has published more than 60 articles in peer-reviewed international scientific journals

Keynote speakers

Skirmantas Kriaučionis

Skirmantas Kriaučionis is an Associate Professor in University of Oxford, Ludwig Institute for Cancer Research. After graduating from Vytautas Magnus University he got Darwin Trust Scholarship for doctoral studies in the University of Edinburgh. Post-doctoral work he did in Rockefeller University in New York. Since 2010 he has established a group in Oxford, where his work aims do understand the function of DNA modification in genomes of normal and cancer cells.

The Roles of Chromatin in the Function of Transcription Factors viliuand Gene Expression

Deterministic transitions between repressed and transcribed gene states underpin normal development and cell physiology. Gene activation is achieved through the interaction of transcription factors (TFs) with chromatin, establishing a state permissible for the productive transcription. While *in vitro* TFs typically recognise short DNA sequences, which are abundant in the genome, only small fraction of those sequences is found occupied *in vivo*. The principles behind this discrepancy are not fully understood. We have investigated roles of two chromatin features, which might impact on TF binding – DNA methylation and acetylation of histones. By profiling chromatin accessibility, gene expression and occupancy of five TFs, we demonstrate that transcription factors have evolved district ways to interact with chromatin. Most notably, DNA methylation and deacetylation of histones act as independent mechanisms to supress occupancy of some TFs at distinct, young families of retrotransposons, which maintained TF binding motifs during the course of evolution. Consequently, loss of DNA methylation and histone deacetylase inhibition result in elevated transcription of those retrotransposon families. In conclusion, chromatin acts as important mediator of TF behaviour in the genome by enabling TFs with small recognition motifs to act on genes rather than spurious or retrotransposon sites in the genome.

Peter Hegemann

Peter Hegemann is a Hertie professor for neuroscience and head of Experimental Biophysics at Humboldt-Universitaet zu Berlin. In 2019 he won a Warren Alpert Foundation Prize for the discovery that lead to the new technology - optogenetics. Hegemann's research focused almost entirely on the characterization of natural sensory photoreceptors. Hegemann has characterized behavioral and photoelectric responses of the unicellular alga Chlamydomonas, a work that cumulated in the claim that the photoreceptors for these responses a rhodopsins that unify the sensor and ion channel in one protein. He has finally proven this concept by identifying the light gated channel channelrhodopsin, and its functionality in animal cells. His group characterized this protein in molecular detail by a wide range of biophysical techniques which lead to the deciphering of the ion channel mechanism, including gating and ion selection. This work was the basis for the discovery of Optogenetics, a technology where light activated proteins – first of all channelrhodopsin - allow to control selected cells of large networks as the animal brain with unprecedented precision in space and time just by application of light. The Hegemann group also works on light-activated enzymes which further expand the optogenetic applications to important biochemical pathways.

Multicomponent Optogenetics ←→ Sensing is not Understanding

Activation or inactivation of a living cell, tissue or animal, either naturally or artificially, initiates small or larger long-term changes within the living system (The biological uncertainty principle). In most cases the researcher intends to learn about the living system without being aware about its own – in many cases -destructive action. Illumination of a cell is probably one the least destructive action one may undertake. We study natural sensory photoreceptors mostly from green algae with respect to the original function within the algal context but also modify these photoreceptors and employ them in host cells to mani-pulate host processes ideally non-invasively. For a long time the membrane voltage has been the main host parameter and we engineered - supported by spectroscopic and structural information - light-gated channels and light-driven pumps in many direction with respect to color sensing, ion selectivity and kinetics, and converted ion pumps into ion channels or vice versa to understand the principle differences. More recently we focused on second messengers as cAMP and cGMP by employment of fungal photo-activated cyclases. The combi-nation with cyclic nucleotide gated (cNG) channels generated multi-component optogenetic systems with large amplifications providing ultra high sensitivity in host cells. We keep in mind Max Plancks concept: "Understanding precedes application" and we begin to learn that "manipulation always causes distraction from natural behavior". Moreover, large signals do not mean natural responses, it may be just the opposite.

Marius Bauža

Marius Bauža is a senior research fellow in O'Keefe group (which received the Nobel Prize in Physiology or Medicine in 2014). He has a background in physics (Vilnius University) and engineering (University College London) and for the last several years has used his expertise to study how the brain works. He takes part in developing, testing and using Neuropixels silicon probes. These high-density probes can record neural activity from an unprecedented number of cells and could potentially open a new era of chronic and acute electrophysiology recordings from awake animals. Marius has already used these probes to show that grid cell pattern is more local than previously thought. Patterns of simultaneously recorded grids deform in sync, suggesting that the grid cell system could still act as a universal spatial metric. He is actively participating in studying the neural basis of spatial cognition in the hippocampal and parahippocampal formations. In addition, he is participating in other cutting edge research projects, such as the Honeycomb Maze behavioural test platform.

There and back again: how do animals navigate?

How do we know where we are and how do we navigate to other locations? What are the neuronal mechanisms allowing us to quickly form map-like representations of the environments and then flexibly use this knowledge? Such computations are enabled chiefly by the brain region called hippocampal formation where multiple types of neurons responsible for representing space are found. I will describe the properties of major classes of spatial cells and their role in spatial navigation and present the recent advances in the technology, that will further our understanding of how neural networks help animals to survive and navigate in complex environments.

Jonas Cicėnas

Jonas Cicėnas currently is a senior scientist/bioinformatician Proteomics Center, Institute of Biochemistry, Vilnius University Life Sciences Center. Jonas Cicėnas' research interests are focused on cell signaling (protein kinases in particular) and its role both in normal cells as well as in diseases, such as breast, prostate cancer and leukemia. He has 21 years' experience in cell signaling (kinases), cancer biology, proteomics, bioinformatics and biostatistics. In the past Dr. Cicėnas worked a lot in the field of biomarker discovery and other clinics-related topics of cancer research and published several publications on the prognostic role of protein phosphorylation. He worked in Lithuania, Germany, USA, and Switzerland. He received his PhD in biochemistry in 2004 at the University of Basel. In 2011-2015 Dr. Cicėnas was a biocurator and kinase expert at the CALIPHO group, Swiss Institute of Bioinformatics, where the main focus of his research interests was on protein kinases, cancer and proteomics. Jonas Cicėnas is also a founder of MAP Kinase Resource, an online knowledge platform and database dedicated to mitogen-activated protein kinases. He is the editor of the related journal "MAP Kinase" and also writes popular science articles.

Kinases, Cancer and OMICS

Kinases are likely the most interesting family of proteins to study. The human genome contains 518 protein kinase genes, 478 of which belong to the classical protein kinase family and 40 are atypical protein kinases. Phosphorylation is one of the critical mechanisms for regulating different cellular functions, such as proliferation, cell cycle, apoptosis, motility, growth, differentiation, etc. Deregulation of kinase activity can result in dramatic changes in these processes. Moreover, deregulated kinases are frequently found to be oncogenic and can be central for the survival and spread of cancer cells, Therefore, it is clear now that these are the most convenient drug targets, so there has been done a lot of basic and clinical research on this family of proteins. "OMIC " are the groupf of the newest and most promising approaches to asses many features of the cell/organ/organism including kinases as well as the proteins they phosphorylate. However, these techniques produce huge amount of data, which sometimes are difficult to interpret. Bioinformatics is the most helpful method, which can help to interpret and define further work flow for the data, obtained by proteomics, transcriptomics or genomics techniques. We will present a several "OMICS" approaches to study protein kinases as well as bioinformatic solutions which help the interpretation of the data

Joan Taylor

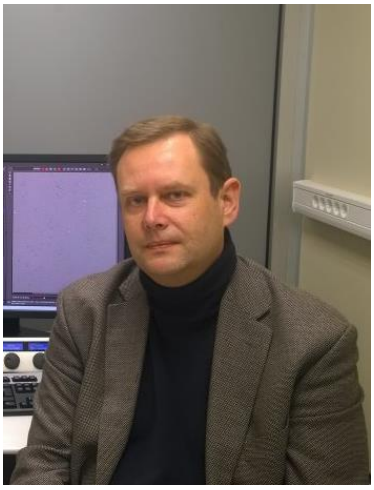
Prof Taylor has led the development of a totally implantable artificial pancreas for the treatment of insulin dependent diabetes and the setting up of an exercise physiology lab in DMU for the study of exercise physiology in diabetes patients.

Novel insulin technologies

The focus here is on the delivery of insulin by formulations that provide an alternative to subcutaneous injection and that rely mainly on gelatinous carrier materials. This will progress to the ideal of delivering an automated closed loop system, first putting this into context with biological and electronic means, but then discussing the evolution of new insulins. The presentation will then explore the use of gel technologies, with an accept on their compatibility with insulin, protection characteristics and their ability to provide a predictable basal dose. At this point we divert to look at gel structure and metrics, the kind of molecular linkages that can be used to form gels and the several gels designs that are already possible for potential delivery orally, by other mucous surfaces and by devices.

The next step is to consider the requirements for design of a gel that could provide on demand boost doses and examine the molecular mechanisms that have been used to date. The talk will finish with an explanation of our own work that involves delivery of insulin to diabetic pigs, using an implantable but refillable peritoneal device that holds a glucose-sensitive gateway gel governing output from an insulin reservoir.

Augustas Pivoriūnas



Augustas Pivoriūnas graduated from Vilnius University Medical Faculty in 1998 (M.D.) and received Ph.D. (2004) in Biochemistry from the Institute of Biochemistry in Vilnius. As a holder of Marie Curie fellowship (Contract Nr: HPMT-GH-00-00130-04) he spent 2 years (2002-2004) at the Department of Medical Microbiology, Linköping University, Sweden. He joined the Department of Experimental Medicine at the Institute of Experimental and Clinical Medicine in Vilnius as research scientist in 2005. From 2010 to 2019 he served as a senior research scientist at the State Research Institute Centre for Innovative Medicine (SRICIM) in Vilnius and from 2012 he was appointed as a Head of the Department of Stem Cell Biology. From 2014 he also serves as a Deputy director for Scientific Affairs at the SRICIM and from 2019 holds position of Chief research fellow. Teaching activities: lecture courses (Tissue engineering and Bioregenerative technologies) at the Vilnius Gediminas Technical University and Vytautas Magnus University. From 2014 Augustas Pivoriūnas is the President of the Lithuanian Association of Stem Cell Researchers. Augustas Pivoriūnas is a co-founder and shareholder of the joint stock company Exosomica.

Augustas Pivoriūnas leads an active group of scientists and PhD students and his research interests focus primarily on extracellular vesicles (EVs) derived from different types of adult stem cells and their applications in basic research and cell-based therapies. In recent years he studies neuroprotective effects of EVs derived from human dental pulp stem cells (DPSCs) using *in vitro* and *in vivo* models of neurodegenerative diseases. His group was among the first to demonstrate that EVs can rescue human dopaminergic neurons from 6-hydroxydopamine (6-OHDA)-induced apoptosis *in vitro* (Cytotherapy. 2015 Jul;17(7):932-9). More recently, his group together with partners from the University of Latvia demonstrated for the first time that intranasal administration of EVs derived from the DPSCs can effectively suppress 6-OHDA-induced gait impairments and normalize tyrosine hydroxylase expression in the striatum and in the substantia nigra of experimental rats (Stem Cells Transl Med. 2019 May;8(5):490-499).

Extracellular vesicles as a new mode of intercellular communication and potent novel therapeutic tools against neurodegenerative diseases

During the lecture I will introduce the audience into the rapidly expanding field of extracellular vesicles (EVs). Then some hot topics, such as problem of heterogeneity of EVs, lack of appropriate markers, problem of quantification and therapeutic dosage will be discussed. During the second part of the seminar I will present and discuss our recent data about neuroprotective properties of EVs using in vitro and in vivo models of Parkinson's disease.

Matthias Hentze

Matthias Hentze is currently the Director of the European Molecular Biology Laboratory (EMBL) and Co-Director of the Molecular Medicine Partnership Unit (MMPU) in Heidelberg (Germany). Following medical studies in Germany and the U.K., and his qualification as a medical doctor, he obtained his postdoctoral training at the NIH (USA) in the late eighties, when he and his colleagues discovered "iron-responsive elements" initiating his interests in RNA biology (translation, mRNA stability, NMD, miRNAs) and diseases of iron metabolism (anemias, hemochromatosis, degenerative diseases). Recent work by the Hentze group has uncovered hundreds of new RNA-binding proteins, including many metabolic enzymes. Their current work uncovers new functions for RNA in the direct regulation of protein function ('riboregulation') and elucidates connections between metabolism and gene regulation.
Prof. Hentze is a co-founder of the MMPU, a joint interdisciplinary and translational research unit of the Medical Faculty of Heidelberg University and the EMBL, which bridges between medicine and molecular biology. Matthias Hentze's research contributions have been recognized in numerous ways including Germany's most prestigious scientific award, the Gottfried Wilhelm Leibniz Prize in 2000, the 2007 Lautenschläger Research Prize of Heidelberg University, and the 2015 Feodor Lynen Medal of the German Society for Biochemistry and Molecular Biology.

A New Continent of the RNA World

We recently reported that the RNA-binding proteome of eukaryotes is much larger than previously anticipated. It includes hundreds of proteins with well characterized functions in cell biology (e.g. metabolic enzymes, autophagy, cell-cell interactions etc.) without defined roles in RNA biology, termed 'enigmRBPs' (for enigmatic RNA-binding proteins). The grand challenge is now to understand their physiological functions (Hentze et al., Nature Rev. Mol. Cell Biol., 2018).

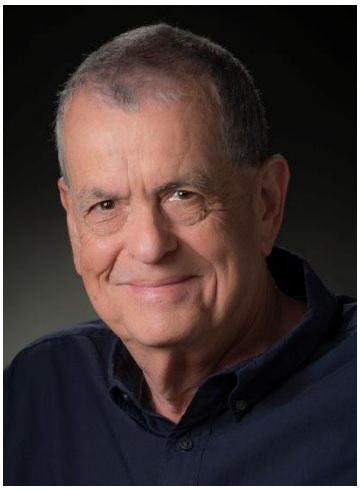
As evident from the aconitase/iron regulatory protein-1 paradigm, metabolic enzymes can 'moonlight' as RBPs and control the posttranscriptional fate of specific target RNAs. Recently, we reported the discovery that an enigmRBP (p62/sequestosome-1) whose known biological function as an autophagy receptor is directly regulated by a non-coding RNA, i.e. subject to 'riboregulation' (Horos et al., Cell, 2019). Riboregulation represents an unexpectedly direct way for genomes to control protein functions by expression of riboregulatory RNAs.

I will discuss ongoing work on riboregulation of metabolic enzymes.

Hentze, M.W., A. Castello, T. Schwarzl and T. Preiss, A brave new world of RNA-binding proteins. Nature Rev. Mol. Cell Biol. 19, 327-341 (2018).

Horos, R., M. Büscher et al., The small non-coding vault RNA1-1 acts as a riboregulator of autophagy. Cell 176, 1054-1067 (2019).

Aaron Ciechanover



Aaron Ciechanover is currently a Distinguished Research Professor in the Faculty of medicine at the Technion - Israel Institute of Technology in Haifa, Israel. He received his M.Sc. (1971) and M.D. (1973) from the Hebrew University in Jerusalem. He then completed his national service (1973-1976) as military physician, and continued his studies to obtain a doctorate in biological sciences in the Faculty of Medicine in the Technion (D.Sc.; 1982). There, as a graduate student with Dr. Avram Hershko and in collaboration with Dr. Irwin A. Rose from the Fox Chase Cancer Center in Philadelphia, USA, they discovered that covalent attachment of ubiquitin to a target protein signals it for degradation. They deciphered the mechanism of conjugation, described the general proteolytic functions of the system, and proposed a model according to which this modification serves as a recognition signal for a specific downstream protease. As a post-doctoral fellow with Dr. Harvey Lodish at the M.I.T., he continued his studies on the ubiquitin system and made additional important discoveries. Along the years it has become clear that ubiquitin-mediated proteolysis plays major roles in numerous cellular processes, and aberrations in the system underlie the pathogenetic mechanisms of many diseases, among them certain malignancies and neurodegenerative disorders. Consequently, the system has become an important platform for drug development. Among the numerous prizes Ciechanover received are the 2000 Albert Lasker Award, the 2003 Israel Prize, and the 2004 Nobel Prize (Chemistry; shared with Drs. Hershko and Rose). Among many academies, Ciechanover is member of the Israeli National Academy of Sciences and Humanities, The European Molecular Biology Organization (EMBO), the American Academy of Arts and Sciences (Foreign Fellow), the American Philosophical Society, the National Academies of Sciences (NAS) and Medicine (NAM) of the USA (Foreign Associate), the Pontifical Academy of Sciences at the Vatican, the Chinese Academy of Sciences (CAS; Foreign Member), the Russian Academy of Sciences (Foreign Member), and the German Academy of Sciences (Leopoldina).

The Revolution of Personalized Medicine: Are We Going to Cure all Diseases and at What Price?

With the thirty years added to our life span in the 20th century compared to the life span people enjoyed merely 120 years ago, the question is whether the trend is going to continue, and how long we are going to live. Much is dependent on modern medicine - new devices, our ability to replace inactive tissues with young functional ones, and m0odern drugs. Many important drugs such as penicillin and aspirin were discovered by serendipity. Other major drugs like statins - the cholesterol biosynthesis inhibitors, were discovered using more

advanced technologies, such as screening of large chemical libraries. One disadvantage of screening is that the mechanism of action of the drug we are seeking is typically unknown at the time of its discovery, and the process largely is random, dependent on the chance to discover one active compound in a collection of millions. Another disadvantage is the model on which the screen is based - cultured cells or inbred animals – which do not faithfully reproduce the disease in humans. Thus, we have started to realize that patients with apparently "same" diseases – breast or prostate cancer, for example - respond differently to similar treatments. This difference stems from the fact that: (i) human beings carry different genetic repertoires and behave differently to different pathogenic processes; and (ii) that the apparent "same" disease" can evolve in different patient from completely different mechanisms to have different molecular bases. Thus, breast or prostate cancers appear can now be sub-divided to smaller distinct classes according to their molecular origins. As a result, we are exiting the era where the treatment of many diseases is "one size fits all", and enter a new era of "personalized (or precise) medicine", where the treatment is tailored according to the patient's molecular/biochemical profile. This era will be characterized initially by the development of technologies to sequence individual genomes, transcriptomes, proteomes and metabolomes, followed by identification and characterization of new disease-specific molecular markers and drug targets, which will be then followed by design of novel, mechanism-based drugs to these targets. This era will be also accompanied by complex bioethical problems, where genetic information of large populations will become available, and protection of privacy will become an important issue

Luca Mazzitelli

Luca Mazzitelli studied plant biotechnology at University of Napoli Federico II and ventured to Scotland for his PhD in plant sciences at University of Dundee and Scottish Crop Research Institute where he characterized the molecular mechanism of bud dormancy in raspberry plant.

Luca joined Qiagen in 2010 as PCR Array sales specialist covering Italy and Commercial Partners. His focus was to achieve sales targets and to provide also post-sales technical support to his customers. Over the past four years Luca developed Qiagen's NGS franchise as a sales specialist, which is a background he now lends to 10X Genomics.

Luca works now as Science & Technology advisor supporting Distributors in EMEA area .

Biology at High Resolution with 10X Genomics: from single cell applications to spatial transcriptomics

Breakthrough technologies such as single-cell analysis, and newly emergent technologies such as spatial analysis, allow scientists to observe and measure complex biological processes, signaling pathways, and phenotypes at massive scale and resolution. It's now possible to capture large-scale single cell information about chromatin state, gene expression, cell-surface proteins, genetic perturbations, and more.

10x Genomics develops technologies that illuminate the details and dynamics of this complexity.

We will discuss how our single cell analysis solutions empower researchers to characterize all cell types of an organism, to study the cellular and molecular mechanisms underlying both normal function and disease states and to decipher the epigenetic regulation unraveling cell type-specific regulatory networks. Besides, 10x Genomics offers the ability to perform comprehensive characterization of the immune response and immune cells, including simultaneous detection of cell surface proteins, antigen specificity, as well as immune repertoire, through single cell and Feature Barcoding technology.
Furthermore, our Visium spatial transcriptomics is groundbreaking technology that will allows scientists to measure gene activity from a tissue section and map where the activity is occurring. Preserving spatial context while identifying distinct groups of cells offers critical information about the relationship of cellular function, phenotype, and location in tissue microenvironments.

Tomaš Paleníček

Dr Tomáš Páleníček has worked as a neuroscientist and psychiatrist at the National Institute of Mental Health, Czech Republic since 2001. In his PhD studies, he focused on animal models of psychosis, studying the neurobiology of schizophrenia. However, he has always been interested in psychoactive substances. During his career, he has been a principle investigator on several projects examining psychedelics in animals, later joining in the human ketamine research, and finally becoming a principle investigator of the first healthy volunteer trials on psilocybin and cannabis in the Czech Republic. Today, he is a leader of preclinical and clinical research teams focusing on the research into psychedelics. Recently he has also co-founded a "PSYRES foundation" which has the intention to support the psychedelic research in the Czech Republic

The neuropsychological effects of psilocybin: focus on cognitive processing and brain activity, implications for treatment

A magic mushroom main psychoactive component, a drug called psilocybin, is currently gaining lots of attention. Psilocybin is a hallucinogenic drug that acutely alters state of consciousness and, to some extent, mimics the symptoms of psychosis typically present in schizophrenia. Contrary to the legal status as a dangerous drug, psilocybin administered in a controlled setting increases human wellbeing and also shows a unique profile to treat several neuropsychiatric disorders. Recently the US Food and Drug Administration (FDA) labelled psilocybin used in two large clinical trials a "breakthrough therapy" for treatment resistant depression (TRD) as well as a major depressive episode. Recently there are more than 30 clinical trials with psilocybin registered worldwide for the treatment of depression, anxiety, obsessive-compulsive disorder, addiction and cluster headache. In my talk I will summarize the mechanism of the effects of psilocybin in healthy volunteers and patients in the context of cognitive processing, brain activity and potential therapeutic effect.

This work was supported by projects VI20172020056, GACR no.: 18-16218S, MH CZ—DRO (NIMH-CZ, 00023752), grant LO1611 from the MEYS CR under the NPU I program, PROGRES Q35, AZV 17-31852A and 260388/SVV/2018.

Alina Urnikytė

Dr. Alina Urnikytė a researcher in the filed of population genetics at the institute of Biomedical Sciences of the Faculty of Medicine of Vilnius University.

She obtained her bacherols degree in biotechnology (UVIC, Spain) and masters digree in medical genetics (Vilnisu university).

During her PhD she reinforced the knowledge and skills in the field during the traineeship in Evolutionary Population Genetics Lab of the Department of Experimental and Health Sciences at the Universitat Pompeu Fabra in Barcelona.

Latest research of the scientist helped answer questions about whether the Lithuanian genome is different from the genome of other peoples of Europe and the rest of the world, about whom we are closer to, peoples from the East or the North, and about how our genes have been affected by changing living conditions.

Tema: Inferring microevolutionary processes in local human populations

The analysis of geographically specific regions and the characterization of fine-scale patterns of genetic diversity may facilitate a much better understanding of the microevolutionary processes affecting local human populations. Here we generated genome-wide high-density SNP genotype data in 425 individuals from six geographical regions in Lithuania and combined our dataset with available ancient and modern data to explore genetic population structure, ancestry components and signatures of natural positive selection in the Lithuanian population. Our results show that Lithuanians are a homogenous population, genetically differentiated from neighbouring populations but within the general expected European context. Moreover, we not only confirm that Lithuanians preserve one of the highest proportions of western, Scandinavian and eastern hunter-gather ancestry components found in European populations but also that of an steppe Early to Middle Bronze Age pastoralists, which together configure **the genetic distinctiveness of the Lithuanian population. Finally,** among the top signatures of positive selection detected in Lithuanians, **we identified several candidate genes** related with diet (*PNLIP*, *PPARD*), pigmentation (*SLC24A5*, *TYRP1* and *PPARD*) and the immune response (*BRD2*, *HLA-DOA*, *IL26* and *IL22*).

Oral presentations

Amniotic fluid - the untapped source of stem cells

Aistė Zentelytė[1], Veronika V. Borutinskaitė[1], Rūta Navakauskienė[1]

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

Human amniotic fluid stem cells (AFSCs) are considered as a rather novel alternative source of stem cells with potential applications in cell therapy and regenerative medicine. Amniotic fluid containing AFSCs is obtained during amniocentesis procedure which is considered to be safe for both the fetus and the mother. AFSCs are of fetal origin and display high proliferation capacity as well as differentiation potential to cell lineages from all three germ layers. The goal of this study was to characterize AFSCs isolated from both healthy and fetus-affected gestations and to evaluate and compare the differentiation potential of stem cells. AFSCs were obtained from amniocentesis samples using two-stage isolation protocol and expanded in monolayer culture. Cells were characterized and were positive for mesenchymal markers (CD44, CD90, CD105) and expressed genes associated with pluripotency (Oct4, Sox2, Nanog, Rex1). Under appropriate conditions AFSCs differentiated towards adipogenic, osteogenic, neurogenic and myogenic lineages, as determined by morphological changes and expression of lineage-specific genes. Differentiation processes were also characterized epigenetically and were linked to distinctive expression of miR-17, miR-21, miR-34a and mirR-146a, decreased levels of acetylated H4 and H3K9, tri-methylated H3K4 and H3K9 and upregulated levels of H3K27me3. The levels of HDAC1, DNMT1 and PRC1/2 proteins (BMI1/SUZ12) were reduced as well in all differentiations. There were no significant differences in the expression levels of investigated epigenetic marks between undifferentiated AFSCs derived from normal and fetus-affected gestations and between those differentiated towards adipogenic, osteogenic, neurogenic and myogenic lineages. The expressional changes of histone marks and miRNAs that occurred during differentiation indicate subtle epigenetic regulation, yet more detailed studies in epigenetic mechanisms are required for a better understanding of AFSCs differentiation in fetus-diseased conditions and their usage in therapeutic application and prenatal disease research.

In vitro modeling of Alzheimer's disease: potential role of astrocyte-derived extracellular vesicles

Karolina Kriaučiūnaitė, Agnė Pociūtė, Aida Kaušylė, Alexei Verkhratsky, Augustas Pivoriūnas

State Research Institute Centre for Innovative Medicine

Alzheimer’s disease (AD) is the most common age-related neurodegenerative disorder worldwide, with an estimated ~20 % lifetime risk in 65-year-old individuals. The brain changes may begin 20 years before the symptoms of AD arise. Among various hypothesis of AD onset, over the last decade “blood-brain barrier (BBB) breakdown” attracts intense attention of scientist. BBB is a highly

selective layer of unique brain microvascular endothelium surrounded by other resident cells. Crosstalk between endothelial and astroglial cells play the key role in supporting physiological BBB function. Despite direct interaction between cells, an important role play extracellular vesicles (EVs), which are phospholipid bilayer enclosed particles carrying biologically active molecules between cells in autocrine, paracrine and even endocrine way. In my presentation I will introduce our newest results obtained using an in vitro model of BBB. Co-culturing human endothelial cells with wild type (WT) or three AD-related mutations (APPswe/Tau-P301L/ PS1-M146V) carrying mouse astrocytes, we revealed that diseased astroglial cells could weaken barrier function. We showed impaired transendothelial electrical resistance (TEER) and reduced tight junction proteins expression of BBB endothelial cells co-cultured together with diseased astrocytes. Next, we investigated EVs secreted by WT- and AD-astrocytes and its effect on BBB. Results showed also strong positive effect of WT-astrocyte-secreted EVs on TEER of endothelial cells. In contrast, AD-astrocyte-secreted EVs reduced tight junction expression and increased inflammation-related intracellular signaling within endothelial cells. Altogether these findings suggest novel role of astrocyte-derived EVs in healthy and AD effected BBB.

New approach for genome-wide single-base resolution profiling of 5-hydroxymethylcytosine

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5-hydroxymethylcytosine (5hmC), the oxidative product of 5-methylcytosine (5mC) mediated by ten-eleven translocation enzymes, is found in many mammalian tissues and cell types, although with diverse levels of abundance contrary to 5mC. Emerging evidence reveal that 5hmC serves as not only an active DNA demethylation intermediate but also as a stable epigenetic DNA modification linked to various biological processes, including development and pathogenesis. Different technologies have been developed to profile 5hmC genome-wide, with the most prominent being bisulfite-based approaches, which are able to reach a single-base resolution. However, these methods cause a significant DNA loss due to degradation, limiting their application in analysis of precious clinical DNA samples, and require deep whole-genome sequencing, thus are very expensive.

We developed a bisulfite-free cost-effective approach for 5hmC profiling at a single-nucleotide resolution, named hmTOP-seq (5hmC-specific tethered oligonucleotide-primed sequencing), which is based on the direct sequence readout primed at covalently labeled 5hmC sites from an in situ tethered DNA oligonucleotide. Firstly, hmTOP-seq was validated on a small model bacteriophage genome indicating quantitative, single-base resolution detection of 5hmC. Next, we attempted to assess the new approach on mammalian genomic DNA and verified its ability to profile 5hmC in mouse embryonic stem cell (mESC) DNA. Our method employs T4 phage β-glucosyltransferase, which has no sequence specificity, thus hmTOP-seq allowed 5hmC identification in non-CG context

in mESC DNA. As our approach targets both DNA strands individually, it has a capacity to detect subtle differences in the strand-specific CG hydroxymethylation. hmTOP-seq analysis determined the association between 5hmC levels at the coding strand of gene bodies and gene expression. Also, we were able to assess the strand-specific 5hmC distribution around the exon-intron junctions. Altogether, hmTOP-seq is a valuable cost-effective high-resolution technique for characterization of epigenetic 5hmC profiles.

Transfection of CHO-K1 Cells Using Nanosecond Electroporation

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Chinese hamster ovary (CHO) cells are very often used for mammalian protein expression in biotechnology. DNA transfection into eukaryotic cells could be performed by using biological, chemical or physical methods. Yet, due to its simplicity, one of the physical methods, such as micro-millisecond electroporation, is applied more often than the others. However, electroporation is an electric pulse dependent phenomenon, thus the efficiency of the methodology is highly dependent on the applied protocol. In this study, we present a new modality of high frequency (1 MHz) nanosecond range electro-transfection protocols. Green fluorescent protein (GFP) encoding plasmids were used to evaluate transfection efficiency by flow cytometry and cell viability by alamarBlue assay. The cell vitality and percentage of transfected cells were tested using various parameters: DNA concentration (0.0125-8 mg/ml), cell numbers (0.108-0.218 x10^6) per well of 48 well plate, electroporation buffer composition (8 different buffers were tested) and incubation time (0-60 minutes) in electroporation buffers before dilution with growth media. We found out that the best transfection conditions are: DNA concentration (1 mg/ml), cell numbers (0.162x10^6 cells per well) and incubation time (10 min), which has shown both, the best transfection efficiency and cell viability. Afterwards, various high-frequency (1 MHz) electroporation pulse parameter combinations of electric field (2-15 kV/cm), duration (300-700 ns) and number of pulses (n=100-1000 pulses) were tested for electro-transfection. As a result, 2 kV/cm x 700 ns and 5 kV/cm x 300 ns with pulse number of n=250 have shown more than 20% higher transfection efficiency and 30-50% better cell viability than standard 1.5 kV/cm x 100 µs procedure. Consequently, nanosecond electroporation could find further biotechnological applications, especially for transient transfections in vitro or future in vivo drug delivery, where high-efficiency rates are indispensable.

Protein engineering of Geobacillus lipolytic enzymes - from enzyme fusions to directed evolution

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As humanity continues to increase in population size, so does the demand for various products and services. Meeting these demands creates both a larger environmental impact, as well as higher economic costs. Innovation in industrial processes is one of the ways to reduce both cost and impact to the environment. Microbial lipolytic enzymes (lipases and carboxylesterases) are biocatalysts that can catalyze both the hydrolysis and synthesis of fatty acid esters and can be presented as attractive innovative tools for various industries. These enzymes are active in organic solvents, do not require cofactors, are regio- and stereo- selective, can hydrolyse a wide spectrum of lipidic substrates. Lipases and carboxylesterases are applied for the production of biofuel, disposal of waste, manufacturing of detergents and emulsifiers, in leather, paper and pharmaceutical industries.
Lipolytic enzymes produced by Geobacillus bacteria are a possible source of such enzymes, with attractive properties like high thermoactivity and thermostability. Our studies presented protein engineering experiments to improve activity, kinetic and physicochemical characteristics of Geobacillus lipolytic enzymes to create new attractive biocatalysts and for deeper insight into structure - function relationships of these enzymes. New lipolytic enzymes created using site directed mutagenesis, protein fusion, and DNA-shuffling/random mutagenesis strategies will be discussed.

Poster Presentations

Biochemistry/molecular biology

1. The antimicrobial activity of geobacillin 26: artificial vs bacterial cell membrane

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Geobacillin 26 is a heat-labile, high molecular weight antibacterial protein from a thermophilic Gram-positive bacteria Geobacillus stearothermophilus 15 and it has a narrow antibacterial spectrum against other thermophilic bacteria. Its mode of action differs from similar bacteriolysins. In recent studies it was proven, that geobacillin 26 is not a cell wall degrading enzyme, but its specific mode of action is unknown [1]. Bacteriocins could potentially be used in the post-surgical control of infectious bacteria, so it is important to understand the working mechanisms of various bacteriocins, including geobacillin 26, which has a great potential in industry where contamination with thermophilic bacteria is unwanted.

The aim of this study was to determine the mode of action of geobacillin 26 using Atomic Force Microscopy (AFM). AFM is a surface sensitive technique which allows to visualize three-dimensional topographic views of a specimen under physiological conditions. Thus we established protocols to immobilize and visualize directly the activity of protein on bacterial and artificial cell membranes (tBLM – tethered bilayer lipid membrane).

Bacterial cells of sensitive strain Parageobacillus genomospecies 1 NUB36187 (9A11) were spread on NB-agar plate and incubated overnight at 55 °C. After the incubation biomass from the plate was transferred to NB medium and incubated overnight at 55 °C. Next morning the cell suspension was inoculated to the NB medium (55 °C) in the ratio 1:50. The cell suspension adjusted to OD (600 nm) of 0.6 and then affected with geobacillin 26. The cell suspension was washed with PBS (pH 7.4) buffer two times. Suspension was spread out on poly-L-lysine modified mica. Surface topography and force curves (elasticity and adhesion properties) of bacteria were analyzed.

The artificial cell membranes were prepared as described elsewhere [2]. To imitate bacterial cell membrane, tBLMs were formed from 1,2-dioleoylphosphatidylglycerol/1,2-dioleoyl-sn-glycero-3-phosphoethanolamine 7/3 multilamellar vesicle solutions. The effect of geobacillin 26 on artificial bacterial membrane was detected via time-lapse capturing.

[1]Vaičikauskaitė M., Ger M., Valius M., Maneikis A., Lastauskienė E., Kalėdienė L., Kaunietis A. Geobacilin 26 – high molecular weight bacteriocin from a thermophilic bacterium. International Journal of Biological Macromolecules 2019 (141): 333-344.

[2]Ragaliauskas T., Mickevicius M., Rakovska B., Penkauskas T., Vanderah D.J., Heinrich F., Valincius G. Biochimica et BiophysicaActa (BBA)-Biomembranes 2017 (1859): 669-678.

2. Polymorphism of prion protein amyloid fibril

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Protein aggregation into amyloid fibrils is linked to multiple neurodegenerative disorders, such as Alzheimer's, Parkinson's or Creutzfeldt-Jakob disease [1]. Usually each disease is related to aggregation of different protein or peptide, but structural polymorphism in amyloid aggregates of the same protein in vivo can lead to different pathologies. It is believed that formation of structurally distinct amyloid fibrils is related either to the changes in protein amino acid sequence or to the different conditions of aggregation.

We have studied sixty samples of mouse prion protein amyloid aggregates formed at three different conditions. Thioflavin T fluorescence assay revealed that polymorphism of amyloid fibrils is not only environment-dependent, but may also be observed between the samples aggregated at identical conditions. The structural differences between samples were confirmed by the assessment of the secondary structure by Fourier transform infrared spectroscopy and morphological variability observed by atomic force microscopy

3. Study of theInteraction of S100A9 Protein Tethered Lipid Bilayer Membranes

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S100A9 protein belongs to the S100 family of protein and is important factor in the regulation of most cellular processes and immune response [1]. It is associated with the development of cancer cells and neurodegeneration. S100A9 protein is involved in the amyloid-neuroinflammatory cascade in Alzheimer's disease [2]. In this work the interaction between S100A9 and membrane was studied and tethered lipid bilayer membranes (tBLM) [3] were used as simplified membrane model for these studies.

The aim of this work was to form tBLM and to optimize their electrical properties in order to use them in the study of the interaction of S100A9 protein with phospholipid bilayer and its mechanism of action. By using Electrochemical Impedance Spectroscopy and Dynamic Light Scattering methods, was shown that smaller S100A9 oligomers are more toxic to the membrane compared to larger aggregates.

1.Markowitz J, Carson WE. Review of S100A9 biology and its role in cancer. Biochimica et Biophysica Acta (BBA) - Reviews on Cancer. 2013; 1835(1):100–9.
2.Wang C, Klechikov AG, Gharibyan AL, Wärmländer SKTS, Jarvet J, Zhao L, Xueen J, Shankar SK, Olofsson A, Brannstrom T, Mu Y, Graslund A, Morozova-Roche LA. The role of pro-inflammatory S100A9 in Alzheimer's disease amyloid-neuroinflammatory cascade. Acta Neuropathologica. 2013; 127(4):507–22.
3.Budvytytė R, Valinčius G, Niaura G, Voiciuk V, Mickevičius M, Chapman H, Goh HZ, Shekhar P, Heinrich F, Shenoy S, Losche M, Vanderah DJ. Structure and Properties of Tethered Bilayer Lipid Membranes with Unsaturated Anchor Molecules. Langmuir. 2013; 29(27):8645–56.

4. Hypoxia dependant alternative mRNA isoform formation of neurodegenerative disease related genes

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Neurodegenerative diseases such as Alzheimer's and Parkinson's are most common neurological disorders associated with old age[1]. Neurodegeneration is characterized by progressive loss of neuron function. This leads to impaired cognitive and motor abilities [1]. Many genetic and environmental factors can cause neurodegeneration [2]. Hypoxia (lack of oxygen) is one of the factors that promote the development of neurodegenerative diseases [2]. Due to lack of oxygen hypoxia inducible factors (HIF) are stabilized in the cells. HIFs activate transcription of genes which are needed for cells to survive in a low oxygen environment [3]. Alternative pre-mRNA splicing is another mechanism responsible for cellular adaptation to stress conditions (hypoxia) [4]. Data from the literature states that several genes which are involved in Alzheimer's and Parkinson's disease pathology can be alternatively spliced [5]. In this work, we have investigated the influence of hypoxia on neurodegenerative disease associated gene alternative pre-mRNA splicing.

[1]Merelli, A., Rodríguez, J. C. G., Folch, J., Regueiro, M. R. & Lazarowski*, A. C. and A. Understanding the Role of Hypoxia Inducible Factor During Neurodegeneration for New Therapeutics Opportunities. Current Neuropharmacology http://www.eurekaselect.com/158926/article (2018).
[2]Zhang, F., Niu, L., Li, S. & Le, W. Pathological Impacts of Chronic Hypoxia on Alzheimer's Disease. ACS Chem. Neurosci. 10, 902–909 (2019).
[3]Nakayama, K. & Kataoka, N. Regulation of Gene Expression under Hypoxic Conditions. Int J Mol Sci 20, (2019).
[4]Sena, J. A., Wang, L., Heasley, L. E. & Hu, C.-J. Hypoxia regulates alternative splicing of HIF and non-HIF target genes. Mol. Cancer Res. 12, 1233–1243 (2014).
[5]Mills, J. D. & Janitz, M. Alternative splicing of mRNA in the molecular pathology of neurodegenerative diseases. Neurobiology of Aging 33, 1012.e11-1012.e24 (2012).

5. Harnessing the Diversity of Cas9 Orthologs for Genome Editing

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The Cas9 protein from CRISPR (Clustered Regularly Interspaced Palindromic Repeats)-Cas (CRISPR Associated) bacterial defense systems has been adopted as a robust and multifaceted genome editing tool. The Cas9 RNA guided DNA endonuclease can be directed to cleave, nick or bind a specific site in the chromosomal DNA just by changing the guide RNA sequence. Cas9-based tools have been used to edit genomic DNA, modulate gene expression, visualize genomic loci in cells and deaminate nucleotide bases. However, for Cas9 to bind a given target, a short nucleotide sequence motif, termed PAM, is required. This PAM constraint as well as insufficient specificity are major obstacles for Cas9 genome editing. Thus, analysis of natural Cas9 orthologs could offer an increased diversity of PAM sequences and biochemical properties which may be beneficial to genome editing applications.

Cas9 nucleases are abundant in microbes. To explore this large uncharacterized diversity of Cas9 orthologs, we established a phylogeny-guided bioinformatic selection approach and developed biochemical screens based on cell-free recombinant protein expression and interrogation of plasmid libraries containing randomized PAM sequences for the rapid characterization of novel Cas9 proteins and identification of PAM requirements. Guide RNAs for each Cas9 ortholog were designed *in silico* by identifying putative tracrRNA (trans-activating CRISPR RNA) coding regions in respective native loci. The examined set revealed nucleases that exhibit a wide range of distinctive T-, A-, C- and G-rich PAM preferences, ranging from two to more than four nucleotides, as well as generate staggered-end breaks or require longer spacers to function robustly. Our results indicate that the natural diversity of Cas9 orthologs provides a source of various PAM recognition sequences and other potentially desirable properties that may be used to expand the genome editing toolbox

6. Characterization of Multilamellar Lipid

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Biological membrane is responsible for multiple physiological functions, so its research is very important. However, due to membrane complexity, these studies are a serious challenge. Model membrane system is used to make it easier. The most common model is tethered bilayer lipid membrane. Tethered bilayer lipid membrane can be formed using multillamelar lipid vesicles. This technique is new and there is not much information about the effect of multillamelar lipid vesicles on tBLM formation.

The aim of this work was to find sizes of various multillamelar lipid vesicles and check their ability to form tethered bilayer lipid membrane using electrochemical impedance spectroscopy.

The results have shown that when the concentration of cholesterol and the total concentration of both lipids (DPPC and cholesterol) is increasing multilamellar lipid vesicle size is getting bigger. The size of multilamellar lipid vesicle is decreasing when the concentration of DOPC is rising. Number of defects in tethered membrane depends on cholesterol concentration. Also, tethered bilayer membrane can be formed when DOPC concentration is 0,25 mM and bigger. The size of liposomes is independent from time and they are suitable to use for 2 weeks.

7. Novel miniature CRISPR-Cas14 activity against DNA

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Small and robust CRISPR-Cas nucleases are highly desirable for genome editing applications. Being guided by an RNA to cleave targets near a short sequence termed a protospacer adjacent motif (PAM), Cas9 and Cas12 offer unprecedented flexibility, however, smaller more compact versions would simplify delivery and extend application. Recently, a new class 2 system encoding a miniature (529 amino acids) effector, Cas14a1, has been shown to exclusively function as a PAM-independent single stranded DNA nuclease [1]. Using biochemical methods, we shown that a T-rich PAM sequence triggers double-stranded DNA cleavage by Cas14 proteins [2]. Finally, we demonstrate the ability of Cas14a1 to target and cleave cellular human chromosomal DNA. Although frequencies were low, our results provide the first evidence that the miniature Cas14 family can be harnessed for genome editing.

8. Additional Thioflavin-T Binding Mode in Insulin Fibril Inner Core

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Amyloidogenic protein aggregation into fibrils is linked to several neurodegenerative disorders, such as Alzheimer's or Parkinson's disease. An amyloid specific fluorescent dye thioflavin-T (ThT) is often used to track the formation of these fibrils in vitro. Despite its wide application, it is still unknown how many types of ThT binding modes to amyloids exist, with multiple studies indicating varying numbers. In this work we examine the binding of ThT to insulin fibrils generated at pH 2.4 and reveal a possible inner core binding mode which is not accessible to the dye molecule after aggregation occurs.
Methods: Insulin fibrils were prepared by incubating 100 or 200 μM insulin solutions (pH 2.4, 100 mM NaCl, 100 mM phosphate buffer) with or without additional ThT at 60°C without agitation for 24 hours. For each ThT concentration, excitation-emission matrices were scanned and used to determine both the maximum ThT fluorescence intensity and the position of the highest intensity peak. Absorbance measurements were used to determine the amount of free and bound ThT present in solution after sample centrifugation.
Results and conclusions: Insulin fibrils formed with ThT added before aggregation display an additional ThT binding mode, which is not accessible to ThT molecules after the fibrils are fully formed. ThT bound in this mode possesses a much higher quantum yield when compared to other bound dye molecules.

9. Investigation of Anti-CRISPR Proteins of a Type I-F CRISPR-Cas

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CRISPR-Cas is a diverse prokaryotic defense system that provides adaptive immunity against foreign nucleic acids. Bacteriophages develop different approaches to evade CRISPR-Cas protection in an evolutionary bacterial-phage arms race. One of the strategies is phage-encoded small proteins, named anti-CRISPR proteins (Acr), that inhibit CRISPR-Cas protection and enable phage evasion. To evaluate the effect of anti-CRISPR on I-F type CRISPR-Cas from *Aggregatibacter actinomycetemcomitans* D7S-1 bacteria (Aa-CRISPR-Cas), proteins from ten previously identified Acr families (AcrIF1-10) were tested. Employing in vivo experiments we show that proteins of AcrIF6 and AcrIF9 families inhibit type I-F Aa-CRISPR-Cas system. Then we purified AcrIF6 and AcrIF9 proteins to assay their action in vitro. We demonstrate that both AcrIF6 and AcrIF9 interact with type I-F CRISPR-Cas interference complex, called Cascade, which binds target DNA and triggers its degradation by an accessory protein – Cas2/3 helicase/nuclease. AcrIF6 interaction with the Cascade prevents its binding to target DNA, while AcrIF9 somehow changes the Cascade-DNA interaction. More detailed mechanisms remain to be discloses.

10. Plasma microRNA Profiles as a Potential diagnostic Biomarker of Rheumatoid Arthritis

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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. MicroRNAs (miRNAs) are present in human plasma and known as a potential non-invasive biomarker for RA detection. The use of appropriate predictive biomarkers may improve the efficiency of RA therapy.

The aim of this study is to highlight the findings on miRNAs expression, compare it between healthy individuals and RA patients and to discuss their potential as biomarkers for diagnostic purposes.

In this study, we used quantitative PCR (qPCR) to evaluate miRNA expression levels of three miRNAs (miR-22, miR-27b, miR-155) in 42 RA patients samples and 43 healthy controls. We detected statistically significant differences in gene expression of two miRNAs (miR-22 ($p=0.0309$) and miR-155 ($p=0.0197$)) between healthy individuals and those that develop RA indicating a potential biomarker role for at-risk individuals. In addition, our results showed statistically significant differences of miR-22 ($p=0.0304$) and miR-27b ($p=0.0058$) between patients who had biological therapy and who did not.

In conclusion, miR-22 and miR-155 could be used as a potential diagnostic biomarker of Rheumatoid Arthritis.

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11. Functional analysis of bacterial RNA 5’ end decapping enzyme NudC

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Nudix hydrolase superfamily is widely distributed in nature and can be found in such classes of organism as bacteria, archaea, eukaryotes or viruses. These proteins have been categorized as “housecleaning enzymes” which carry out the hydrolysis of many different **nu**cleoside **di**phosphates linked to an **x** moiety. The substrates of Nudix enzymes usually are cell signaling molecules, metabolic intermediates and potentially toxic compounds the concentration of which need to be modulated during the cell cycle. Recently it was shown that *E. coli* Nudix hydrolase NudC facilitates RNA degradation by hydrolysing its newly found 5’-end modification, nicotinamide adenine dinucleotide (NAD), resulting in the release of nicotinamide mononucleotide and 5'-monophosphorylated RNA. What is more, enzyme prefers NAD-capped RNA over NAD^+ or NADH as the cleavage of 5’-NAD-RNA is carried out more efficiently. Knowing that other members of Nudix superfamily carry out their functions while interacting with additional proteins we raised the hypothesis that NudC may also form a protein complex while removing the 5’-NAD cap. Combining methods of protein purification under mild conditions, mass spectrometry as well as bacterial adenylate cyclase two-hybrid system, we were indeed able to find the NudC interaction partner. Northern blot and functional analysis of 5’-NAD modified and unmodified RNA molecules revealed the possible effects of proteins on both types of RNA substrates. Collectively these findings not only strengthened the hypothesis that both proteins function together but also revealed NudC function reaching beyond regulation of only 5’-NAD modified RNAs. We hope that these results will broaden our knowledge on the mechanism behind RNA degradation in bacteria.

12. Acetyltransferase CheA in Acinetobacter baumannii stress response

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Acinetobacter baumannii is a gram-negative opportunistic pathogen, causing pneumonia, bacteremia and urinary tract infections in immunocompromised patients [1]. Due to its ability to quickly acquire antibiotic resistance, form biofilms on plastic surfaces and persist desiccation, *A. baumannii* was able to spread in the hospitals worldwide as well as in Lithuania. Rapidly increasing *A. baumannii* resistance to most antibiotics is a serious threat, thus more research is needed to understand the virulence and survival mechanisms of this bacterium and find new potential targets for antimicrobial treatment.

GNAT N-acetyltransferases are enzymes widely distributed among eukaryotic and prokaryotic organisms. These proteins usually transfer an acetyl group from acetyl-CoA to a large array of

substrates, ranging from proteins and peptides to small molecules such as aminoglycosides [2]. In bacteria, GNAT acetyltransferases are associated with response to reactive oxygen species, toxins, iron acquisition and other cellular stress inducing factors. Moreover, it has been proved that some GNAT acetyltransferases transfer acetyl group to the aminocyclitol ring of a wide variety of aminoglycoside antibiotics and contibute to antibiotic resistance in bacteria [2].

In this work, we analysed the role of conservative *A. baumannii* N-acetyltransferase CheA in bacterial physiology, concentrating on the virulence and survival related traits. We have constructed an *A. baumannii ΔcheA* mutant and compared its properties with the wild type *A. baumannii* isolate.

1. Gonzalez-Villoria, A. M. & Valverde-Garduno, V. Antibiotic-Resistant Acinetobacter baumannii Increasing Success Remains a Challenge as a Nosocomial Pathogen. J Pathog 2016, (2016).
2. Favrot, L., Blanchard, J. S. & Vergnolle, O. Bacterial GCN5-Related N-Acetyltransferases: From Resistance to Regulation. Biochemistry 55, 989–1002 (2016)

13. Dependence of the in vivo activity of viral endoribonuclease RegB on ribosomal protein S1

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Neurodegenerative diseases are one of the most widely spread disorders in the world. Sadly, despite the intensive research, the understanding of the disease mechanisms is quite moderate and all available therapies are only symptomatic. Alzheimer's disease has attracted the most of attention, because it is the most common disorder affecting around 50 million people worldwide and this number is expected to increase in the near future. It was determined that the neurofibrillary tangles formed from microtubule-associated protein Tau are the hallmark of this disease and other tauopathies. Therefore, it is imperative to understand the mechanisms affecting this process and to find the best way to tackle them. However, in order to carry out such experiments it is important to obtain Tau protein with good yield and purity. In this work, we used SUMO-fusion technology1, to produce Tau isoform 2N4R, which allowed to reduce the time of purification and resulted in higher protein purity and yield.

For further experiments polyanion heparin has been used as amyloid-like protein aggregation inducer in vitro. In order to understand mechanism behind the complexity of protein Tau aggregation, different conditions were examined. Since protein Tau is classified as intrinsically disordered protein, the aggregation rate is highly dependent on different pH values changing its total net charge and solubility due to high proportion of polar and charged amino acids in protein sequence2. Moreover, high sodium chloride concentrations are expected to heavily affect aggregation rate3. Also, we presume that pre-formed protein Tau fibrils can induce aggregation without using polyanion heparin what would explain misfolding of monomeric protein Tau in healthy recipient neuron cells in the brains4. All performed

aggregation kinetics were followed using Thioflavin T fluorescence assay at a range of recombinant Tau protein and heparin concentrations.

1. Peroutka III R.J., Orcutt S.J., Strickler J.E., Butt T.R. (2011). SUMO Fusion Technology for Enhanced Protein Expression and Purification in Prokaryotes and Eukaryotes. In: Evans, Jr. T., Xu MQ. (eds) Heterologous Gene Expression in E.coli. *Methods in Molecular Biology (Methods and Protocols)*, vol 705. Humana Press
2. Tedeschi, G., Mangiagalli, M., Chmielewska, S., Lotti, M., Natalello, A., & Brocca, S. (2017, September 8). Aggregation properties of a disordered protein are tunable by pH and depend on its net charge per residue.
3. Goto, Y., Adachi, M., Muta, H., & So, M. (2018). Salt-induced formations of partially folded intermediates and amyloid fibrils suggests a common underlying mechanism. *Biophysical reviews*, *10*(2), 493–502.
4. Nizynski, B., Nieznanska, H., Dec, R., Boyko, S., Dzwolak, W., & Nieznanski, K. (2018). Amyloidogenic cross-seeding of Tau protein: Transient emergence of structural variants of fibrils. *PloS one*, *13*(7), e0201182.

14. Targeting Trypanosoma cruzi chaperone Hsp90 in Chagas disease

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Parasitic protozoan organisms contribute to the high burden of infectious diseases that are a causing factor of severe morbidity and mortality in both developing and developed countries. *Trypanosoma cruzi*, which is the subject of our research, is the causing agent of Chagas disease, also known as American trypanosomiasis. The disease is characterized by cardiac, neurologic and digestive tract pathologies that can lead to sudden death. Although mostly widespread in Latin America, due to international immigration the disease threatens people living in other areas as well. There is no existing vaccine against Chagas disease and many of the current drugs have unwanted side effects. Furthermore, the ability of parasites to quickly develop resistance mechanisms renders many drugs ineffective, so novel treatment options are urgently needed[1,2].

Heat shock protein 90 (Hsp90) is a dimeric molecular chaperone, which is involved in many eukaryotic cell pathways ensuring proteostasis. The chaperone has a role in folding, maturation and degradation of select client proteins. As well as other protozoan parasites, *T. cruzi* relies on its functionality for survival, stage differentiation and adaptation to stressful conditions during infection. The fact that healthy human cells are significantly less sensitive to partial Hsp90 inhibition than parasitic protozoa makes it an attractive drug target for treatment of the Chagas disease[3,4].

We aim to develop antiparasitic drugs based on Hsp90 inhibition. To achieve that, we first had to acquire a viable protein model for ligand binding assays. Since full length proteins are often difficult to obtain, we chose to work with isolated N-terminal domain of Hsp90, which binds ATP molecules and Hsp90-selective inhibitors of interest[5]. Fluorescence thermal shift assay was used to assess protein stability and determine binding affinities for the Hsp90 inhibitors.

1. Fletcher, S. M.; Stark, D.; Harkness, J.; Ellis, J. Enteric Protozoa in the Developed World: A Public Health Perspective. Clin. Microbiol. Rev. 2012, 25 (3), 420–449. https://doi.org/10.1128/CMR.05038-11.
2. Sales Junior, P. A.; Molina, I.; Fonseca Murta, S. M.; Sánchez-Montalvá, A.; Salvador, F.; Corrêa-Oliveira, R.; Carneiro, C. M. Experimental and Clinical Treatment of Chagas Disease: A Review. Am. J. Trop. Med. Hyg. 2017, 97 (5), 1289–1303. https://doi.org/10.4269/ajtmh.16-0761.
3. Shonhai, A.; G. Maier, A.; M. Przyborski, J.; L. Blatch, G. Intracellular Protozoan Parasites of Humans: The Role of Molecular Chaperones

in Development and Pathogenesis. Protein Pept. Lett. 2011, 18 (2), 143–157. https://doi.org/10.2174/092986611794475002.
4. Schopf, F. H.; Biebl, M. M.; Buchner, J. The HSP90 Chaperone Machinery. Nat. Rev. Mol. Cell Biol. 2017, 18, 345.
5. Gewirth, D. T. Paralog Specific Hsp90 Inhibitors – a Brief History and a Bright Future. Curr. Top. Med. Chem. 2016, 16 (25), 2779–2791.

15. Tagging A. baumannii Type VI Secretion System Components with a Green Fluorescent Protein

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Acinetobacter baumannii is a Gram-negative opportunistic pathogen responsible for hospital-acquired nosocomial infections. It is a successful pathogen due to its ability to resist desiccation, disinfectants and major antimicrobials. Previously identified A. baumannii two-component signal transduction system BfmRS was shown to be responsible for regulating virulence-related traits such as biofilm production, resistance to antibiotics, type VI secretion system (T6SS) regulation, survival in human ascites fluid and serum. T6SS is also related to A. baumannii virulence and responsible for inter-bacterial competition and bacterial interactions with eukaryotic cells. Secretion systems are usually regulated by two-component signal transduction systems. However, T6SS regulation and BfmRS role in it are not fully understood. Therefore, in this work, we aimed to fluorescently label T6SS components in A. baumannii.

Markerless gene deletion technique was used to generate ΔbfmRS, Δhcp, and ΔbfmRSΔhcp mutants. The total protein content of mutants was visualized using SDS-PAGE. The inter-bacterial competition assay was performed by incubating mixed bacterial strains at the aggressor (A. baumannii) and prey (E. coli) ratio 10:1, respectively. Components of T6SS (Hcp and TssB) were fused with a green fluorescent protein by the PCR-based overlap extension method. Labeled proteins were tracked by a fluorescent microscope with a 600x-1000x magnification range.

Protein secretion profiles of A. baumannii clinical strain and its ΔbfmRS mutant revealed that the mutant displayed a reduction of Hcp protein, which is essential for the assembly of the T6SS apparatus. However, competition assays showed that loss of bfmRS did not impair the killing phenotype. To evaluate the assembly state of T6SS in the mutant, Hcp protein was fluorescently labeled with a green fluorescent protein in N- and C- termini. However, fluorescent labels per se impaired killing phenotype and Hcp tracking did not reveal any information about the state of T6SS. Therefore, we then fluorescently labeled sheath protein TssB and evaluated T6SS activity.

Fluorescently labeled T6SS with Hcp protein was non-functional. However, T6SS component TssB looks like a promising candidate to track T6SS in A. baumannii.

16. Analysis of metallo-ß-lactamases from Chryseobacterium spp. of soil origin

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VU, GMC, Biomokslų institutas, Biochemijos ir molekulinės biologijos katedra

Antibiotics have been used in clinical settings since 1941 to treat bacterial infections. Due to reckless use of antibiotics, many pathogenic bacteria are becoming more resistant to them, causing hardly treatable diseases. Most widely used antibiotic class is ß-lactams, the compounds targeting bacterial cell wall synthesis by inhibiting transpeptidation of peptidoglycan, resulting in death of the cell. Resistant bacteria often have enzymes called ß-lactamases that degrade these antibiotics. Genes of various ß-lactamases can be easily transferred from one bacteria to the other, accelerating the spread of resistance.

Many soil microbes are naturally exposed to antibiotics and thus, have developed resistance to the compounds over millions of years. Some soil dwellers can also cause infections to patients with a suppressed immune system, thus becoming opportunistic pathogens. For example, *Chryseobacterium* genus consists of common soil bacteria, which can also cause urinary tract infections, sepsis or bacteraemia. *Chryseobacterium indologenes*, the most virulent species in the genus, have started to be associated with urinary tract infections in 1996 but nowadays more species are discovered to cause a variety of diseases, spreading from Taiwan, the primary source of *Chryseobacterium* infections. *Chryseobacterium* are known to have a genus specific IND ß-lactamase, responsible for their resistance to ß-lactams.

In our previous study we have discovered two ß-lactamases from *Chryseobacterium* spp.: IND-like and an unknown metallo-ß-lactamase (MBL). Both of them gave significant resistance to ß-lactam antibiotics when transferred to Escherichia coli. MBL gene homologues are found in almost half sequenced *Chryseobacterium* genomes and the proteins share ~40% similarity to IND ß-lactamases, which are more common and found encoded in almost 60% of sequenced *Chryseobacterium* genomes. The aim of this study is to characterise these novel ß-lactamases, as none of the MBL homologues have been characterised yet and less than a half of IND ß-lactamases have their biochemical activities determined.

17. Gallic Acid oxidation products possess anti-amyloid properties

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Protein aggregation into highly structured, beta-sheet rich fibrils is associated with multiple neurodegenerative diseases, affecting millions of people worldwide[1]. The screening for anti-amyloid compounds has led to many different molecules entering clinical trials with an extremely low success rate. Tea extracts are known for their beneficial effects on health and several compounds have been shown to possess anti-amyloid properties[2]. The effect of these molecules is usually determined in neutral pH, without considering the fact that compounds undergo oxidation at neutral or higher pH.

In this research we compare the inhibition effect of gallic acid and its oxidation products (GAO) on insulin amyloid formation. The gallic acid does not affect the insulin aggregation while GAO increases the aggregation lag time. Furthermore, the global fitting of the kinetic data suggests that GAO affects the aggregation nucleation state.

1. Tooba NazShamsi, Athar T, Parveen R, Fatima S. A review on protein misfolding, aggregation and strategies to prevent related ailments. International Journal of Biological Macromolecules. 2017;105(1):993-1000. doi.org/10.1016/j.ijbiomac.2017.07.116
2. Fernando WMADB, Somaratne G, Goozee KG, Williams S, Singh H, Martins RN. Diabetes and Alzheimer's Disease: Can Tea Phytochemicals Play a Role in Prevention? *J Alzheimer's Dis*. 2017;59(2):481-501. doi:10.3233/JAD-161200

18. The Role of Target Sequence Length for DNA Interference in the Type I-F CRISPR-Cas System

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CRISPR (Clustered Regularly Interspaced Short Palindromic Repeats) – Cas (CRISPR Associated) is the immune system of bacteria or archaea that provides resistance against invasive genetic elements. Proteins encoded by *cas* genes together with a mature crRNA molecule that carries a sequence (spacer) of extracellular nucleic acid origin form an effector complex, which destroys foreign nucleic acids. In type I CRISPR-Cas systems, ribonucleoprotein complex, termed Cascade (CRISPR-associated complex for antiviral defence), recognises and binds intruding DNA, which has (i) a spacer-complementary sequence, named protospacer, and (ii) a protospacer adjacent motif (PAM). Cascade binding to DNA target triggers a Cas3 helicase/nuclease, which destroys the intruder

.

In type I-E systems, Cascade-DNA target interaction is destabilised by mutations at the PAM distal end of the protospacer. Otherwise, the influence of mutations at the protospacer distal end for a type I-F system, which is the phylogenetically closest for type I-E, was not yet investigated. In this work, we show the importance of these nucleotides for interference in the type I-F system from *Aggregatibacter actinomycetemcomitans* D7S-1 bacteria. Mutations at the PAM distal end of the protospacer do not inhibit interactions between the Cascade complex and DNA target. Cascade complexes have a similar affinity for truncated protospacers and form R-loops the length of which depends on spacer-protospacer complementarity. However, R-loop of at least 32nt is necessary to trigger Cas2/3 protein for target DNA destruction.

19. Effects of Essiantial Oils and Their Compounds on Mealworms (Tenebrio Molitor L.) Larvae

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Entomophagy is not a new phenomenon in our society. Insects have been eaten by humans from prehistoric times. Nowadays people eat insects not only as an exotic snack, but in some soceties insects are the main food source. According to the Food and Agriculture Organization of the United Nations [1], population growth, urbanization and the increase of human population of average income has increased global demand for food products, in particular those with great source of protein. One of the main aspects why insect eating is an alternative to the animal meat is because insects are very nutritious, they multiply fast and do not contaminate the environment. Currently the insect farms are popular and attempt to include insects in the daily diet of humans.

Mealworms (Tenebrio molitor L.) are easily cultivated, have a relatively large increase in biomass, are nutritious and a great source of protein. Because of these characteristics mealworms are the best example of insect food for humans. It is very important to increase the effectiveness of the collection of insect larvae biomass for the insect breeders. One of the possibilities could be the manipulation of insect larvae behavior using natural repellents. Essential oils as natural repellents could be perfectly used for controlling insect behavior. The current thesis describes the impact of natural essential oils on the behavior of mealworm larvae. The results showed that the best repellents for the mealworm larvae were the essential oils of thyme (Thymus vulgaris L.) and mint (Mentha spicata L.). The effect of active compounds of essential oils was also tested. The best repellency was recorded for 100 mM concentration of citronelool and 100 mM of linalool. It has been observed that in control plates without stimulus, the larvae spend most of their time on the periphery, not in the central zone.

20. Impact of Magnetic Nanoparticles (CoFe2O4, MnFe2O4 and Fe3O4) On Lepidium sativum L.

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Magnetic nanoparticles (NPs) such as Co and Mn ferrite or Fe oxide have received increasing attention due to their widespread therapeutic and agricultural applicability, useful for removing metals and metalloids from water. The production and use of magnetic NPs have been expanding considerably due to their unique properties such as size and size dispersity, shape, crystallinity, surface decoration and hybrid derivatives, therefore, the possibilities of the intentional or accidental releasing of these substances into the environment increases. However, the potential toxicity of these NPs is still unknown. Thus, the aim of the study was to assess the effects of synthesized magnetic NPs ($CoFe_2O_4$, $MnFe_2O_4$, Fe_3O_4) on garden-cress (Lepidium sativum L.) morphological and physiological parameters. The relative growth of the roots, biomass of root and seedlings, amounts of chlorophylls a and b, carotenoids and level of malondialdehyde were measured. The dependence of morphological and physiological parameters of garden-cress on tested NPs and concentrations was demonstrated. Results obtained in this study can be significant in assessing the

environmental impact of magnetic NPs, which display fates and properties substantially different from those of traditional chemicals, addressing ecotoxicological issues directly related to human health due to a possibility of transfer of these NPs from aquatic to terrestrial ecosystems.

21. Molecular and Morphological Investigations of Sarcocystis Sp. from the Common Raven (Corvus Corax)

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Parasites of the genus *Sarcocystis* are intracellular protozoan with an intermediate-definitive host life cycle based on a prey-predator relationship. In general, granivorous, insectivorous and omnivorous birds are intermediate hosts for *Sarcocystis* species, while carnivorous birds serve as definitive hosts for these parasites. However, sarcocysts of *Sarcocystis* species are found in the muscles of the carnivorous birds too. In the period of 2015-2019, having examined 23 leg muscles of common ravens in Lithuania, sarcocysts were detected in 19 birds (82.60%). *Sarcocystis* spp. were characterized using light microscopy (LM) and first internal transcribed spacer (ITS-1) region sequences analysis. By LM, one morphological types of cysts (I) were distinguished according to morphometric parameters of sarcocyst and structure of the cyst wall. Sarcocysts were microscopic, ribbon-shaped, have thick cyst wall, which reached up to 1.5 µm; also have lancet-shaped 6.1–9.0 × 1.2–3.0 µm bradyzoites. Ultrastructurally, the sarcocyst wall was wavy with small elevations of the primary cyst wall, had no protrusions; reached up to 1.8 µm. These sarcocysts had type-1f tissue cyst wall. According to 18S rRNA, 28S rRNA genes and ITS-1 region sequences, it was shown that *Sarcocystis* sp. is a genetically separate species. On the basis of these genetic markers, *Sarcocystis* sp. was most closely related to *S. cornixi* (89.50%), which parasitize birds and are characterized by type-1g of sarcocyst wall. Phylogenetic results obtained could be helpful suggesting definitive hosts of *Sarcocystis* species examined.

22. Construction of carbonic anhydrase active site mutants (Thr199Val in CA II, Val201Thr in CA XIII) demonstrate amino acid effect on the position and affinity of primary fluorinated benzensulfonamides

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In most cases, sulfonamide inhibitors of human carbonic anhydrases (CA) form a coordination bond between the sulfonamide amino group and the catalytic Zn(II), located at the active site of the protein 1,2. However, our crystallographic studies of CA XIII in a complex with two primary fluorinated benzensulfonamide inhibitors revealed unexpected results: two crystal structures were obtained where sulfonamides bound upside down exposing their sulfonamide group towards the solvent.

We hypothesized that substitutions of one certain amino acids in the protein active site (CAII-Thr199Val, CAXIII-Val201Thr) would significantly affect the binding mode and affinity of inhibitors. We expected that the mutated CA II would mimic CA XIII and vice versa. These recombinant mutant proteins were generated to explain such strange structural behavior of compounds and also to analyze the importance of substituted amino acids for the ligand positioning.

Active site mutant proteins were generated utilizing PCR-mediated site directed mutagenesis, expressed in E.coli and purified. Binding affinities of native and recombinant proteins were evaluated by the fluorescent thermal shift assay (FTSA). Three X-ray crystallographic structures of CAII-T199V with bound ligands were solved and analyzed.

Measurements obtained by FTSA exibited stronger binding affinities between CAII-Thr199Val and ligands with a similarity to CA XIII. Weaker CAXIII-Val201Thr interaction was thought to resemble CA II binding affinities determined by utilizing same ligands. The alternate positions and possible conformational changes of several ligands were also determined after crystallization of mutated CAII-Thr199 protein.

Our results indicate that one amino acid substitution of particular amino acids in the protein active site can significantly influence protein-ligand interaction and thermodynamic parameters. Unfortunately, our hypothesis that substitution of particular amino acids would cause compound reorientation was not confirmed. Reorientation of ligands was not common and we think that opposite ligand orientation was an artifact observed only due to acidic pH (4.0) used for protein-ligand complex crystallization

1. Maren, T. H. Carbonic anhydrase: chemistry, physiology, and inhibition. Physiological Reviews 47, 595–781 (1967).
2. Baranauskienė, L. & Matulis, D. Overview of Human Carbonic Anhydrases. in Carbonic Anhydrase as Drug Target (ed. Matulis, D.) 3–14 (Springer International Publishing, 2019). doi:10.1007/978-3-030-12780-0_1.

23. Identification of new antimicrobial peptides from thermophilic bacteria

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Bacteriocins comprise a huge family of ribosomally synthesized peptides. They are heat-stable, produced by various bacteria and have antibacterial activity towards closely related strains, although there are an increasing number of bacteriocins reported to have broad range antimicrobial activity. Interest in bacteriocin research has gained great momentum

due to its potential as both a natural food preservative and as next-generation antibiotics targeting the multiple-drug resistant pathogens. They are especially attractive for various applications. Thermophilic bacilli are a potential contaminant in various industries that maintain higher temperatures (40-65°C) in the manufacturing process as food industry. Bacteriocins acting against thermophilic bacteria could be a solution to this problem. Thermophilic bacteria proteins are usually thermostable, therefore bacteriocins derived from these bacteria could be also thermostable, even with a higher thermostability than those encoded in mesophilic bacteria This study aimed to synthesize and characterize new antibacterial peptides. We analyzed genomes of thermophilic bacteria species and identified gene clusters encoding potential bacteriocins. One novel bacteriocin (circularin-like) was encoded in Geobacillus thermoleovorans strain. It is post-translationally modified head-to-tail cyclized peptide, whose N- and C-termini are linked by a peptide bond. Another one (lacticin-like) was found in genome of Parageobacillus thermoglucosidasius. It is leaderless bacteriocin and do not contain unusual post-translational modifications. We have cloned the bacteriocin biosynthesis genes into expression vectors and performed their heterologous biosynthesis in Escherichia coli to obtain active antibacterial peptides. The synthesized bacteriocins will be purified, characterized and evaluated for their antibacterial activity against various bacteria strains including pathogens.

24. Tick-borne pathogens in passerine birds

Asta Karalavičiūtė

Ticks are one of the major vectors of disease transmitted in the world. The pathogens they carry and the diseases they cause are rapidly spreading due to bird migration, increasing climate change, socio-economic causes and human conditions. Migratory birds have a major influence on the transmission of tick species and their pathogens over long distances and helping to spread to new geographical areas. During the spring and autumn migrations of 2016 and 2018, blood samples of 212 passerine birds were collected at "Ventės ragas" ornithological station in Lithuania. Total birds blood samples were screened for presence of epidemiologically important pathogens such as Anaplasma spp., Borrelia spp., Babesia spp. and Rickettsia spp. Multiplex real-time PCR analysis was performed using msp2 gene (98 bp) for Anaplasma spp., 23S rRNA gene (77 bp) for Borrelia spp., 18S rRNA gene (218 bp) for Babesia spp. and gltA gene (338 bp) for 546 bp Rickettsia spp. According to multiplex real time PCR analysis A. phagocytophilum were identified in 33,0 % (70/212) of birds, Borrelia spp. – 21,2 % (45/212). Babesia spp. and Rickettsia spp. pathogens were not identified. PCR products which was positive for Anaplasma spp. and Borrelia spp. were sequenced and analyzed using the Mega software. Sequence analysis showed the presence of A. phagocytophilum and B. valaisiana DNAs in birds. These studies shown that migratory birds might be important for the dispersal of A. phagocytophilum and B.valaisiana in Lithuania territory.

25. Crystallisation of Streptococcus thermophilus Csm6 HEPN domain

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CRISPR-Cas systems protect prokaryotes against viruses and other foreign nucleic acids. This protection is specific due to crRNA molecules that recognize the targets and guide the effector complex to them. On binding invading RNA, type III CRISPR-Cas system use Cas10 subunit to degrade DNA and to produce cyclic oligoadenylates (cOA). cOA acts as a secondary messenger which activates non-specific RNA cleavage by type III associated ribonuclease Csm6. Csm6 family proteins consist of a CARF domain that binds cOA and HEPN ribonucleolytic domain, which cleave RNA in a sequence independent manner. In this study we aimed to obtain crystals of HEPN domain of Csm6 from Streptococcus thermophilus (St) for structural studies. We cloned and purified StCsm6 HEPN domain, performed screening of crystallisation conditions and obtained crystals which diffract X-rays up to 1.5 Å resolution. These crystals will be used to determine the structure of HEPN domain. Understanding the stucture of StCsm6 HEPN domain could contribute to the understanding of regulation mechanism of ribonuclease and to design of new cOA-controllable proteins.

26. Amyloidophilic Molecule Interactions on the Surface of Amyloid Fibrils: Cooperative Binding and Fluorescence Quenching

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Protein aggregation into amyloid fibrils is associated with neurodegenerative disorders, such as Alzheimer's, Parkinson's, or prion diseases. Amyloidophilic dye molecules can be used as potential aggregation inhibitors and some of them can be applied to track amyloid formation. In this work, the interaction of amyloidophilic molecules on the surface of protein amyloid fibrils were investigated using absorption and fluorescence spectroscopy. Insulin, lysozyme and mouse prion protein amyloid fibrils were prepared at 60°C (final conc. 300 μM). The absorbance spectra and excitation-emission matrices (EEM) of the samples were measured by mixing the fibrils, dye and PBS (final conc. 100 μM) in range from 200 to 800 nm. In many cases, amyloidophilic molecules, such as ThT, CR, Dapoxyl, ANS or MB assist each other in binding to amyloid fibrils, but this does not increase the fluorescence intensity of ThT, ANS, Dap. Often, there is a noticeable decrease in fluorescence.

27. Streptococcus thermophilus CRISPR-Cas Adaptation Studies in vivo

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Prokaryotes are the most abundant cellular organisms on Earth. Nevertheless, there are at least 10 bacteriophages for each prokaryotic organism that can serve as a host. Over the course of evolution, prokaryotes have evolved a variety of defence mechanisms in order to avoid or combat the invading bacteriophages and thus prevent viral infection. CRISPR-Cas is a widespread prokaryotic defence system in which Cas proteins provide adaptive antiviral immunity. CRISPR-Cas mechanism is divided into three stages: 1) adaptation (viral DNA is selected, cut and integrated as a spacer into the CRISPR region in the bacterial chromosome), 2) expression and processing (spacer DNA is transcribed into CRISPR RNA (crRNA)) and 3) interference (crRNA is used as a guide to identify invading DNA which is then cut by Cas nucleases). The best characterized stage is interference, as exemplified by Cas9 effector nucleases that have revolutionized the field of genome editing. By contrast, the adaptation stage in many CRISPR-Cas systems remains poorly understood. We are interested in the spacer acquisition stage of Streptococcus thermophilus Type IIA CRISPR-Cas system. Recently it was demonstrated that Cas1, Cas2 and Csn2 proteins of this system form several different DNA-bound complexes, but the exact role of these assemblies in adaptation remains elusive (Wilkinson et al. 2019 MolCell). Based on these structures, we are performing site-directed mutagenesis of Cas genes in S. thermophilus genome and are testing the effect of these mutations on spacer acquisition in vivo.

28. Colight: Novel Optogenetic Tools For Modular Bacterial Control

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Varying both protein levels and expression timing in biological systems would deepen the understanding of how cellular pathways function and could prove useful biotechnological applications. Notably, optogenetic approaches enable this level of dynamic control. The variability of the stimulus light allows for a specific triggering of cellular events in a non-invasive and highly resolving spatiotemporal fashion. However, significant factors stopping the progress include:

1. Complicated two-component or one-component system design.
2. Lack of other than blue light-responsive domains.
3. Absence of tools in other than the transcription level.

To address these problems, we created a collection of novel multi-level optogenetic tools for dynamic bacterial control.

29. Aggregation of recombinant Tau protein isoform 2N4R dependance on different environmental conditions

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Neurodegenerative diseases are one of the most widely spread disorders in the world. Sadly, despite the intensive research, the understanding of the disease mechanisms is quite moderate and all available therapies are only symptomatic. Alzheimer's disease has attracted the most of attention, because it is the most common disorder affecting around 50 million people worldwide and this number is expected to increase in the near future. It was determined that the neurofibrillary tangles formed from microtubule-associated protein Tau are the hallmark of this disease and other tauopathies. Therefore, it is imperative to understand the mechanisms affecting this process and to find the best way to tackle them. However, in order to carry out such experiments it is important to obtain Tau protein with good yield and purity. In this work, we used SUMO-fusion technology[1], to produce Tau isoform 2N4R, which allowed to reduce the time of purification and resulted in higher protein purity and yield.

For further experiments polyanion heparin has been used as amyloid-like protein aggregation inducer in vitro. In order to understand mechanism behind the complexity of protein Tau aggregation, different conditions were examined. Since protein Tau is classified as intrinsically disordered protein, the aggregation rate is highly dependent on different pH values changing its total net charge and solubility due to high proportion of polar and charged amino acids in protein sequence[2]. Moreover, high sodium chloride concentrations are expected to heavily affect aggregation rate[3]. Also, we presume that pre-formed protein Tau fibrils can induce aggregation without using polyanion heparin what would explain misfolding of monomeric protein Tau in healthy recipient neuron cells in the brains[4]. All performed aggregation kinetics were followed using Thioflavin T fluorescence assay at a range of recombinant Tau protein and heparin concentrations.

1. Peroutka III R.J., Orcutt S.J., Strickler J.E., Butt T.R. (2011). SUMO Fusion Technology for Enhanced Protein Expression and Purification in Prokaryotes and Eukaryotes. In: Evans, Jr. T., Xu MQ. (eds) Heterologous Gene Expression in E.coli. Methods in Molecular Biology (Methods and Protocols), vol 705. Humana Press
2.Tedeschi, G., Mangiagalli, M., Chmielewska, S., Lotti, M., Natalello, A., & Brocca, S. (2017, September 8). Aggregation properties of a disordered protein are tunable by pH and depend on its net charge per residue.
3.Goto, Y., Adachi, M., Muta, H., & So, M. (2018). Salt-induced formations of partially folded intermediates and amyloid fibrils suggests a common underlying mechanism. Biophysical reviews, 10(2), 493–502.
4. Nizynski, B., Nieznanska, H., Dec, R., Boyko, S., Dzwolak, W., & Nieznanski, K. (2018). Amyloidogenic cross-seeding of Tau protein: Transient emergence of structural variants of fibrils. PloS one, 13(7), e0201182.

30. A novel design for an implantable artificial pancreas

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Type 1 diabetes (T1D) is an auto-immune disease that destroys the pancreatic cells that produce insulin; those diagnosed with conventional T1D would die without injected insulin given several times daily.

An artificial pancreas should test, calculate and administer minute doses frequently and continually, so that the

administered insulin is corrected in real time to the blood glucose at any given time. However present designs of artificial pancreas are external and do not achieve full needs of adjusting the insulin dose, being hazardously slow to respond; also tubing, wiring and alarm systems can cause practical and social problems.

We have created novel implantable artificial pancreas which is invisible from outside the body, conveniently refillable by syringe and can deliver insulin components and doses faster and more securely.

In this study we have measured in vitro performance of our implantable artificial pancreas pigs' model. A standard diffusion experiment was set up in the lab where the glucose-specific gel was mounted inside an insulin reservoir. Fluorescent insulin labelled with FITC was used to measure the diffusion coefficients and match these with the rheology parameters for a range of glucose concentrations.

The in vitro and in vivo work we have done indicates that our modified implanting procedure using an omental pocket prevents adhesive peritonitis. However for human use, the engineering of such artificial pancreas would have to be medically, legally and commercially acceptable to guarantee leak-proof operation in delivery and in refilling. This should be achievable, following some principles of current pacemaker design.

31. ImmunoCap 250 for Diagnostics of Allergy and Autoimmunity

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Allergy and autoimmunity are rapidly increasing world-wide. The identification of clinically relevant antibodies in patient serum is crucial for diagnostics of allergy and autoimmunity. ImmunoCap 250 by Thermo Fisher is a singleplex assay system currently used for diagnostics in clinical labs.

In this study we have investigated is current ImmunoCap tests for patients with allergy to egg and peanut and patients with autoimmune coeliac disease, which is characterised by

sensitivity to gluten from dietary wheat, barley and rye would be improved by using specific allergen or antigen components. We explored if EliA IgA Gliadin could complement to current diagnostic tests for coeliac disease as well as if egg and peanut allergen component use could be more advanced than current whole allergen testing.

Blood samples (31 for allergy and 37 for coeliac disease) were collected into gel vacutainers and then centrifuged at 3000rpm for 10 minutes to separate serum. Samples were stored at -20°C until testing using the ImmunoCAP 250. The samples were used in line with the hospital laboratory protocols, rules and ethical procedures of both DMU and NGH. Samples were incubated with peanut (Ara h 1, 2 and 8) and egg white (Gal d 1, 2 and 3) allergens or with human recombinant tissue transglutaminase (tT) and synthetic deaminated gliadin peptides to identify specific antibody-mediated reactions for allergy or autoimmunity respectively.

Our study indicated that it would possibly be of greater benefit to use the EliA IgG GliadinDP test alongside the EliA IgA Celikey assay, as this should increase sensitivity and potentially remove the need for total IgA testing. Endomysial antibodies are still required as a confirmatory test in those patients with positive EliA IgA Gliadin test. For allergy samples, we found that component diagnosis may complement current diagnostic tests rather than replace them.

* Crystallisation of Streptococcus thermophilus Csm6 HEPN domain

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CRISPR-Cas systems protect prokaryotes against viruses and other foreign nucleic acids. This protection is specific due to crRNA molecules that recognize the targets and guide the effector complex to them. On binding invading RNA, type III CRISPR-Cas system use Cas10 subunit to degrade DNA and to produce cyclic oligoadenylates (cOA). cOA acts as a secondary messenger which activates non-specific RNA cleavage by type III associated ribonuclease Csm6. Csm6 family proteins consist of a CARF domain that binds cOA and HEPN ribonucleolytic domain, which cleave RNA in a sequence independent manner. In this study we aimed to obtain crystals of HEPN domain of Csm6 from Streptococcus thermophilus (St) for structural studies. We cloned and purified StCsm6 HEPN domain, performed screening of crystallisation conditions and obtained crystals which diffract X-rays up to 1.5 Å resolution. These crystals will be used to determine the structure of HEPN domain. Understanding the stucture of StCsm6 HEPN domain could contribute to the understanding of regulation mechanism of ribonuclease and to design of new cOA-controllable proteins.

32. Qualitative and Quantitative Analysis of Bacteriocin-Encoding Genes in Two Paenibacillus sp. Strains from Krubera-Voronja Cave

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Bacteriocins are ribosomally synthesized antimicrobial peptides produced by bacteria to inhibit or kill closely related strains. These compounds may be used as food preservatives or become a potential drug candidate by replacing antibiotics in the age of multi-drug resistance. Species of the genus *Paenibacillus* are known to produce a variety of such antimicrobial compounds. This feature makes these organisms decent candidates for bacteriocin research.

To detect potential bacteriocin clusters in draft genome sequences of *Paenibacillus* sp. strains 28ISP30-2 and 23TSA30-6 from Krubera-Voronja Cave we used a web-based software tools BAGEL3 and BLAST. Specific PCR primer pairs were designed for the multiplication of identified bacteriocin or bacteriocin-related peptides encoding genes using PRIMER BLAST tool. Afterwards, four growth curves were drawn (two for each strain) to evaluate the influence of nutrient availability for the growth of the investigated bacteria cultures. Using this data the target points for total RNA extraction were chosen and the reverse transcription PCR was performed in order to evaluate the qualitative expression of genes of interest. The resulting cDNA was screened for the genes of interest with the earlier engineered primers. The final stage of the study was to conduct a quantitative gene transcription analysis by applying the real-time PRC method.

The results we received reveal that three classes of bacteriocin-encoding genes are being transcribed in *Paenibacillus* sp. strains 28ISP30-2 and 23TSA30-6. Collectively, in both strains isolated from the Krubera-Voronja cave, 8 genes for bacteriocins and their modifying proteins were found to be transcribed. These include 3 lanthipeptide genes (most actively transcribed in the exponential growth phase), 2 sactipeptide genes (transcribed intensively regardless of strain or medium), 3 lasso peptide genes (transcription is more intense and tends to last longer in strain 23TSA30-6). What is more, the quantitative analysis showed a great difference in transcription intensity between the genes of interest. Genes encoding sactipeptides and lasso peptides tend to be transcribed approximately 1000 or in some cases even 10000 times more intensively than the lanthipeptide encoding genes. This analysis revealed that the investigated strains have a fine potential to produce a variety of antimicrobials signifying that paenibacilli from Krubera-Voronja Cave might be a good source for novel bacteriocin discovery.

This work was supported by the Research Council of Lithuania (grant No. S-MIP-17-21).

33. Physicochemical characterization of immobilized lipolytic GD-95RM, GDEst-95 and GDEst-lip enzymes

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Lipases and esterases are enzymes with broad pH, temperature and substrate tolerance. Because of their regio- and stereospecificity and substantial activity in organic solvents, lipases and esterases have been recognized as very useful biocatalysts in industrial applications, such as the production of pharmaceuticals, leather, detergents, foods and medical diagnostics. Currently, hidrolases, including lipases and esterases, are leading enzymes in the world's industrial biotechnology sector. However, the commercial application of these enzymes is limited because of their production costs. Design of improved lipolytic enzymes via protein engineering is not enough to develop efficient biocatalyst systems for industrial applications. The most important aspects of such biological tools are stability and reusability. Nowadays, immobilization of enzymes is commonly used to achieve these properties. One of the most effective enzyme immobilization techniques is enzyme entrapment in calcium alginate hydrogel. In this study, GD-95RM[1], GDEst-95[2] and GDEst-lip[2] microbial lipolytic enzymes were immobilized, their characteristics were analyzed and compared with non-immobilized enzymes. Results show increased thermostability of all immobilized enzymes. Furthermore, after immobilization GD-95RM was able to perform hydrolysis reactions in higher temperatures compared to non-immobilized enzyme. The substrate specificity was also evaluated.

Acknowledgments

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References

1. Druteika G. Analysis of Structure-Function Relationship in Geobacillus Lipases and Design of Lipolytic Enzymes with Improved Characteristics via Different Mutagenesis Strategies, Bachelor thesis, 2019
2. Gudiukaite R, Sadauskas M, Gegeckas A, Malunavicius V, Citavicius D. Construction of a novel lipolytic fusion biocatalyst GDEst-lip for industrial application. J Ind Microbial Biotechnol 2017;44(6):799e815.

34. Molecular characterization of four broad-temperature range myoviruses infecting Pantoea sp.

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Plants are usually populated by a complex microbial communities composed of algae, fungi, bacteria and their viruses. The genus Pantoea is one of the predominant taxa in plants and it comprises both pathogenic and beneficial species that can survive as epiphytes or endophytes on their hosts, but sometimes can cause infections in humans. Therefore, broad-temperature range bacteriophages could be useful for the prevention and therapy in both plants and humans or for food preservation.

We present here the report on the molecular characterization of four broad-temperature range bacteriophages (AAM22, SSEM1, PSKM and AAM37) that have similar virion morphology. Transmission electron microscopy images showed that all phages were members of the family Myoviridae, and had isometric heads (diameters ranging from 63 to 67 nm), and contractile tails from about 90 to 118 nm in length. Efficiency of plating test revealed that phages AAM22, SSEM1, PSKM and AAM37 could form plaques in the temperature ranges of 4–30, 4–34, 4–35, and 4–40 °C, respectively. The genomes of all aforementioned phages are relatively small, ranging from 49744 bp (phage AAM22) to 54982 bp (phage SSEM1). Bioinformatics analysis revealed that the number of probable protein encoding genes in the genomes of aforementioned phages ranges from 82 (phage PSKM) to 104 (phage SSEM1) but no genes for tRNA have been detected. The G+C content of genomes varied from 43.9% (phage SSEM1) to 53.1% (phage PSKM).

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35. Application of Klebsiella phage vB_KleM-RaK2 self-assembling protein gp041 for the generation of hybrid structures

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Viruses and virus-like particles show an inherent propensity to self-assemble into higher-order hierarchical structures. They provide an ideal building scaffold for the design of nanostructured materials. Using nanoparticles as the base platform, a variety of tissue-specific ligands or other molecules may be attached or genetically displayed on the particle surface. Chimeric proteins constructed in this manner can be used for a variety of applications, including delivery vehicles, targeted toward imaging and treatment of diseases and as scaffolds that interact with the local environment, which can be utilized for vaccines, immunotherapy and tissue engineering[1].

The aim of this study was to construct hybrid proteins based on the self-assembling structural protein gp041 from vB_KleM-RaK2 *Klebsiella* sp. bacteriophage, and to analyze the nanostructures formed by this protein, thus expanding current knowledge and exploring the biotechnological potential of self-assembling nanostructures based on bacteriophage proteins.

Our work was focused on the construction and synthesis of hybrid structures derived from truncated tail sheath protein gp041. The protein was fused in-frame to the fluorescent proteins GFP, YFP, mCherry, or dsRed. Four hybrid protein variants were developed during this work: 041Δ200_GFP, 041Δ200_YFP, 041Δ200_mCherry, and 041Δ200_dsRed. All constructed proteins were successfully synthesized in *E. coli* BL21 (DE3) cells. Moreover, three of them self-assembled into tubular structures (confirmed by transmission electron microscopy) and had a fluorescent activity, confirmed by stimulated emission depletion microscopy. In addition, Förster resonance energy transfer (FRET) was observed when 041Δ200_GFP and 041Δ200_mCherry were expressed together, showing, that both fluorescent proteins were assembled into a single nanotube.

In conclusion, the results indicate that gp041-based hybrid proteins self-assemble *in vivo* into nanotubular structures, and the fluorescence provided by the fused proteins allows detection of the formed structures by fluorescence microscopy.

1. Wen AM, Steinmetz NF. Design of virus-based nanomaterials for medicine, biotechnology, and energy. Chem. Soc. Rev. Royal Society of Chemistry; 2016;45(15):4074–126.

36. Canis familiaris allergen Can f 6 and Penaeus monodon allergen Pen m 4: expression, purification and analysis

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Allergy is an under-recognized public health concern with a steadily rising number of affected individuals. Since the late sixties, serological tests for allergen-specific IgE antibodies used natural allergens, either their individual components or an extract, to identify allergies. Notably, the composition and amount of an allergenic extract strongly affect the results of such assays.

The use of single allergenic molecules has introduced a new area of high-resolution molecular allergy diagnostics. The application of recombinant protein technologies is based on the ease of production scale-up, high protein yields, and cheap production.

This research aimed to assess the allergenicity of several recombinant allergens fused to a highly soluble partner – maltose-binding protein (MBP) and to determine whether MBP is capable of functioning not only as a general molecular chaperone but does it also affect the allergens' IgE-binding capacity.

In this study we analyzed two different allergens: the dog, *Canis* familiaris, allergen Can f 6 and the black tiger shrimp, *Penaeus monodon*, allergen Pen m 4. Both recombinant allergens were synthesized with and without MBP and purified under native conditions by Ni-NTA affinity chromatography. To test the antigenic properties of rCan f 6 and rPen m 4 allergens – an enzyme-linked immunosorbent assay was evaluated for testing blood serum samples from patients positive for dog and shellfish allergies, respectively.

This study has shown that rCan f 6 and rPen m 4 allergens expressed in *Escherichia coli* are probable candidates for the diagnostic of their respective allergies and that MBP not only promotes the production of soluble recombinant allergens but also enhances their antigenicity.

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37. Characterization of Bacillus sp. infecting bacteriophage vB_BacS_KLEB27-1

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In this study we present the report on the molecular characterization of a lysogenic bacteriophage vB_BacS_KLEB27-1 (below referred to by its shorter common laboratory name, KLEB27-1), active on *Bacillus australimaris*. Based on the results of TEM analysis, phage KLEB27-1 belongs to the family *Siphoviridae* and has an isometric head (B1 morphotype) about 68 nm in diameter and a non-contractile flexible tail about 132 nm in length. The host range determination test revealed that out of 28 bacterial strains tested, only *Bacillus australimaris* isolate KR4M-27 was sensitive to KLEB27-1. Plating tests revealed that phage can form plaques in the temperature range of 18 to 38°C. The 65.037 bp genome of KLEB27-1 has a G+C content of 46% and contains 117 probable protein encoding genes and no genes for tRNA. Comparative sequence analysis revealed that 11 out of 117 KLEB27-1 ORFs encode unique proteins that have no reliable identity to database entries. Based on homology to biologically defined proteins, 40 ORFs of KLEB27-1 have been given a putative functional annotation, including genes coding for structural proteins as well as those associated with lysogeny, phage-host interactions, DNA metabolism and morphogenesis. Bioinformatic analysis revealed that most of KLEB27-1 ORFs have >95% identity (in amino acid level) to proteins of *Bacillus* spp. On the other hand, phylogenetic analysis, based on the alignment of the essential structural and functional genes, revealed that phage KLEB27-1 has no close phylogenetic relationship to phages annotated to date. Thus, based on the results of comparative genome sequence analysis conducted during this study,

bacteriophage KLEB27-1 cannot be assigned to any genus currently recognized by ICTV and potentially represents a new one within the family of *Siphoviridae*.

38. The strategy for foreign protein epitope display on vB_EcoS_NBD2 bacteriophage-originated polytubes

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Highly repetitive display of foreign peptides or proteins on the surface of virus-based nanoparticles (VNPs) is known to induce a high immune response. While most of VNPs have different characteristics and may accommodate different antigens on their surface, a low diversity of self-assembled rod-shaped VNPs is observed. Previously we demonstrated, that the recombinant tail tube protein gp39 of vB_EcoS_NBD2 (NBD2) bacteriophage self-assembles into flexible polytubes in S. cerevisiae. The length of polytubes varies from 0.1 μm to >3.95 μm, while the diameter is ~12 nm. To test the feasibility of novel polytubes as a foreign epitope platform, recombinant yeast-expressed protein gp39 was used. To determine the regions of the protein, important for self-assembly into polytubes, a set of mutant proteins lacking amino- or carboxy-terminal end was created. Also, we employed a library of different origin and various length peptides as well as proteins to be genetically fused to the carboxy-terminal region of the recombinant protein gp39. A 6x His-tag or selected epitopes of a honey bee (Apis mellifera) major allergen Api m 4, Hantaan virus nucleocapsid protein as well as full-length green fluorescent protein (eGFP) constituted inserts spanning from 6 to 238 amino acids. Additionally, short glycine-serine linker between protein gp39 and fused epitopes was used as a spacer. Different efficiency of the synthesis and self-assembly properties was observed. The composition of amino acids of the epitope plays more important role than the length of inserted epitopes as evidenced by PAA gel electrophoresis and electron microscopy. Here, the recombinant protein gp39 fused with full-length eGFP (gp39_C-eGFP) self-assembled into short polytubes and retained eGFP functional activity as evidenced by fluorescence microscopy. We demonstrate, that flexible polytubes formed by the recombinant protein gp39 can be exploited as a scaffold to present epitopes or proteins consisting of 238 amino acid residues.

39. Synthesis of Art v 3 allergen from Artemisia vulgaris and allergen Bet v 4 from Betula verrucosa in Esherichia coli and their characterization

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Allergic diseases are affecting the lives of many people worldwide. Globally, 300 million people suffer from asthma, about 200 to 250 from food allergies, one-tenth of the population

suffers from drug allergies and 400 million people from rhinitis[1]. Allergens are recognized as the proteins that induce immunoglobulin E (IgE) responses in humans. These proteins come from a range of sources including pollen, fungi, insects, domestic animals, food products and have many different biological functions[2]. Allergens used for diagnosis and immunotherapy are mostly produced by extraction from primary sources. The usefulness of commercial whole-allergen extracts for skin testing is limited by many factors: they are difficult to standardize and have variable content of major and minor allergens. Sometimes important allergen components are even not present in the extracts. Furthermore, several studies found that these extracts can be contaminated by allergens from other sources, which can lead to false-positive results[3]. Single recombinant allergen components could be used in allergy diagnostic assays and immunotherapy. Moreover the tests based on whole-allergen extracts could be supplemented with recombinant allergen components. These applications would improve diagnosis and treatment options for allergic patients.

In the present study, two recombinant pollen allergen components of *Artemisia vulgaris* (common mugwort) and *Betula verrucosa* (European white birch) were investigated. *Artemisia vulgaris* elicits allergic reactions in 10-14% of pollinosis patiens in Europe[4]. Birch pollen is the most dominant tree pollen in Central and Northern Europe and is a major cause of allergic rhinitis and, possibly, asthma symptoms[5]. Artemisia vulgaris 3 and Betula verrucosa 4 allergen components were synthesized in *E. coli*, purified under native conditions using Ni^{2+} affinity chromatography and tested in an indirect enzyme-linked immunosorbent assay with specific IgE positive human blood serum samples.

1. Pawankar R. Allergic diseases and asthma: a global public health concern and a call to action. *World Allergy Organ J.* 2014;7(1):12.
2. Platts-Mills TAE, Woodfolk JA. Allergens and their role in the allergic immune response: Allergens and the allergic immune response. *Immunol Rev.* 2011;242(1):51-68.
3. Curin M, Garib V, Valenta R. Single recombinant and purified major allergens and peptides. *Ann Allergy Asthma Immunol.* 2017;119(3):201-9.
4. Gadermaier G, Hauser M, Ferreira F. Allergens of weed pollen: An overview on recombinant and natural molecules. *Methods.* 2014;66(1):55-66.
5. Biedermann T, Winther L, Till SJ, Panzner P, Knulst A, Valovirta E. Birch pollen allergy in Europe. *Allergy.* 2019; 74: 1237– 48.

40. Isolation and characterization of bacteria and bacteriophages from gypsum karst lakes

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In this study we present a number of bacteria and bacteriophages isolated from water samples of unique sulfate-type gypsum karst lakes Kirkilai and Ramunėlis located near Biržai, Lithuania. Based on the results of partial 16S rRNA sequencing, 27 culturable bacterial strains were identified including bacteria from the genus *Aeromonas*, *Agrobacterium*, *Bacillus*, *Chryseobacterium*, *Duganella*, *Flavobacterium*, *Paracoccus*,

Pararheinheimera, *Pseudaeromonas*, *Pseudomonas*, *Shewanella and Stenotrophomonas*. Nine bacteriophages were isolated using aforementioned strains as the host for phage propagation and phage growth experiments. TEM analysis revealed that most of the phages (*Bacillus* phage KLEB27-3, *Pararheinheimera* phages KLER1-1 and KLER1-2, *Pseudaeromonas* phage KLEP7 and *Pseudomonas* phage KLEP17-4) belong to the family *Myoviridae*. *Aeromonas* phage KLEA5, *Bacillus* phages KLEB27-1 and KLEB30-3S are the members of the family *Siphoviridae* and *Paracoccus* phage KLEP18-1 is a podovirus. Most of aforementioned phages have isometric heads of about 60-65 nm in diameter, while with a head in diameter of ~108 nm and a long, contractile tail (~285 nm) *Bacillus* phage KLEB27-3 is the biggest phage isolated. Plating tests revealed that all phages can form plaques in the temperature of 22°C. Phylogenetic analysis, based on the comparison of essential structural and functional genes, revealed that the vast majority of the phages (except KLEB30-3S), have no close phylogenetic relatedness to other viruses published to date and potentially represent new genera within the order Caudovirales. Thus, the data presented in this study will not only expand our knowledge of the diversity of bacteriophages but also leads for a better understanding of almost unexplored communities of bacteria and viruses in the unique sulfate-type gypsum karst lakes.

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41. Biodegradation of polyurethane

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Polyurethanes are the fifth most used plastic in Europe, making up to almost 8 % of total plastic manufactured in Europe[1]. Due to its specific features such as - universality and durability, polyurethane is widely used in many areas of everyday life and is produced in main industrial sectors: building materials, automobile seats, furnishings, mattresses, surface coatings and adhesives. However, the advantages provided by these polymers are correlated with the problem caused by its accumulation after the end of its use. The degradation of polyurethane waste is very complicated as it degrades slowly, the processing is limited to only several technologies, and its destruction may release toxic compounds. One of the ways to ensure sustainable and environment clean technology is biodegradation. Biodegradation is biologic degradation process by employing microorganisms and their enzymes. The chemical structure of polyurethane is the key factor why some polyurethanes are easily degradable and others are hardly degradable or undegradable at all. Polyurethanes based on polyester polyols (PS-PU) due to their ester bonds that are sensitive to hydrolysis are more tend to biological degradation than polyether polyurethane (PE-PU)[2].

The aim of this study is to investigate the biodegradation of polyurethane by biotechnological methods. The first goal was to isolate and identify microorganisms with capability to degrade polyurethane. The second goal was to investigate the enzymes responsible for this specific degradation and to perform their gene cloning in expression systems. The obtained results in more detail will be presented during the poster session.

1. https://www.plasticseurope.org/application/files/6315/4510/9658/Plastics_the_facts_2018_AF_web.pdf
2. **Magnin A.**, Pollet E., Perrin R., Ullmann Ch., Persillon C., Phalip V., Averous L., ***2019***, Enzymatic recycling of thermoplastic polyurethanes: Synergistic effect of an esterase and an amidase and recovery of building blocks, *Waste Management*, 85, 15, 141-150. doi: org/10.1016/j.wasman.2018.12.024

42. Investigation of enzymatic hydrolysis of plant proteins

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The worldwide demand for proteins is progressively expanding due to the strong growth in the quality of life[1]. Insufficiency of proteins is causing physical weakness, dizziness, impaired coordination and increasing the risk of unconsciousness. The extremely low level of proteins in the body can lead to anemia, can reduce body's resistance to infectious diseases, etc.[2]. Sufficient quantity of proteins can be obtained from products containing plant or animal origins. Plant proteins have an ability to provide high levels of proteins without the high content of fats that is often associated with animal proteins[1]. In addition, plant proteins are the source of water-soluble fibers, vitamins, high level of essential amino acids, which are not synthesized by the human body and should therefore be obtained with food. However, plant proteins, as nutrient ingredients, are not widely used because of its complex isolation and processing[3]. Hydrolysis increases protein solubility, improves the functions of nutrient proteins and helps to eliminate antinutritional components. Also, protein hydrolysates, which mainly consists of dipeptides, tripeptides and free amino acids, are more readily absorbed by the body. Hydrolysates, which are easily digestible, can be applied to a variety groups of populations, for example, athletes, lactating and pregnant women, the elderly, children, also patients with eating disorders after injuries or burns[4].

By this study we have investigated the enzymatic hydrolysis of plant proteins. The alfalfa flour protein was as an investigation object. The activities of commercial proteases and the efficiency of protein pretreatment for hydrolysis was determined. More detailed results will be presented during the poster session.

1. Ahnen Rylee T., Jonnalagadda Satya S., Slavin Joanne L. (2019) Role of plant protein in nutrition, wellness, and health. *Nutrition Reciews.* DOI: 10.1093/nutrit/nuz028

2. Wu Guoyao (2016) Dietary protein intake and human health. *Food & Function,* 7/3: 1251-1265. DOI: 10.1039/c5fo01530h

3. Elmadfa Ibrahim ir Meyer Alexa L. (2017) Animal Proteins as Important Contributors to a Healthy Human Diet. *Annual Review of Animal Biosciences*, 5: 111-131. DOI: 10.1146/annurev-animal-022516-022943

4. Wouters Arno G. B., Rombouts Ine, Fierens Ellen, Brijs Kristof, Delcour Jan A. (2016) Relevance of the Functional Properties of Enzymatic Plant Protein Hydrolysates in Food Systems. *Comprehensive reviews in food science and food safety,* 15/4. DOI: https://doi.org/10.1111/1541-4337.12209

43. Screening of New Biological Control Agents Against Fusarium sp

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Fusarium is a large genus of filamentous fungi widely distributed in soil and often associated with plant diseases such as crown rot, head blight, and scab on cereal grains. Fusarium head blight (FHB), a wellknown crop disease, is caused by different Fusarium species and can result in yield loss and reduced grain quality[1]. Micromycetes are plant pathogens, some Fusarium species can cause root and stem rot, vascular wilt or fruit rot. It is a phylogenetically diverse genus and some species have already emerged as opportunistic pathogens[2]. It was brought to concern that Fusarium micromycetes synthesize wide

spectrum of various mycotoxins and it can also cause life - threatening diseases such as hyalohyphomycosis, mycotic keratitis and onychomycosis in humans[3]. Currently, used control measures are not efficient, because it is impossible to apply fungicides directly to the roots of infected plants. The use of harmful chemical fungicide eliminates not only pathogens but beneficial microbes as well. Thus, the use of such fungicide is health threatening. This study was aimed to find new and effective Fusarium micromycetes biocontrol measures. Lyzobacter capsici and Staphylococcus saprophyticus could provide an efficient way to deal with the problem. In this study, two bacterial strains were monitored for the best growth conditions to increase biomass yield. A big concern was the identification of the synthesis of active antifungal compounds – growth phases and location (intracellular, extracellular). Thin–layer chromatography was performed to separate antifungal compounds. The antifungal activity tested against two Fusarium strains using spore suspepension. The isolated bacteria and/or isolated active compounds could potentially be used for field studies in the future or even coping with the problem on worldwide means.

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1. Edel-Hermann V, Brenot S, Gautheron N, AimÃ© S, Alabouvette C, Steinberg C. Ecological fitness of the biocontrol agent Fusarium oxysporum Fo47 in soil and its impact on the soil microbial communities: Application of Fo47: ecological fitness and risk assessment. FEMS Microbiol Ecol. 2009;68(1):37-45. doi:10.1111/j.1574-6941.2009.00656.x
2. Chetouhi C, Bonhomme L, Lasserre-Zuber P, et al. Transcriptome dynamics of a susceptible wheat upon Fusarium head blight reveals that molecular responses to Fusarium graminearum infection fit over the grain development processes. Funct Integr Genomics. 2016;16(2):183-201. doi:10.1007/s10142-016-0476-1
Mayayo E, Pujol I, Guarr J. Experimental pathogenicity of four opportunist Fusariurn species in a murine model. :4.

44. Optimization of Conditions for Genetic Transformation of Thermophilic Bacteria

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LithuaniaThermophilic bacteria are considered to be useful biotechnological objects, since this type of bacteria has a lot of possible uses in various industrial fields, where these microorganisms serve as a source of many thermostable enzymes as well as biotechnologically produced metabolites. However, due to the lack of reliable genetic transformation systems for thermophiles, industrial application remains a challenging approach. Thus, optimization of genetic transformation of thermophilic bacteria is highly required.
The aim of this research was to broaden current knowledge of genetic manipulation of thermophilic bacteria in order to find the method of transforming the three bacterial strains - Geobacillus stearothermophilus 15, Aeribacillus pallidus 8 and Parageobacillus toebii DSM 14590T. Shuttle vectors used in this study were methylated and non-methylated,

containing replication initiation proteins from different species of thermophilic bacteria. Unfortunately, we did not obtain any transformants, which confirms that transformation efficiency is not strongly dependent on these factors.
Further studies concentrate on the vector methylation using recombinant restriction-modification (R-M) systems encoded by the thermophilic bacteria strains. Using the Restriction Enzyme Database (REBASE), we identified the R-M systems in the thermophiles and transferred these genes to the Escherichia coli strains. The extracted shuttle-vectors are later used for genetic transformation of thermophiles. We hope that using the heterologous expression of R-M system genes from thermophilic bacteria will allow exogenous DNA to avoid restriction by the host R-M system.
Overall, we believe that this work has a great potential and could be useful in future researches associated with genetic engineering of thermophilic microorganisms.

45. New linear azol(in)e containig antimicrobial peptide identification in thermophilic bacterium

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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-translationaly modiefied 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.

1. R.H. Perez, T. Zendo, K. Sonomoto, Novel bacteriocins from lactic acid bacteria (LAB): various structures and applications, Microb. Cell Fact. 13 (2014) S3.

46. Functional Analysis of Genes Involved inthe Biosynthesis of Bacteriocin Geobacillin 26

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Bacteriocins are antibacterial peptides or proteins, which are synthesized in ribosomes. Bacteriocins have antibacterial activity against bacteria that are closely related to the producer strain. In our research we are studying a specific gene cluster related to bacteriocin geobacillin 26, which is found and produced by a thermophilic Gram-positive bacterium Geobacillus stearothermophilus 15. Geobacillin 26 is heat-labile, 26 kDa bacteriocin. Little is known about other genes: geo26B and geo26C, encoded in the gene cluster of geobacillin 26. Our study suggests that they might be related to the immunity and transportation/secretion of the bacteriocin. To confirm it, our study is focused on cloning these genes into plasmids and their coexpression with geobacillin 26 in Escherichia coli and thermofilic Parageobacillus genomospecies 1 NUB36187 bacteria. Moreover, using these constructs we will evaluate the expression levels of geo26B and geo26C genes in E. coli and thermofilic P. genomospecies 1 NUB36187 bacteria using green fluorescent protein. Overall, very little is known about high molecular weight bacteriocins as geobacillin 26 and how other genes might be associated with immunity and secretion of geobacillin. Nevertheless, geobacillin 26 as an antibacterial agent has a great potential in medicine or food industry.

47. Effect Of Probiotic Compositions On Growth, Antioxidant Activity And Productivity Of Root Crops

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Last few decades have shown that consumption of chemical fertilizers in agronomy has improved crop productivity. However it has negative response on the environment and soil microbiological variety. Problems mentioned above have encouraged scientists to look for new alternatives of chemical fertilizers. One of it is plant probiotics that are able to improve soil and plant microbiota.

The application of symbiotic bacteria in agriculture has demonstrated positive effects on soil fertility and yield quality (e. g. higher amount of vitamin C in root crops, which are important part of human's diet).

Carrots (Daucus carota L.) were treated with two different probiotic compositions - Naturgel and ProbioHumus in ecological farm in Lithuania. The root crops were collected at the end of September. Biometric parameters of carrots were measured after collecting yield. The results showed that symbiotic bacteria have no significant impact on yield quantity. ProbioHumus-treated carrots reached 139.9 g mass, the mass of Naturgel-treated carrots was 136.9 g, non-treated vegetables 125.8 g.

The amount of monosaccharides was established. ProbioHumus and Naturgel combination had positive effect on monosaccharide amount of tested crops. The value of sugars was 8% higher comparing to ProbioHumus-treated plants, and 40% higher comparing to non-treated plants. Antioxidant activity of root crops was evaluated according to their free radical

scavenging using DPPH test. Plants treated with the mix of two probiotic components showed higher antioxidant activity by 12.6 % more than ProbioHumus-treated and by 23.8% more than non-treated plants. The amount of vitamin C in root crops was measured using HPTLC method. ProbioHumus- treated crops, disclosed better results comparing to control – by 18% more, and by 6% more comparing to ProbioHumus and Naturgel complex treatment. All these results suggest, that applying probiotics or mixture of few compositions in agriculture will led to better yield quality and stimulates us to experiment more with plant symbiotic microorganisms.

48. Studies and Application of Third Generation Biosensors Created Using TRGO Fractions and Glucose Dehydrogenase

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This year the biosensor market is expected to reach $22.68 billion. The increasing demand for inexpensive and portable analytical devices, which require low-sample consumption and real-time response, has greatly raised the novelty in the design of biosensors. Researchers have especially focused on new materials, which could improve miniaturization and portability of mentioned analytical devices. This can be achieved by integrating carbon materials, which are characterized by unique properties, into analytical systems. One of these materials - graphene, which after modification could help to create third-generation biosensors that use direct electron transfer (DET) between the enzyme and the electrode. Design of biosensors acting on DET principle do not require any intermediate mediating materials, so technologies using such biosensors is becoming less complicated and cost-effective.

This study aims to create third-generation biosensors based on thermally reduced graphene oxide (TRGO) fractions and *pyrroloquinoline quinone-dependent glucose dehydrogenase (PQQ-GDH)* from Acinetobacter calcoaceticus sp. and to investigate efficiency as well as substrate selectivity of biosensors. While PQQ-GDH possesses substrate specificity to wide range of carbohydrates, the enzyme could be very promising for creation of new technologies for investigation of diseases related to release of carbohydrates.

TRGO fractions were synthesized from graphite oxide, by following thermal reduction and fractionation process. Properties of TRGO fractions were characterised by x–ray diffraction and thermogravimetric analysis. The biosensors were constructed using membranes, made from PQQ-GDH immobilised into layer of TRGO. The main characteristics of proposed biosensors, such as sensitivity, limit of detection, linear part of the calibration curve, substrate selectivity, were determined. Subsequently biosensors were attempted to be used for detection of maltose in urine of patients suffering with severe pancreatitis.

The research was supported by Research Council of Lithuania (Project No. 09.3.3-LMT-K-712-16-0125).

49. Yeast Diversity and Prevalence in Lithuanian Freshwater

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Yeasts are single-celled eukaryotic microorganisms found in environment: on plant surfaces, in soil, air and water. Microbiological safety of water is usually assessed by the amount of indicator bacteria present in the water. Although in the last few decades fungi are becoming frequently recognized as causative agents of various infections. Possible reasons may be the lack of knowledge of the fungal load in water, divergent cultivation methods, heterogeneous mechanisms of fungal pathogenicity and consequently the low number of reports connecting fungal presence in water and the occurrence of diseases in humans. The objective of the present work was to identify and compare the diversity of yeast species in human bathing areas in Lithuania. In the course of our research, water samples were collected from human bathing areas and applied for direct isolation of microorganisms as well as for metagenomic-based identification. Isolated colonies with yeast-like morphology were used for molecular identification. Not all yeasts found in water are cultivable under laboratory conditions. So, we extracted total gDNA from concentrated water samples, amplified internal transcribed spacer regions by PCR, inserted resulting DNA fragments into cloning vector and sequenced. It was shown that the most common yeast genera found in freshwater are Rhodotorula, Cryptococcus, Candida and Aureobasidium. They are associated with anthropogenic pollution and some of them are opportunistic pathogens, such as Rhodoturula glutinis, Aureobasidium pullulans, etc. The greatest diversity of yeast species was found in Nemunas and Neris rivers.

50. Transcriptional Analysis of Non-ribosomal Peptide Synthetase and Polyketide Synthase Genes in Two Paenibacillus sp. Strains from Krubera-Voronja Cave

Juknevičiūtė Gabrielė, Kuisienė Nomeda

Non-ribosomal peptide synthetases (NRPS) and polyketide synthases (PKS) are multimodular enzymes which synthesize nonribosomal peptides, polyketides and their hybrid compounds. These bioactive substances are important, because they have antimicrobial, antifungal, antiparasitic or antitumor features which could be very useful in medical applications. In this study we analysed two genomes belonging to *Paenbacillus* sp.: *Paenibacillus sp.* 23TSA30-6 and *Paenibacillus sp.* 28ISP30-2. These strains were obtained from Krubera-Voronja cave which is one of the deepest caves in the world. Oligotrophic environment suggests that microorganisms in this cave might possess novel bioactive substances. Also, our previous studies with these strains revealed their potential for unknown bioactive compounds synthesis.

The aim of this study was to evaluate how NRPS and hybrid NRPS-PKS gene transcription is affected by the carbon availability in the growth medium and by the growth phase of bacterial culture. For this reason we plotted a growth curves for both strains. Also, based on the previously made genome analysis, where we identified NPRS and hybrid NRPS-PKS genes, we made specific primers for further transcriptional analysis.

In this study we tested 14 specific primers for NRPS and hybrid NRPS-PKS. Our PCR-based screening revealed that all genes are expressed in transition or stationary phase at any carbon availability. Further transcriptional analysis confirmed our PCR-based results and also showed that genes in *Paenibacillus sp.* 23TSA30-6 and *Paenibacillus sp.* 28ISP30-2 strains are expressed differently on distinct growth phases and carbon availability.
This work was supported by the Research Council of Lithuania (grant No. S-MIP-17-21).

51. Synthesis analysis of bacteriophage vB_KleM-RaK2 recombinant tail sheath protein gp41 in Saccharomyces cerevisiae and Pichia pastoris yeast

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Virus-like particles (VLPs) are multiprotein structures that mimic the conformation of authentic native viruses but lack the viral genome. VLPs are expected to gain widespread use in numerous fields, such as *in vitro* diagnostics, vaccines, and therapeutic modalities. However, little information regarding self-assembly of phage tail tube proteins is available. Therefore, the need to synthesize new structures of different morphology as well as properties is increasing.

Previously it was demonstrated, that recombinant tail sheath protein gp41 of myovirus vB_KleM-RaK2 (RaK2) self-assembles into rigid tubular structures (polysheaths) with an uneven surface morphology in *Saccharomyces cerevisiae*. According to TEM observations, the length of polysheaths varied from 90 nm to 1 μm, having the width from 27 nm to 43 nm. However, the synthesis efficiency of the recombinant protein gp41 in *S. cerevisiae* was low and reached only about 8 % of the total cell protein level. In order to synthesize recombinant protein gp41 in the preparative amounts, *Pichia pastoris* protein synthesis system was employed. Here, *P. pastoris* expression vector encoding gp41 protein was constructed. The synthesis efficiency of the recombinant protein gp41 was determined after 24, 48 and 72 hours after induction in *P. pastoris* Mut^+ phenotype. According to PAA gel electrophoresis, the 100 kDa size protein band corresponding to the molecular mass of gp41 protein was not observed. In the future, the model eGFP protein will be synthesized in parallel to ensure appropriate growing conditions. Additionally, to improve recombinant protein gp41 synthesis, *P. pastoris* with a phenotype of Mut^S will be used for its lower grown rate and slower methanol utilization pathway.

52. Corylus avellana allergen Cor a 2 expression optimization in Escherichia coli

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The prevalence of food allergies has increased in recent decades and is now recognized as a substantial public health burden in developed countries. There are many types of food allergens, but the most common of them are found in peanuts, tree nuts, cow's milk, and fish. Nuts are a well-defined cause of food allergy, which can lead to severe systemic and even fatal reactions in the human body.

Nowadays, allergic diseases are detected using extracts from primary allergen sources. This type of detection increases the possibility of cross-reactivity because allergen extracts are composed of many components, the majority of which are irrelevant for the allergic reaction and allergy diagnostics. The use of recombinant allergens can increase assay sensitivity and analytical specificity. For the high-level production of recombinant proteins, the most widely used host is Escherichia coli due to its easy manipulation and scale-up process.

In this work, the hazelnut, Corylus avellana, Cor a 2 allergen was expressed in three different E. coli strains: BL21(DE3), Rosetta (DE3) and ArcticExpress (DE3). The recombinant protein was also fused to a highly soluble partner - the maltose-binding protein (MBP), which promotes solubility and influences the proper folding of its fusion partners.

The study aimed to find the most suitable conditions for the high-level production of the recombinant allergen rCor a 2. Interestingly, while the expression level of rCor a 2 protein increased when fused to MBP, it did not have an impact on rCor a 2 protein solubility. Overall, none of these strains and conditions were suitable to reach the aim of this project.

A natural progression of this work will be to explore a eukaryotic protein expression system for the synthesis of rCor a 2 and to compare the antigenic properties of the purified rCor a 2 proteins.

Genetics

53. MiRNA analysis in blood of women diagnosed with gestational diabetes

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Gestational diabetes mellitus (GD) is a chronic hyperglycemia which occurs during the second trimester of pregnancy and usually disappears after delivery. The prevalence of this disease is constantly growing in Lithuania and worldwide and correlates with the type 2 diabetes mellitus (T2D) as GD causes higher risk of T2D for the mother and her child. Therefore, it is necessary to elucidate not only the molecular mechanisms of the GD development, but also its association with T2D in order to prevent its development after pregnancy.

As epigenetic mechanisms are the most promising in this research field, the aim of this study was to analyze 6 microRNAs (miR-16-5p, miR-27a-3p, miR-152-3p, miR-155-5p, miR-222-3p, and miR-518d-5p) in whole-blood samples of women diagnosed with GD.

In total, 33 women diagnosed with GD and 5 healthy women were included in the study. The blood samples were taken at the 24-28 weeks of gestation (N = 38) and at 6-12 weeks after delivery (N = 10). The selected miRNAs were quantified by means of quantitative PCR (using TaqMan-based assays) after reverse transcription (RT).

Although the miRNA levels did not differ between the GD and non-GD pregnant women (all $p > 0.05$) during the gestational period, higher amounts of miR-16-5p, miR-152-3p, and miR-155-5p (all $p < 0.05$) were detected in blood collected after delivery as compared to the samples taken during the pregnancy. The miR-16-5p level in blood during gestation correlated positively with the pre-gestational body-mass index (BMI; $p = 0.030$), while miR-152-3p and miR-155-5p levels after delivery were associated with age ($p = 0.034$ and $p = 0.015$, respectively). Various associations were also observed between miRNA abundance levels and diabetes-related biochemical parameters.

The present study revealed that changes of miR-16-5p, miR-152-3p, and miR-155-5p abundance in blood are associated with GD and might be used as a potential biomarker for GD follow-up.

54. Genetic evaluation of Medicago sativa populations and cultivars using ISSR markers

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Genetic evaluation of Medicago sativa populations and cultivars using ISSR markers

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Due to the everchanging world and its climate, where living conditions are becoming more extreme, people are taking increased interest in cultivated animal fodder, and their adaptivity. Research is ongoing to locate new properties of such fodder, which would help cultivars become more resistant to harsh climates and bad weather, grant immunity to newly found diseases and, lastly, increase crop yields. All these newly found properties would help feed the ever-growing human population and the domesticated animals we keep.

The subject of this research is *Medicago sativa* (also known as alfalfa or lucerne) cultivars and populations. The *Medicago sativa* cultivars are grown on large territories as important forage crops, due to their high productivity, abundance of protein, good digestibility, which is very valuable as fodder for livestock, and theability to fix atmospheric nitrogen by forming symbiosis with nitrifying microorganisms.

In this study, ISSR (Inter-Simple Sequence Repeat) marker technique was used, 6 different lucerne cultivars and 6 naturally growing lucerne populations were studied.

The goal of this study is to evaluate the genetic diversity of *Medicago sativa* cultivars from different countries, as well as populations found in Lithuania, using ISSR primers.

The genetic diversity parameters (*I, He, P, FST*) were assessed using softwares GenAlEx and TreeCon. The highest mean percentage of polymorphic loci (*P*) and the highest expected average heterozygosity (*He*) were found in Lithuanian lucerne populations.

UPGMA cluster analysis revealed that naturally growing geographically separated populations of *Medicago sativa* form discrete groups on the dendrogram. However, the overlap of individuals of cultivars from neighboring regions was detected. Our results indicate that the genetic differences are more expressed in naturally growing populations than of the cultivars we studied.

55. Induction Of Nuclear Abnormalities In Flounder (Platichthys Flesus), Herring (Clupea Harengus Membras) And Atlantic Cod (Gadus Morhua Callarias) Collected From The Gotland Basin Of The Baltic Sea (2011–2017)

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After the World War II, at least 40,000 tons of chemical munitions (CW) were dumped largely in the Lille Belt, near the Bornholm Island and in the Gotland basin of the Baltic Sea. In southern part of the Gotland Basin were transported and dumped 2,000 tons of CW (consisting of approximately 1000 tons of chemical warfare agents (CWAs)

payload)[1]. Frequencies of eight nuclear abnormalities reflecting environmental genotoxicity and cytotoxicity, were examined in 605 specimens of herring (*Clupea harengus membras*), flounder (*Platichthys flesus*) and cod (*Gadus morhua callarias*) collected between 2011 and 2017 at 47 study stations located in the Gotland Basin of the Baltic sea. The highest levels of geno- and cytotoxicity were recorded in herring caught at stations located in CW dumping sites. Genotoxicity levels were found to be lower at stations located further away from the known pollution by CWAs. Exceptionally high total genotoxicity (ΣGentox) risk was found for herring collected from 23 out of 29 stations, for flounder caught at 17 out of 19 stations and for cod caught at 6 out of 12 studied stations. There is a necessity to monitor genotoxicity impacts of CWAs on marine organisms and to determine the ecological significance in the areas of CW dumpsites.

1. HELCOM, 2013. Chemical munitions dumped in the Baltic Sea. Report of the Ad Hoc Expert Group to Update and Review the Existing Information on Dumped Chemical Munitions in the Baltic Sea (HELCOM MUNI). Balt. Sea Environ. Proc. 142, 36–56.

56. Kluyveromyces lactis and Pichia pastoris - promising yeast expression systems for efficient production of ß-carbonic anhydrase from Bacillus mojavensis

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Over the last few decades, the application of enzymes in industrial processes has increased remarkably. Enzymatic processes are applied in baking, brewing, detergents, pharmaceuticals, textiles, leather, paper and other industrial sectors. These processes have exposed essential advantages over chemical processes such as high specificity, eco-friendliness, biodegradability and cost-effectively.

Mainly, recombinant enzymes are produced by expression systems with expectation for high production rate and yield. The most commonly used expression hosts are bacteria and yeast. The latter one has a wide range of advantages - they accomplish proper post-translation modifications, fast growth, GRAS (generally recognised as safe), simple genetic manipulations. Furthermore, expression vectors containing inducible promoters, resistance markers, secretion signal peptides ensure efficient production and secretion of targeted proteins [1, 2].

This study focused on the development of expression systems for efficient production of *Bacillus mojavensis* ß-carbonic anhydrase in *Pichia pastoris* (*Komagataella phaffii*) and *Kluyveromyces lactis*. The unique feature of this enzyme is an ability to catalyze reversible CO_2 hydratation and the fixed atmospheric CO_2 can be converted into various useful products (acrylates, polycarbonates, stable polymers, *etc.*)[3, 4]. The obtained results of ß-CA expression in the yeast systems will be presented in more detail during the poster session.

1. Singh R, Kumar M, Mittal A, Mehta PK. Microbial enzymes: industrial progress in 21st century.*3 Biotech*. **2016**; 6(2):174. doi:10.1007/s13205-016-0485-8

2. Vieira Gomes AM, Souza Carmo T, Silva Carvalho L, Mendonça Bahia F, Parachin NS. Comparison of yeasts as hosts for recombinant protein production. *Microorganisms*. **2018**; 6(2):38. doi:10.3390/microorganisms6020038

3. Sakakura T, Choi JC, Yasuda H. Transformation of carbon dioxide. Chem. Rev. 2007; 107, 2365–2387. doi:10.1021/cr068357u

4. Beckman EJ. Making polymers from carbon dioxide. Science. 1999; 283, 946–947. doi:10.1126/science.283.5404.946

Neuroscience

57. NLRP3 Inflammasome Activation by Amyloid-beta Oligomers in Microglia Cells

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Alzheimer's disease (AD) is the most prevalent neurodegenerative disorder causing memory loss and deficits in further cognitive domains. While key pathological hallmarks have been identified, the precise mechanisms of disease progression remain unclear to date. AD is associated with the accumulation of amyloid-ß (Aß) and the formation of neurofibrillary tau tangles. Furthermore, inflammation, before thought to be a pure bystander reaction, has now been shown to contribute to AD progression. Aß itself represents a danger associated molecular pattern, which is recognized by pattern recognition receptors present on the surface of microglia, the brain's principle innate immune cell. One of the key players in this process is the NLRP3 inflammasome, which is strongly activated in AD patients and AD-relevant animal models. The question is if Aß oligomers, that are more neurotoxic compared to fibrils, could activate NLRP3 inflammasome in microglia cells. In our study we used different size Aß preparations: small oligomers and protofibrils. Their structure was confirmed by atomic force microscopy. Cells were treated with Aß and inflammasome activation, represented by caspase-1 cleavage, IL-1ß production, and ASC speck formation was analysed. Both protofibrils and low molecular weight Aß aggregates induced a significant increase in IL-1ß, which is a strong mediator of inflammation, release. Inflammasome activation was also confirmed with ASC speck formation and active caspase-1 detection. We also used specific inflammasome inhibitor MCC950 which completely blocked Aß induced response. Our results show that the inflammasome is activated not only with fibrils forming Aß aggregates, what was observed before, but also with lower molecular weight Aß oligomers, highlighting microglia together with Aß as a potent neuroinflammation drivers.

58. Effect of L-type Ca2+ channel blocker verapamil on Nitellopsis obtusa cells

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Nitellopsis obtusa is a freshwater and brackish water macroalgae belonging to Characeae family. Nitellopsis obtusa cells are macroscopic, making the algae one of the most convenient model systems to investigate how plants and other photosynthetic organisms adapt to changing environmental conditions on the level of single cells. As secondary messengers, Ca 2+ ions are vital for regulating various cellular processes, such as cell metabolism. Verapamil is a well-known animal L-type Ca 2+ ion channel blocker, but the knowledge is limited about its effect on plant cells, especially on properties of plasma membrane. To analyze how verapamil affects algae cells, cell viability, velocity of cyclosis and dynamics of plasma membrane potential were investigated. For our experiments, different concentrations of verapamil were dissolved in APW (artificial pond water). Tests of cell viability showed that 1 mM verapamil solution was lethal by inducing loss of cell turgor pressure after 0,5 hour. Effect of 0,1 mM verapamil solution was variable: loss of turgor was observed after 1-3 hours. Velocity of cyclosis was observed when a cell was placed in a verapamil solution and compared to velocity in APW. It was found that in some cells velocity of cyclosis was temporarily decreased. In other cells no initial decrease in velocity was observed, but cyclosis stopped in 1-2 hours. 1 mM verapamil solution also depolarized cell membrane potential, inducing spontaneous generation of action potentials, eventually leading to complete membrane depolarization and cell death. All these results indicate that verapamil in Nitellopsis cells acts not only as a Ca 2+ ion channel blocker. For example, decrease of velocity of cyclosis is induced when Ca 2+ ions bind to actin- myosin complexes in cytoplasm, indicating increased cytoplasmic Ca 2+ concentration. Plasma membrane depolarization and lethality of verapamil solution also cannot be explained only by its action as a Ca 2+ channel blocker.

59. What visual half-field paradigm can say about processing of foreign language and visual working memory?

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The visual half field paradigm is a well-established measure to evaluate functional hemispheric laterality of cognitive functions. It is well know that verbal stimuli presented in the right visual field (RVF) are processed faster and more accurate, indicating that the left cerebral hemisphere (RVF/LH) is dominant in language processing. Meanwhile, knowledge about working memory laterality is limited. We investigated hemispheric asymmetry of foreign language and visual working memory (VWM) processing by assessing and evaluating participants' behavioral metrics, links between language and VWM, effect of handedness Methods. 74 volunteers performed the translingual lexical decision task (TLDT), VWM task and completed the Edinburg Handedness Inventory. Each trial in TLDT consisted of two 100 ms duration stimuli, either word or non-word, that were presented simultaneously for both

the RVF and LVF. Participants had to decide whether a meaningful word was presented in the LVF or RVF or non-word in either side. In VWM task, trials consisted of four types of displays: a fixation display, a cue display (arrow indicating in which VF the change may appear), a memory sample display (from 3 to 5 letters in each VF), and a memory test. Participants had to recall and decide if there was a change of letter in the RVF or LVF depending on the cue. Accuracy in TLDT task was higher for RVF/LH than LVF/RH (p < 0.05) and performance laterality index correlated positively with handedness (r = 0.233, p = 0.046). In the VWM task, memory capacity and accuracy were higher, and reaction times were faster in the RVF/LH than in LVF/RH (p < 0.05). Our study showed that greater memory capacity was linked with higher accuracy in both visual fields in VWM task (r > 0.603, p > 0.001) and higher accuracy in the LVF/RH in TLDT (r = 0,292, p = 0.012). However, there was no significant relationship between laterality index in TLDT and VWM task. Conclusions. Our study showed that the left hemisphere is dominant for foreign language and VWM processing. There was no direct correlation between laterality indexes in language detection and VWM tasks.

60. Rutin attenuates the cytotoxic stress in SH-SY5Y cells caused by the aggregation of ß-amyloid

Robaya Akter

School of Life Sciences

Neurodegenerative diseases embrace a group of conditions that interrupt the neurons' function in the brain and or spinal cord resulting in dementia. The two most prevalent disorders affecting the older generation are Alzheimer's Disease (AD) and Parkinson's disease. However, the present study will be focusing on the pathophysiology of AD and possible treatment. Dementia is a condition that worsen with time, that affects the memory and learning regions including the hippocampus, basal forebrain, inferior and frontal cortex. The symptoms include difficulty in remembering events and feeling disoriented in unfamiliar places. AD is associated with abnormal accumulation of beta-amyloid and neurofibrillary tangles in the brain. The mechanism of neural death in the disease remain unclear, however it has been postulated that is due to apoptosis. Recent studies demonstrated that rutin, an olive biophenol exert numerous biochemical and pharmacological activities including protection against free-radicals and effects on immune and inflammatory cell functions. The aim of the study is to demonstrate that rutin attenuates the damage caused by Aß and to determine the apoptotic mechanism involved in the cell death caused by Aß. The project was based on a cell-line investigation in which we differentiated neuroblastoma cells (SH-SY5Y) in a complete nutrient media. The neuroblastoma cells were exposed to Aß at different concentration showing a decrease of cell activity and morphological changes with the increase of cytotoxic Aß analysed with the MTT assay. However, SH-SY5Y cells treated with rutin presented improved cell activity suggesting beneficial effects of olive biophenol. Moreover, the caspase assay demonstrated the programmed cell death of neural cells cause by cytotoxic Aß in the brain is mediated by the activation of caspase pathways. In conclusion,

the present findings can be considered a pilot study for further pharmaceutical investigation using natural olive biophenols as candidate treatment against AD.

61. Screening of compounds that may affect ethanol sensitivity in Drosophila Melanogaster

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Introduction: Drinking alcohol has toxic effects on human health. World Health Organisation estimated that alcohol intake accounts for 5.1 % of global diseases and injury and is responsible for 10% of all deaths worldwide among people aged 15-49 years old. Environmental and genetic factors contributes to alcohol susceptibility rendering addiction patterns challenging to be identified in simple animal model systems. However, ethanol tolerance can be induced in a wide variety of animal populations to study genes and drugs enhancing or interfering with ethanol sensitivity. Drosophila melanogaster were used in previous studies to explore how drugs could alter the sensitivity to ethanol. Drosophila is a useful model because it expresses many genes that are conserved in mammalian species. The aim of the project is to screen a set of neuro-active compounds for their potential effect on alcohol tolerance in Drosophila. Methods: To deliver and screen the effects of GABAB agonist, Guvacine (GABA transporter inhibitor), Midazolam (benzodiazepine that potentiates GABA effect on GABAA receptor), Naltrexone and Nalmefene (opiate receptor antagonists), CAFE (Capillary Feder) apparatus was used. Subsequently, sensitivity to ethanol was measured: 500 µL of 100% ethanol was delivered to flies to measure the amount of time needed for 50% of the flies to be sedated (ST50). Flies were fed with the drug of interest for 24 hours before the ethanol exposure. Results: Data indicate that administration of GABAB agonist SKF 97451 reduces time of sedation by increasing sensitivity to alcohol. Therefore, GABAB receptor could be used as a potential pharmacological target for the treatment of alcohol use disorder (AUD). Conclusion: Drosophila are an inexpensive and useful model organism to identify the molecular components participating in addictive behaviours. However, drugs need to be tested on other models to establish repetitive mechanisms of addiction. The ultimate goal of the research is to test four drugs to observe changes in alcohol sensitivity.

Cancer research

62. P53 knockout by CRISPR-Cas9 system in HCT116 cell line highlighted importance of p53 activation to chemotherapy drug response

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Tumor suppressor p53 protein is a transcription factor that enables cell cycle arrest, senescence, and apoptosis induction under various cell stresses. TP53 is also known as the most crucial tumor suppressor gene that stops cell division or initiates cell death in response to DNA damage, thereby preventing an injured cell from reproducing. Mutations of this gene eliminates a key cellular fail-safe mechanism and is a step leading to cancer development. Also, p53 mutation occurs in approximately 40%-50% of sporadic colorectal cancer cases. Patients with mutant p53 gene are often resistant to current therapies, conferring poor prognosis. Therefore, recent interests in human colorectal cancer have highlighted the necessity to conduct studies of p53 to better understand the role of this gene in drug resistance. To evaluate the biological and clinical relevance of p53 loss, CRISPR/Cas9 was used to delete the p53 gene in the human colorectal cancer cell line HCT116. Genetic engineering technology such as clustered regularly interspaced short palindromic repeats CRISPR/Cas9 system provides a powerful tool for developing disease models and determining gene functions. We have attempted to generate CRISPR/Cas9 system to target tumor protein 53 to establish p53 knockout cell line for cancer resistance research. For this research we chose HCT-P and HCT-Oxa cells, where HCT-P is parental human colon carcinoma cell line and HCT-Oxa is human colon carcinoma resistant to chemotherapeutic drug oxaliplatin. After cells were targeted with CRISPR/Cas9, we cloned these cells into subclones. Each subclone was analyzed for the presence of p53 via Western Blot. P53 -/- subclones were pooled together to establish HCT-P-p53 -/- and HCT-Oxa-p53 -/- cell lines. Resistance to oxaliplatin of these cell lines were evaluated using flow cytometry. We found, that p53 protein is responsible for acquired resistance to oxaliplatin in HCT-P cells. However, p53 knockout in HCT-Oxa cells did not make any difference considering resistance.

63. Gene expression analysis of histone modifying genes in prostate cancer

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Histone modifying enzymes participate in regulation of various cellular processes which are impaired in solid tumors, including prostate cancer (PCa). However, studies specifically focusing on their expression profile in relation to cancer aggressiveness are lacking. Furthermore, mechanisms responsible for their own epigenetic regulation are not thoroughly described. The aim of this study was to analyze expression changes of histone modifying genes in PCa of different levels of aggressiveness and to evaluate the role of promoter DNA methylation as a potential mechanism of epigenetic regulation. In total, 50 PCa and 24 non-cancerous prostate tissues (NPT) were included in the study. Gene expression was analyzed by means of quantitative PCR after reverse transcription using RT 2 Profiler PCR Arrays (Human Epigenetic Chromatin Modification Enzymes gene set; Qiagen)

or TaqMan-based single assays of selected targets. For the methylation analysis, extracted genomic DNA was bisulfite-modified and methylation-specific PCR was used to analyze promoter methylation status of the genes. Array-based gene expression analysis showed that ESCO2 and MLL5 were upregulated in PCa as compared to NPT, while ASH1l, DZIP3, KDM5D and several other genes were upregulated in NPT (all P < 0.0500). Higher expression of 11 genes, related mostly to histone methylation, were identified in indolent, i.e. slowly or not progressing, tumors (all P < 0.0500). Furthermore, HDAC9 expression levels were higher in cases having TMPRSS2-ERG fusion transcript (P = 0.0357) and correlated with prostate-specific antigen (PSA) level (P = 0.0489). Down-regulation of KMT5A, ASHL1, MLL5, and others were associated with rapid biochemical progression of the disease. Promoter DNA methylation analysis of the selected microRNA host genes, known to modulate expression of KMT5A, KDM3A, KDM5D and PHF8, did not indicate the involvement of this epigenetic regulatory mechanism in progression of PCa. This study showed that expression of various histone modification enzymes was impaired in PCa, which could be used as a molecular tool for PCa differentiation into prognostic groups and treatment individualization.

64. Autophagy and Akt signaling promotes resistance to ferroptosis in pancreatic cancer cells

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Pancreatic cancer is the fourth leading cause of cancer-related deaths in European population[1]. In most cases poor clinical outcome results from resistance to mainstream cancer treatment modalities, such as conventional chemotherapy. About fifteen years ago a new anticancer drug erastin was discovered, which was lethal to cancer cells bearing oncogene KRAS mutations, such as pancreatic ductal adenocarcinoma (PDAC)[2]. Soon, a new cell death type induced by erastin was identified and named ferroptosis. Now it is known that ferroptosis is caused by accumulation of free cellular iron and a successive rapid membrane lipid oxidation[3].

In this study we examined a link between ferroptosis and another cell death related process - autophagy. We showed that inhibition of protein complex mTORC1 enhances PDAC cell resistance to erastin, in addition, inhibition of autophagy - related ULK1 kinase sensitizes cells back to ferroptic stimulus. Also, we determined that active Akt kinase decreases cell sensitivity to erastin. Thus, we propose a hypothesis that autophagy and Akt signaling promotes resistance to ferroptosis in pancreatic cancer cells.

1. Capurso G, Sette C. Drug resistance in pancreatic cancer: New player caught in act. EBioMedicine. 2019;40:39-40.
2. Dolma S, Lessnick SL, Hahn WC, Stockwell BR. Identification of genotype-selective antitumor agents using synthetic lethal chemical screening in engineered human tumor cells. Cancer Cell. 2003;3(3):285-96.
3. Dixon SJ, Stockwell BR. The hallmarks of ferroptosis. Annu. Rev. Cancer Biol. 2019; 3:35-54.

65. New Urine Based Genetic Test Model for Prostate Cancer

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Prostate cancer (PCa) is one of the most prevalent types of cancer in Lithuania and the rest of the world. In recent decades, the high increase of PCa incidence has been highly linked with the wide use of serum prostate-specific antigen (PSA) test for early detection. However, due to low specificity, PSA test shows little benefit in mortality rates and causes overtreatment. New diagnostic and prognostic tests for PCa are in great need to replace the current diagnostic techniques. In recent years, new non-invasive genetic tests for PCa based on gene expression in urine samples have been developed and are already used in the clinic, however the urine samples for these tests are obtained after DRE and stored with RNA stabilizers, wich inflates the cost of such tests.
In our study, qPCR was used to determine *PSMA, PCA3, TMPRSS2:ERG, AR* and *HOXC6* expression in 94 PCa urine sediment samples, obtained without DRE and stored without RNA stabilizers. *PSMA*, *PCA3* and *HOXC6* expression was significantly different between pT2 and pT3 stages. *PSMA* expression distinguished patients with clinically significant (Gleason score ⩾7) and early stage (Gleason score 6) disease, this data was used to create *PSMA*, *PCA3*, *TMPRSS2:ERG*, *AR* and *HOXC6* urine sediment PCa genetic test model (AUC = 0.805, sensitivity = 0.897, specificity = 0,733). In conclusion, the studied genes are perspective PCa biomarkers, which may be used to create a new test for PCa diagnosis.

66. Detection of Mutations in the Mitogen-Activated Protein Kinase Pathway (MAPK) in Human Melanoma

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Melanoma is a malignancy originating from melanocytes of the skin with a high propensity to metastasize. Hot spot mutations of the oncogenes *BRAF* and *NRAS* lead to constitutive signalling of the MAPK pathway and thereby enhance tumour growth and promote disease progression. Although genetic alterations in both genes can be detected in approximately 40 and 20% of cases, respectively, the association between lymph node metastasis and presence of *BRAF* mutations in patients with melanoma remains uncertain, hence needs to be elucidated.

The aim of the study was to clarify the incidence of *BRAF* and *NRAS* mutations and compare its manifestations in paired samples of lymphatic metastases and primary melanoma.
BRAF and *NRAS* mutations were assessed using genotyping assays. Overall, detection of *BRAF* mutation status was performed in 52 formalin-fixed, paraffin-embedded paired samples (primary melanoma + lymph nodes) from 26 patients. 11 more patients were tested for *BRAF* mutations only in primary melanoma (n=37). *NRAS* alterations were determined for *BRAF* negative patients only.
57 % of all tested patients (21/37) carried a *BRAF* mutation in primary melanoma, including *BRAF* V600E (7/21) and *BRAF* V600M (1/21). In more than one-third (10/26) of *BRAF* positive patients, good concordance in *BRAF* mutation status was found between paired samples. Discordance in *BRAF* mutation status was found in seven patients whom lymph node metastases were analysed. We identified 4 *NRAS* mutations in primary melanoma samples (n=10) and another 4 in lymph node samples 3 of which were detected in different than first four mutations patients.
In conclusion, our study identified frequency of *BRAF* and *NRAS* mutations in melanoma patients. We also determined that the association between lymph node metastasis and risk factors, such as the presence of the *BRAF* mutations in primary tumour sample in these patients, requires further investigation.

67. Effects of p62 Upregulation for Chemoresistant Colorectal Cancer Cells

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Colorectal cancer is one of the most common malignancies worldwide and the third leading cause of cancer related deaths. Acquired chemoresistance is one of the main causes that limit the efficiency of chemotherapeutic treatment. Autophagy is an important process for cell survival and death, it can promote cell survival after treatment with chemotherapeutic drugs or participate in autophagy dependent cell death. Moreover, autophagy is interconnected with most of cellular stress response pathways, including those controlling immune response and inflammation.

We have found that selective autophagy cargo receptor p62 is upregulated in cells chemoresistant to 5-fluorouracil. During autophagy p62 selects ubiquitin tagged cell components for degradation. Autophagy cargo receptors are degraded during selective autophagy, but if accumulated they can activate NF-κB and Nrf2 transcription factors, which can promote survival of cancer cells. The aim of this study was to evaluate the importance of p62 for chemoresistance and the expression of immune response molecules in colorectal cancer cells HCT116. This was achieved by diminishing p62 expression by siRNA. We have demonstrated that silencing of p62 does not affect cell viability, but it changes the levels of immune response molecules.

68. The in vitro evaluation of “Bystander” effect after Bleomycin electrotransfer

Neringa Barauskaitė

Cancer is a disease that brings a heavy financial and social burden to the society. Therefore, an extensive research has been done during the last few decades in order to perform efficient therapy on cancer patients. A conventional anticancer treatment therapies are immensely effective in treating cancer, however that is not enough. Therefore, alternative treatments have a potential to be exploited. One of such, is an electroporation driven cancer treatment therapy – electrochemotherapy. The process of electroporation occurs when external electric field charge the membrane to the extent of membrane breakdown, hence creating temporal hydrophilic pores in the membrane. Through those temporal hydrophilic pores small hydrophobic molecules (i.e. anticancer drug Bleomycin) diffuse into the cell. The entrance of Bleomycin induces multiple cleavage of genomic DNA, that in turn, leads to apoptotic cell death. The combination of anticancer drug and electroporation resulted in aforementioned electrochemotherapy. Such treatment is already widely used in clinics, however not all processes are well researched. Radiotherapy, as a well-known conventional cancer treatment has a so called "Bystander" effect. Cells that are not affected, but are close to the affected cells, die as a result of apoptosis. In this presented study we analyse the "Bystander" effect presence in the process of electrochemotherapy in vitro, since the effect of Bleomycin to genomic DNA is similarly damaged to the ROS generated in radiotherapy. The cell viability and permeability was evaluated on the cells that are affected and unaffected cells.

69. Drug resistance in Acute Myeloid Leukaemia

Zarin Abu

Acute Myeloid Leukaemia (AML) is a rare blood cancer involving haematopoietic stem cells (HSC) and is caused by the formation of abnormal immature cells in the bone marrow (BM). Reportedly, the risk of acquiring AML increases with age. Therefore, the older population is more susceptible to AML. Symptoms typically arise from BM failure, which drove scholars to investigate the role of the BM microenvironment in AML. Subsequently, many research papers exploring the role of BM components such as fibroblasts, endothelial cells, osteoblasts and many more. Other factors to be taken into consideration were pathways such as VEGF and the CXCL12-CXCL4 axis. Inhibiting or altering pathways is what led to AML treatments. For example, sorafenib, a drug currently used in AML, is a multikinase inhibitor against the VEGF receptor. The WASP protein has a major role in AML. This is because it is highly expressed in haematopoietic cells (HPC) and regulates cell migration in cells present in the myeloid lineage and expresses leukocytes. The most common treatments in AML are currently cytarabine and anthracycline for younger patients and daunorubicin and cytarabine for older patients. Therefore, for my dissertation, we will be exploring the mechanisms of drug resistance in AML. Our hypothesis is that WASP may contribute to the increased migratory phenotype of tumour cells in AML that result in the drug resistance to daunorubicin. Specifically, we will study the possible role of the nuclear localisation signal domain (NLS domain), the nuclear export signal 1 (NES1) domain or the VCA domain of WASP in regulating cell size and actin organisation, cell proliferation/cell cycle progression. This

will be performed with the AML cell line THP-1 devoid of endogenous WASP using CRISPR technology and then, re-expressing one of the following eGFP-WASP recombinant proteins. Conclusions cannot be predicted, as further research is required.

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