The COINS 2026

Life Sciences Conference

Book of abstracts

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KEYNOTE SPEAKERS

Victor de Lorenzo

Programming bacteria for microbiome interventions: the issues and the tools.

The soil bacterium Pseudomonas putida, specifically strain KT2440, has been the starting point for generation of a number of synthetic biology chassis capable of delivering biodegradative activities to contaminated sites with precise spatiotemporal control and/or creating smart consortia fortified in their catalytic properties. This process required a coherent and specialized molecular toolbox, the Standard European Vector Architecture (SEVA), which enables a range of genetic manipulations in environmental bacteria that were previously limited to E. coli and other model species. The often-observed detrimental consequences of extensive genome editing can be swiftly mitigated by subsequent laboratory experimental evolution, which helps restore robustness and normal growth parameters. This approach has led to the creation of surface-naked strains optimized for the ectopic display of nanobodies (and other adhesins), facilitating the assembly of microbial consortia and the engineering of monolayer bacterial skins on solid surfaces. One specific application of such surface-displayed nanobodies involves engineering bacteria to recognize specific surface antigens on target components of a microbiome, thereby depleting them from the community through the injection of a T6SS-delivered toxin. In scenarios where the introduction of a new bacterial member into a pre-existing community is hindered by colonization resistance, it remains possible to endow the microbiome with new traits by engineering HGT using a hyperpromiscuous plasmid derived from RP4.

Diethard Tautz

Genetic functions out of random sequences - what we can learn from the de novo evolution of genes?

Comparative genomic analyses have shown that genes can be created de novo from previously non-coding sequences in the genome. This mechanism has long been thought to be rare, but there are now well-studied cases from all domains of life which suggest that may be a rather frequent process. To better understand how easy it is to generate genetic functions out of random sequences, we have devised experiments in which we expressed random sequence peptides in E. coli and in eukaryotic cells and assessed their influence on the growth trajectories of the cells. We found in both types of experiments that a large fraction of random sequences has an effect on the growth, either negative or positive, supporting the notion that de novo evolution of genes is an evolutionary very active mechanism. I will also discuss what this means for the practical applications, especially for discovering peptides that are of biomedical relevance.

Inese Čakstina Dzerve

Functional precision oncology: methods and implementation in research.

Genomic precision oncology has brought powerful insights into cancer biology, but for many tumours – especially triple-negative breast cancer (TNBC) – DNA sequencing alone cannot tell us which therapies will work. Cancer behaviour under treatment is driven not only by mutations but by dynamic cellular states, stress responses, and survival programs that static molecular data simply cannot predict.

Functional precision medicine (FPM) tackles this challenge by directly measuring how cancer cells respond to perturbations. In this talk, I will focus on two of the most informative tools in the FPM toolkit: high-throughput drug sensitivity testing (DST) and basic and dynamic BH3 profiling (DBP). DST reveals phenotypic vulnerabilities by observing real-time viability under diverse compounds, while DBP measures mitochondrial apoptotic priming – and an early indicator of whether a celli s ready to die or determined to survive. Together, these approaches generate actionable, behaviour-based insights that sequencing alone cannot provide.

As an example, I will share findings from our project (HipTNBC) investigating how TNBC cell lines behave under hypoxia. Surprisingly, these cells maintain strong viability and exhibit low apoptotic priming in low-oxygen conditions. This resilience underscores why functional assays are essential: cancer cells can appear genetically vulnerable but remain phenotypically resistant.

By highlighting how FPM illuminate the true functional state of tumour cells, this keynote will show how integrating dynamic phenotypic measurements into precision oncology can improve therapy selection and help us outsmart cancers that thrive under stress.

Norbert Pardi

Nucleoside-modified mRNA: the past, present and future.

The development of nucleoside-modified mRNA was critical for the success of COVID-19 mRNA vaccines. Dr Pardi will tell about the most critical discoveries that led to the birth of mRNA vaccines. He will also provide information on the current standing of the field and the potential future directions that may result in more mRNA-based medicines for human use.

Joe Millard

Global biodiversity change in the age of the semiconductor: a past, a present, a future.

Life on Earth is experiencing a rapid period of change. Human activities such as land-use, climate change, and over-exploitation are causing a significant restructuring of biodiversity worldwide, likely on the scale of a mass extinction. In parallel, the semiconductive properties of silicon and the scalability of the transistor have led to a revolution in computing, ushering in a new age of scientific progress. This computer revolution has enabled new tools and ways of thinking in ecology, leading to a significant development in our understanding of ecosystems and global biodiversity change. In this talk, I will describe how my own work on global biodiversity change has developed over the past ~decade, as the scope of what's possible with a digital computer continues to increase. I will start with the development of R Shiny apps for processing data for the trade in wildlife, and then come to the present, describing how large multi-modal models represent a step-change for the synthesis of evidence in ecology and conservation. I will then look to the future, describing that combining distinct forms of foundational AI—particularly earth-system models and generative agent-based simulation—will likely unlock new forms of virtual ecology and the simulation of ecosystems in digital space.

Luke Rendell

Changing perspectives on human and cetacean cultures.

While human culture is a dominant ecological force on our planet, we are at a period where understanding of how cultural processes in non-humans is flowering, with little sign of slowing down. In this talk I will describe how Interactions between human and cetacean cultures have changed along with our understanding, focussed on sperm whale cultural variation and ending on an update on our most recent findings. Sperm whales are a key component of pelagic ecosystems, taking significant biomass from mesopelagic waters. They are often characterised as monotonous harvesters of deep water squid, but we are beginning to understand more about the complexity of their behaviour. As a number of long-term projects begin to mature, we are gaining new insights into how cultural processes influence how sperm whale populations are structured and how they use and partition their habitats. I will review these new results from Mediterranean, Caribbean and Pacific populations, and argue that appreciating this behavioural variation will be crucial in understanding their role in pelagic ecosystems.

Cecilia Nilsson

Animal Movements in the Aerial Habitat.

The air is a crucial habitat for a wide range of flying animals, including many birds, bats and insects. It is also important arena for a range of different behaviors, from long distance migration to foraging. However, the air is also difficult to monitor and therefor our understanding of the aerial habitat lags that of terrestrial and aquatic environments. Recent technological advances, including the expanded use of radar, increasingly show us how flying animals use the aerial habitat. This enables us to answer questions about the environmental patterns and ecological processes that shape animals' life in the air, map behaviors and identify areas where human/wildlife conflicts in the air occur. Humans are increasingly affecting the air, through high rising construction, manned and unmanned vehicles, and different types of pollution. By understanding how animals use the airspace we can better understand how these changes affect flying animals. I will discuss the aerial habitat and my work using radar to map animal occurrence and behavior in the air.

Ana Claudia Sima

On the opportunities of Large Language Models for scientific data exploration.

Generative AI and in particular Large Language Models have significantly impacted all areas of science, benefitting in particular data-intensive disciplines, such as bioinformatics. However, Large Language Models – while very powerful – are still imperfect tools, due to hallucinations, knowledge cut-off times and training costs. On the other hand, the rich ecosystem of structured data collected and curated over time by scientific groups can be leveraged as a natural complement to existing LLMs, mitigating their limitations.

In this talk, I will highlight some of the applications of LLMs in bioinformatics, as well as a few of the services developed by the Knowledge Representation Unit at the SIB that leverage LLMs for scientific data exploration. In particular, I will introduce ExpasyGPT (https://www.expasy.org/chat), an open-source platform that enables precise question answering over structured, multi-disciplinary bioinformatics data.

Thomas Hamelryck

AlphaFold’s Bayesian Roots in Probability Kinematics.

The developers of AlphaFold received the Nobel Prize in Chemistry in 2024 for solving the protein folding problem. The journey to this solution began in 2018 with AlphaFold’s first incarnation, when CASP13 (the 13th edition of a biennial competition benchmarking protein structure prediction accuracy) reported “unprecedented progress.” The successors AlphaFold2 (2020) and -3 (2024) delivered increased accuracy and scope of application. However, in contrast to the pragmatic deep learning pipelines of its two successors, AlphaFold1 can be interpreted as a probabilistic model based on a little-known generalization of Bayesian belief updating called Jeffrey conditioning or probability kinematics. Beyond clarifying the epistemic foundations of a paradigm-shifting method in life science and machine learning, probability kinematics offers a principled foundation for building compositional deep probabilistic models based on generalized Bayesian updating, delivering predictions with well-calibrated uncertainties.

Edwin Cuppen

Cancer whole genome sequencing data analysis and mining for improving care outcomes.

Advances in next-generation sequencing technologies now enable whole genome characterization of tumor genomes. This provides unique opportunities for data mining approaches to advance our knowledge on tumor biology and etiology, but also for personalization of cancer treatments. I will talk about the latest developments in sequencing technology, the routine application for cancer diagnostics and state-of-the-art bioinformatic data analyses on a large cohort of more than 7,000 patients, including machine learning for the identification of the tissue of origin for cancers of unknown primary.

SHORT TALKS

Antimicrobial Resistance: From a Global Threat to Laboratory Solutions

Deimantė Tiškevičiūtė

Linea libera
d.tiskeviciute@linealibera.lt

Antimicrobial resistance (AMR) is recognized as one of the most significant global public health threats of the 21st century. Increasing antibiotic consumption, together with the limited development of new antibiotic classes, has accelerated the emergence and spread of resistant bacterial pathogens [1]. In response to the growing AMR burden, the European Union has established targets aimed at reducing antibiotic consumption and limiting the spread of resistant infections by 2030. Achieving these goals requires not only responsible antibiotic use but also reliable laboratory diagnostics capable of accurately detecting resistant microorganisms [2].

Clinical microbiology laboratories play a crucial role in monitoring antimicrobial resistance and guiding appropriate antibiotic therapy. Antimicrobial susceptibility testing (AST), including determination of the minimum inhibitory concentration (MIC), remains a key method for assessing bacterial susceptibility according to standardized guidelines such as those provided by EUCAST.

This presentation will provide an overview of current global and European trends in antibiotic consumption and antimicrobial resistance. Particular attention will be given to laboratory approaches used for resistance detection, including MIC determination and diagnostic solutions for detecting resistantv bacteria.

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Direct bacteriophage suspension sequencing using Oxford Nanopore

Jonas Juozapaitis[1] , Rūta Bajorinaitė[1] , Ema Ežerskytė[1] , Miglė Plioplytė[1] , Goda Grigorianaitė[1] , Dovilė Juozapaitė[1] , Agnė Čiukšytė[1]

[1]UAB SeqVision, Budiniškių 3-9, Vilnius
jonas@seqvision.com

Bacteriophage genome characterization is a critical step in phage research and phage therapy development. Conventional sequencing workflows rely on phage DNA extraction procedures that are time-consuming and extend the overall turnaround time to several days. We have developed a proprietary, extraction-free solution for complete bacteriophage genome sequencing directly from phage suspension using Oxford Nanopore Technology (ONT).

Our approach eliminates the DNA extraction step entirely, streamlining sample preparation while preserving genomic integrity. Through our optimized workflow, phage suspension is processed directly into a sequencing-ready library, enabling complete, high-quality phage genomes to be obtained within 12 to 24 hours from raw suspension. This is a substantial reduction in turnaround time compared to standard methods.

This solution was validated across multiple bacteriophage isolates, consistently yielding complete genome assemblies with sufficient depth and accuracy for downstream analyses including genome annotation. The workflow is designed for reliability and reproducibility across laboratory settings, with particular relevance for time-sensitive applications such as phage therapy and mutant phage characterisation. Our results establish this technology as a powerful and efficient platform for phage genomics, enabling faster, smarter decisions across research and applied contexts.

A Brief Introduction To Advanced Tissue Modeling Enabled by Multi-Material 3D Bioprinting

Lidija Marija Smertinaitė[1]

[1]Laborama UAB, Lithuania
lidija.smertinaite@laborama.lt

Three-dimensional (3D) bioprinting is rapidly developing as a powerful technology for the creation of novel in vitro tissue models. In contrast to conventional two-dimensional cell cultures and animal models, bioprinted constructs allow the spatially controlled arrangement of multiple cell types, biomaterials, and growth factors within defined three-dimensional architectures. [1] Bioprinting can thus recreate key aspects of human tissue microenvironments, including cell–cell interactions, extracellular matrix composition, and structural organization in reproducible and highly customizable systems [2, 3]. By enabling more physiologically representative model systems, 3D bioprinting technology advances the ongoing development of increasingly accurate experimental models in many fields of research, including drug screening, disease modeling, or transplant development, all while supporting efforts to decrease the use of animal models. [4, 5] Continuous progress in bioink, printing strategy, and multi-material bioprinting platform development have only further expanded the range of potential inherent in this application and helped bridge the gap between conventional in vitro models and human biology.

This talk highlights recent developments in 3D bioprinting approaches used to fabricate advanced tissue models and examines their potential to improve ethically sustainable biomedical research workflows that support the transition toward models more reflective of human biology.

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Streamlined Ambient Shipping of Taq and High-Fidelity Polymerases: Reducing Carbon Footprint Without Compromising Performance

Agnieška Mackoit-Končiuvienė[1], Aistė Serapinaitė[1], Rasa Sukackaitė[1], Ernestas Navardauskas[1], Daiva Tumaitienė[1]

[1]Thermo Fisher Scientific Baltics, Lithuania
Email: agnieska.mackoit@thermofisher.com

Climate change and environmental sustainability are no longer peripheral concerns but central drivers shaping innovation, supply chains, and decision-making across the life sciences industry.

European sustainability regulations are rapidly evolving, introducing stricter requirements on chemical safety, packaging recyclability, transport emissions, and eco-design obligations. In parallel, life science customers increasingly expect suppliers to provide solutions that reduce environmental impact without sacrificing product performance, quality or regulatory compliance.

At Thermo Fisher Scientific, sustainability is embedded in our product development strategy through our Design for Sustainability framework and the Greener by Design™ [1] program. We recognize that the greatest opportunity to reduce environmental impact occurs at the earliest stages of product design — from raw material selection, manufacturing processes to packaging and logistics considerations.

Historically, molecular biology enzymes such as Invitrogen™ Platinum™ II Taq Hot-Start Polymerase and Thermo Scientific™ Phusion™ Plus DNA Polymerase were shipped under cold-chain conditions using dry ice and EPS (Expanded Polysterene) packaging. While effective, this approach increased logistical complexity, carbon emissions, and compliance risks related to temperature excursions.

To address these challenges, we evaluated the feasibility of ambient shipping without compromising quality. Based on these studies, selected Taq and high-fidelity polymerases can now be shipped at ambient temperature while maintaining equivalent performance and stability.

This initiative demonstrates that sustainability and scientific performance are not mutually exclusive. By removing cold-chain requirements for shipping of selected enzymes, we reduce environmental impact while delivering the same reliability customers expect.

This case study illustrates how evidence-based product shipment conditions can align regulatory compliance, customer expectations, and environmental responsibility — without compromising assay performance.

Multiomic insights into complex biology of VCI dementia

Karolina Žukauskienė[1], Milda Milčiūtė[1], Sangeetha Vishweswaraiah[2,3], Nadia Ashrafi[2,3], Romana Mimi[2,3], Nazia Saiyed[2,3], Abdullah Khalid[2], Ceyda Buyuker[4], Boran Aksakal[4], Lorena Gomes[2], Robert T. Brooke[5], Migle Gabrielaite[1], Ingrida Olendraitė[1], Vilija Lomeikaitė[1], Juozas Gordevičius[1], Ali Yilmaz[2,3], Stewart F. Graham[2,3]

[1]VUGENE, Vilnius, Lithuania
[2]Corewell Health Research Institute, Royal Oak, MI 48073, USA
[3]Oakland University-William Beaumont School of Medicine, Rochester, MI 48309, USA
[4]School of Medicine, Acibadem Mehmet Ali Aydinlar University, Istanbul, Turkey
[5]Epigenetic Clock Development Foundation, Torrance, California, USA
karolina@vugene.com

Vascular cognitive impairment (VCI) and dementia (VCID) represent a spectrum of cognitive disorders linked to cerebrovascular pathology. While VCI accounts for 20-40% of dementia cases, its complex molecular mechanisms and systemic understanding remain elusive. While metabolomics is frequently essential in characterizing disease-associated biochemical changes, traditional single-omics approaches have limitations in capturing the comprehensive VCI biology landscape. Multiomics integration bridges the gap between genotype and phenotype, offering a powerful framework to uncover interconnected molecular pathways and their impact on VCI pathogenesis.

In this study, we combined knowledge from genomic, epigenomic, transcriptomic and metabolomic landscapes enabling deeper and more comprehensive understanding of the disease. The analysis was performed on Brodmann area 7 brain tissue samples from 19 VCI cases and 21 cognitively normal controls. We have analysed each omic separately (through linear modelling and pathway enrichment analysis) and combined them into multiomic systematic interaction maps.

Multiomics integration revealed widespread molecular disruptions in VCI (Fig. 1), with significant shifts in association networks. Genomic analysis pointed to the Rac GTPase pathway as a key player in VCI, with epigenetic changes disrupting the expression and splicing of related genes. Transcriptomic data showed disruptions in lipid metabolism, oxidative stress, and GTPase activity, while metabo-epigenomic analysis linked Rac GTPase dysregulation to altered diacylglycerol and phosphatidylethanolamine metabolism. These findings provide new insights into the complex interactions driving VCI pathophysiology.

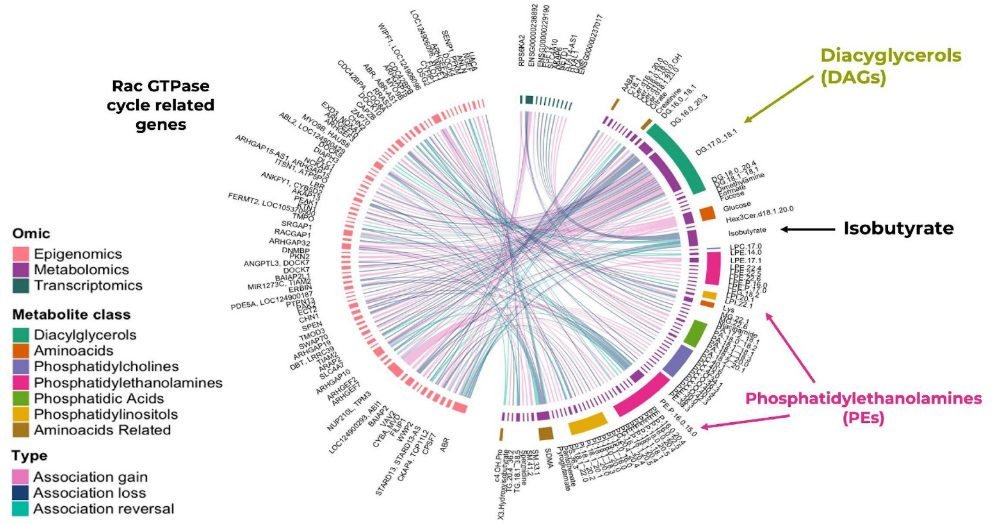


Figure 1. The multiomics circos plot of the Rac GTPase-driven methylation, transcripts and metabolites.

These findings emphasize the power of multiomics integration in revealing complex molecular interactions and metabolic alterations in VCI that remain hidden in single-omics studies. By uncovering Rac GTPase-mediated lipid dysregulation, this study provides novel insights into VCI pathogenesis, paving the way for biomarker discovery and potential therapeutic strategies.

GENETICS

SHORT PROKARYOTIC ARGONAUTES PROVIDE DEFENCE AGAINST MOBILE GENETIC ELEMENTS THROUGH NAD$^+$ DEPLETION

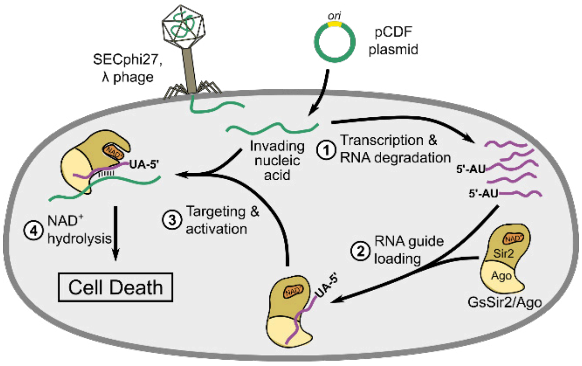
Donata Dakineviciene[1], Mindaugas Zaremba[1], Edvardas Golovinas[1], Evelina Zagorskaite[1], Edvinas Stankunas[1,3], Anna Lopatina[2,4], Rotem Sorek[2], Elena Manakova[1], Audrone Ruksenaite[1], Arunas Silanskas[1], Algirdas Grybauskas[1], Edvinas Jurgelaitis[1] and Virginijus Siksnys[1]

[1]Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
[2]Department of Molecular Genetics, Weizmann Institute of Science, Rehovot, Israel
[3]Present address: Max Perutz Labs, Medical University of Vienna, Vienna Biocenter Campus (VBC), Vienna, Austria
[4]Present address: Division of Microbial Ecology, Center for Microbiology and Environmental Systems Science, University of Vienna, Vienna, Austria
donata.tuminauskaite@gmc.vu.lt

Argonaute (Ago) proteins are present in all domains of life. In eukaryotes, they form the core of RNA silencing pathway by using small RNAs as guides to identify and repress target RNAs [1-2]. Prokaryotic Argonaute proteins (pAgos) show far greater diversity than their eukaryotic counterparts (eAgo). Based on their domain architecture, pAgos are divided into full-length or long pAgos (~40%) sharing conserved N, PAZ, MID and PIWI domain architecture with eAgos, and short pAgos (~60%) composed only of MID and catalytically inactive PIWI domains [3]. Short pAgos are typically organized in operons alongside various effector genes, whose products feature not only an APAZ domain but also distinct effector modules such as Sir2 (SPARSA), TIR (SPARTA), or Mrr (SPARMA). About half of all identified short pAgos are associated with or fused into a single-chain protein with Sir2-APAZ proteins [3]. The Sir2 domain-containing proteins are widely distributed and perform protein deacetylation or ADP-ribosylation functions using NAD$^+$ as a co-factor [4].

Despite increasing insights, many aspects of pAgo mechanisms and their potential cellular roles remain to be elucidated. Here, we aimed to explore whether short pAgos SPARSA system can act as prokaryotic defence system against viruses or plasmids [5]. We selected two short pAgos SPARSA systems, GsSir2/Ago from *Geobacter sulfurreducens* and CcSir2/Ago from *Caballeronia cordobensis*, and expressed them heterologously in *E. coli* cells by using pBAD vectors. Following protein expression and purification by liquid chromatography, we showed that GsAgo forms a stable heterodimeric complex with NAD$^+$-bound Sir2 using SEC-MALS and SAXS. Phage restriction (plaque and liquid culture) and plasmid interference assays indicated that the GsSir2/Ago complex detects invading DNA, triggering activation of the Sir2 subunit. This activation leads to intracellular NAD$^+$ depletion, measured by mass spectrometry in cell lysates, resulting in cell death and inhibition of foreign DNA propagation (Fig. 1). We further reconstituted NAD$^+$ depletion in vitro and demonstrated that the activated GsSir2/Ago complex exhibits NADase activity, hydrolyzing NAD$^+$ into ADPR (measured by a colorimetric NAD/NADH assay). These findings indicate that short Sir2-associated pAgos serve as a defense mechanism against phages and plasmids, highlighting the mechanistic diversity of pAgo systems.

Figure 1. Putative model of GsSir2/Ago defence against mobile genetic elements.



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A2

DIAGNOSTIC SIGNIFICANCE OF *KIF2C*, *KIF14* AND *KIF20A* EXPRESSION IN OVARIAN CANCER

Areta Petrauskaitė[1], Ieva Vaicekauskaitė[1,2], Rūta Čiurlienė[3], Rasa Sabaliauskaitė[1,2]

[1]Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]National Cancer Institute, Vilnius, Lithuania
[3]National Cancer Center, Vilnius, Lithuania
areta.petrauskaite@gmc.stud.vu.lt

Ovarian cancer is the third most prevalent gynecologic malignancy and represents the second leading cause of death among oncologic diseases worldwide [1]. More than 60% of cases are diagnosed at an advanced stage, largely due to the absence of effective early diagnostic tools and the disease's rapid progression, resulting in a five-year survival rate of approximately 45% [2]. Currently, serum cancer antigen 125 (CA 125) is the most commonly applied biomarker in clinical practice, but its diagnostic accuracy is limited by insufficient sensitivity and specificity [3]. The kinesin superfamily consists of conserved, microtubule-dependent motor proteins involved in cell cycle regulation [4]. Given their roles in mitosis and potential links to tumorigenesis, three kinesin genes, *KIF2C*, *KIF14* and *KIF20A*, were investigated as candidate diagnostic biomarkers by analyzing their expression and association with clinical features of ovarian cancer patients.

Reverse transcription quantitative PCR (RT-qPCR) was used to quantify the expression of selected genes in 65 ovarian cancer samples grouped into three histological groups (benign gynecological tumors, high-grade serous carcinomas and other malignant tumors). Statistical analyses assessed correlations between gene expression and patient clinical features, and diagnostic performance was evaluated via receiver operating characteristic (ROC) curve analysis.

Evaluation of gene expression identified significant differences among the study groups, with *KIF2C*, *KIF14*, and *KIF20A* showing higher levels in high-grade serous carcinoma compared with benign gynecological tumors (*KIF2C*, = 0,03; *KIF14*, p = 0,001; *KIF20A*, p = 0,02). ROC analysis demonstrated good discriminatory ability, with areas under the curve of 0,77, 0,91, and 0,82, respectively. These results suggest these genes may serve as potential diagnostic and prognostic biomarkers for ovarian cancer.

In summary, *KIF2C*, *KIF14* and *KIF20A* may represent promising diagnostic and prognostic candidates for ovarian cancer. Variations in their expression levels appear to be associated with tumor histological subtype and could help distinguish malignant from benign samples, as indicated by ROC analysis. Further studies are needed to validate these findings and determine their potential clinical utility.

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A3

SRA GENE FUNCTION IDENTIFICATION IN BARLEY, USING TARGETED MUTAGENESIS

Miglė Remeikytė, Vėjūnė Pukenytė, Eglė Liugaitė, Raimondas Šiukšta

Institute of Biosciences, Life Sciences Centre, Vilnius University, Saulėtekis Ave. 7, LT-10257, Vilnius, Lithuania
migle.remeikyte@gmc.vu.lt

Barley (*Hordeum vulgare*) is the fourth most widely cultivated cereal crop worldwide and serves as an important model for genetic research. [1]. Barley yield is strongly affected by inflorescence architecture, yet many genes regulating flowering remain functionally uncharacterized [2]. *HvSRA* (*SISTER OF RAMOSA3*) gene, a paralog of maize *RA3*, is involved in barley spike development, but its precise function is still unknown [3].

In this study, CRISPR-Cas9-mediated gene knockout was used to investigate the role of *HvSRA* in the barley cultivar 'Golden Promise'. Initially, two binary T-DNA vectors containing a hygromycin resistance cassette, cas9 cassette and two guide RNAs were constructed and cloned in *Escherichia coli* bacteria. After verification by agarose gel electrophoresis and sequencing, the constructs were introduced into *Agrobacterium tumefaciens* and used for T-DNA-mediated transformation of immature barley embryos. After inoculation, embryos underwent callus induction, selection, and regeneration, resulting in twelve independent transgenic T_0 plants. Genomic DNA was extracted from leaf tissue of regenerated plants, and Sanger sequencing of the *HvSRA* target region was performed for ten T_0 lines. Sequence analysis revealed heterozygous mutations in three plants, as indicated by overlapping peaks in sequencing chromatograms. One mutant exhibits branched spike phenotype while the remaining are yet to develop inflorescences. Although the precise mutation types could not be determined at this stage, these results confirm successful targeted editing of *HvSRA*.

Future work will be focused on verifying targeted mutations in the remaining T_0 plants via sequencing and phenotyping, as well as obtaining homozygous *HvSRA* mutants free of T-DNA through recombination.

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ROLE OF EXTRACELLULAR PROTEASES IN THE PATHOGENESIS OF THE OPPORTUNISTIC PATHOGEN *STENOTROPHOMONAS MALTOPHILIA*

Giedre Kukanauskaite, Laurita Klimkaite, Julija Armalyte

Department of Biochemistry and Molecular Biology, Vilnius University
giedre.kukanauskaite@gmc.stud.vu.lt

Stenotrophomonas maltophilia is a gram-negative opportunistic pathogen responsible for hospital-acquired infections among immunocompromised patients worldwide [1]. One of the key mechanisms underlying *S. maltophilia* pathogenicity is the secretion of extracellular virulence factors, including serine proteases StmPr1 and StmPr2. These proteases have been shown to proteolytically degrade essential host structural components, thereby disrupting epithelial barrier integrity and inducing anoikis-cell death [2]. In addition, our preliminary studies have demonstrated that StmPr2 can proteolytically degrade the main component of the human innate immune system, complement protein C3. However, other secreted serine proteases, such as StmPr1, could also contribute to C3 degradation. Furthermore, the specific hydrolysis patterns and regions of C3 targeted by *S. maltophilia*-secreted proteases have not been comprehensively characterized. Therefore, the aim of this study was to generate *stmpr1* and *stmpr2* gene deletion mutants to investigate the role of protease StmPr2 in the degradation of the complement system protein C3 and to identify the regions of C3 hydrolysis following exposure to proteins secreted by *S. maltophilia*.

Methods: Deletion of the *stmpr1* and *stmpr2* genes in a clinical *S. maltophilia* strain was attempted using a double homologous recombination method. Deletion plasmids were constructed using a pUC19_*sacB*-based vector and verified by PCR amplification and restriction enzyme digest analysis. Then, the verified plasmids were transformed into *S. maltophilia* cells to promote homologous recombination for targeted gene deletion. Recombinant clones were screened by colony PCR and agarose gel electrophoresis to verify homologous recombination events. In parallel, to characterize the specific hydrolysis regions of the human complement protein C3, purified C3 was incubated with cell- free culture supernatant containing extracellular proteins secreted by *S. maltophilia*. The resulting C3 degradation products were analyzed by SDS-PAGE, and selected fragments were further examined by mass spectrometry (MS) in the Proteomics Core Facility (EMBL, Germany) to identify regions of proteolytic cleavage of the C3 protein.

Results: Deletion plasmids targeting the *stmpr1* and *stmpr2* genes were successfully constructed. Following transformation into *S. maltophilia* cells, recombinant clones were screened by colony PCR and agarose gel electrophoresis. However, homologous recombination events leading to plasmid integration and precise gene deletion were not detected under the tested conditions. In parallel, incubation of purified human complement protein C3 with cell-free culture supernatant containing proteins secreted by *S. maltophilia* resulted in the formation of several degradation products, as detected by SDS-PAGE gel. MS analysis of selected fragments enabled mapping of identified peptides onto the C3 protein sequence, revealing two putative regions of proteolytic cleavage. Further experiments are needed to identify the precise cleavage sites of the C3 protein.

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GENETIC DIVERSITY ANALYSIS OF THE GENUS *MYRIOPHYLLUM* IN DIFFERENT TYPES OF LITHUANIAN WATER BODIES

Vainius Jacys[1], Jurgita Butkuvienė[1,2]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Saulėtekio Ave. 7, LT-10257 Vilnius, Lithuania
[2]Vilnius University, Botanical Garden of Vilnius University, Kairėnų St. 43, LT-10239 Vilnius, Lithuania
vainius.jacys@gmc.stud.vu.lt

Watermilfoils (genus *Myriophyllum* L.) are a group of aquatic plants that belong to the *Haloragaceae* family, one of the most polymorphic plant taxa. *Myriophyllum* is a species-rich genus with species distributed worldwide [1]. They are characterized by their phenotypic plasticity and local adaptation, which create difficulties with morphological species identification [2]. Interspecific hybridization is also common in this genus and can promote rapid expansion to new habitats, causing significant ecological changes. Studies have noticed that *Myriophyllum spicatum* can hybridize with *Myriophyllum sibiricum*. The resulting hybrids tend to be more competitive and have a broader ecological amplitude, including faster vegetative proliferation [3]. This factor is very important and likely has contributed to a rapid increase in watermilfoils in Lithuanian water bodies, especially smaller ones such as ponds. However, genetic diversity studies of the genus in Lithuania are still limited.

This study evaluated the genetic diversity of *Myriophyllum* populations from eight water bodies: ponds (Šilalė, Gudeliai, Kuršėnai), rivers (Širvinta), and lakes (Rūžas, Balžis, Šventas, Kampiniškis). DNA was extracted from fresh plant material using a modified CTAB method. ISSR-PCR was performed using seven selected primers, and genetic variation was analysed using band scoring.

The selected ISSR primers revealed high levels of polymorphism (>90%). Evaluation of the bands showed that genetic diversity differed among the populations. The Šilalė pond had the least diversity among the analysed populations, while lake Šventas was the most genetically diverse with the highest expected heterozygosity. AMOVA results showed that more genetic variation (57 %) was observed among populations than within populations. Principal coordinate analysis indicated four distinct groups. Most individuals clustered by population, although several genetically distinct individuals were observed, suggesting the potential presence of hybrids.

The results demonstrate genetic differentiation among Lithuanian *Myriophyllum* populations. However, to precisely distinguish the species and potential hybrids additional research is needed. Sequencing of nuclear and chloroplast DNA regions would help with species identification and confirm hybrid individuals.

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A6

EXPRESSION ANALYSIS OF HIPPO SIGNALING PATHWAY COMPONENTS IN OVARIAN CANCER

Kornelia Gurska[2], Rūta Čiurlienė[3], Rasa Sabaliauskaitė[1,2], Ieva Vaicekauskaitė[1,2]

[1] National Cancer Institute, Vilnius, Lithuania
[2] Institute of Biosciences, Life Science Center, Vilnius, Lithuania
[3] National Cancer Center, Vilnius University Hospital Santaros Clinics, Vilnius, Lithuania
kornelia.gurska@gmc.stud.vu.lt

Ovarian cancer is the second most common cancer of the female reproductive organs, second to endometrial cancer [1]. It is associated with a poor survival rate due to its non-specific symptoms and lack of early-stage diagnostic tools. The deadliest subtype of ovarian cancer is high-grade serous ovarian carcinoma (HGSOC), which occurs at 90 % of ovarian malignancies [1].

Understanding the molecular mechanism and the role of the genetic factors in the development of cancer is crucial to define new diagnostic tools. Studies indicate that Hippo signaling pathway, which regulates organ size control, development and regeneration, plays an important role in governing ovarian physiology, fertility, and pathology [2]. The main Hippo factors are four protein kinases YAP, TAZ, MST1/2, LATS1/2, which control cell proliferation by regulating gene expression. Disregulation of this pathway contributes to uncontrolled cell proliferation and tumour progression.

The aim of this research was to evaluate the expression of *YAP1*, *MST1*, *TAZ* and *LATS1* genes in HGSOC samples by comparing it with benign and other gynecological malignancies. We extracted RNA from 42 HGSOC, 14 other and 9 benign tissue samples. The gene expression was analyzed by preparing an RT-qPCR with a reference gene *GAPDH*.

Our investigation showed that genes *YAP1*, *MST1*, *TAZ*, *LATS1* are downregulated in HGSOC and other gynecological cancer samples ($p<0.05$) compared to benign gynecologic tumors. Furthermore, the expression of *TAZ*, *MST1* and *LATS1* decreases as the disease progresses ($p<0.05$) in stage. Also, ROC curve analysis when comparing HGSOC and benign cases showed an AUC of 0.94 for *TAZ*, 0.89 for *LATS1* with a high sensitivity and specificity.

Our research suggests that the components of Hippo signaling pathway would be considered as a potential biomarker for ovarian cancer diagnosis. Further research is needed to validate this suggestion.

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Barley *TPS1* gene knock-out creation using CRISPR – Cas9 system

Eglė Liugaitė, Vėjūnė Pukenytė, Bernadeta Kaminskaitė, Miglė Remeikytė, Raimondas Šiukšta

Institute of Biosciences, Life Sciences Center, Vilnius University, Saulėtekis ave. 7, LT-10257 Vilnius, Lithuania
egle.liugaite@gmc.stud.vu.lt

Barley is the fourth most important agricultural crop and is often used as a model organism for genetic research due to its relatively small diploid genome compared to polyploid species like wheat and rye [1]. As environmental challenges such as climate change intensify, developing barley varieties with enhanced stress tolerance and higher productivity is critical to ensure global food security and agricultural sustainability [1]. This study focuses on modifying the *HvTPS1* gene of barley "Golden Promise" variety using CRISPR – Cas9 system to create a gene knockout. This gene encodes an enzyme involved in trehalose-6-phosphate (T6P) synthesis, which plays a crucial role in sugar metabolism and heat stress responses [2]. It is also thought to influence branching and spike architecture, making it a valuable target for genetic improvement [3]. Little is known about the molecular basis of cereal inflorescence architecture, so studies like this will improve knowledge about inflorescence genetic development pathways, by exporting gene – phenotype relationships.

Previously, a construct was created containing a plasmid with Cas9, hygromycin resistance genes (used for transformed plant selection) and two single-stranded guide RNAs containing 20-nucleotide target sequences. Immature barley embryos were isolated by detaching the seed coat and embryonic axis. Each embryo was inoculated with a drop of the prepared *Agrobacterium* suspension, containing the construct for *HvTPS1* gene knockout. After callus induction 4 T_0 plants were regenerated, DNA sequencing analysis indicated that one regenerated plant carried a frameshift mutation in a heterozygous position. Seeds were collected from all four plants and sown in soil. In total, 31 T_1 generation plants were obtained, and DNA sequencing revealed that 10 plants had no mutation, 12 had heterozygous mutations and 9 were homozygous mutants. Morphometric measurements were performed for all plants and compared with the control group. Growth analysis during the first 14 weeks showed no significant differences between groups. These observations suggest that the *HvTPS1* gene does not have a notable effect on barley height or germination time. In the future, the knockouts will be phenotyped and analysed under stress conditions to investigate the impact of the *HvTPS1* gene on stress response, spike architecture and barley development.

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EFFECTS OF GRNA STRUCTURE ON ASCAS12A ACTIVITY IN E. COLI

Gabija Magelinskaitė[1], Jonas Kubilius[1], John P. Cooper[1], Stephen K. Jones Jr. [1]

[1]Life Sciences Center EMBL Partnership Institute, Vilnius University, Lithuania
gabija.magelinskaite@gmc.stud.vu.lt

CRISPR–Cas-based genome editing displays significant variability in editing efficiency across different targets. We hypothesise that tailoring the entire guide RNA (gRNA) sequence for each target can yield more predictable and stable editing outcomes. Testing this hypothesis requires not only the construction of large and diverse gRNA plasmid libraries, but also functional assays that report on gRNA-dependent cleavage efficiency at scale.

Here, we investigate gRNA activity using an *Escherichia coli* cleavage assay, comparing an original gRNA plasmid library to libraries subjected to Cas-mediated cleavage under induced and non-induced conditions. Cas expression was controlled by IPTG induction during bacterial growth, enabling direct comparison of cleavage-dependent library depletion. Following library propagation, plasmid populations were analysed by high-throughput sequencing to quantify changes in gRNA representation. Overall, our workflow provides a scalable strategy for linking gRNA sequence variation to cleavage outcomes, supporting the development of more predictable CRISPR guide design rules.

A9

ANALYSIS OF THE BARLEY *NUD* GENE IN ABIOTIC STRESS ADAPTATION USING TARGETED MUTAGENESIS

Žygimantas Vareikis, Bernadeta Kaminskaitė, Vėjūnė Pukenytė, Raimondas Šiukšta

Institute of Biosciences, Life Sciences Center, Vilnius University, Saulėtekis ave. 7, LT-10257 Vilnius, Lithuania
zygimantas.vareikis@gmc.stud.vu.lt

Since its domestication in the Fertile Crescent nearly 10,000 years ago [1], barley (*Hordeum vulgare* L.) has played a major role in shaping agriculture. It has a well-established role in human nutrition, and now it ranks as the fourth most widely cultivated cereal crop worldwide [2]. However, the changing environment creates a need for the development of improved barley varieties. Some barley varieties, such as hull-less barley, found in mountainous regions of Asia, possess advantageous traits, including an easily detachable hull and improved nutritional value [3]. One major genetic determinant of this phenotype is loss-of-function of the *Nud* gene, which encodes an AP2/ERF protein; when mutated, *Nud* fails to form the cementing layer between the pericarp and the lemma and palea, causing the hull to detach easily from the grain. Although hull-less barley variety is adapted to harsher environments typical of mountainous regions, some laboratory studies report increased susceptibility to abiotic stress in this variety [4]. The precise role of the *Nud* gene in conferring resistance to abiotic stress therefore remains unclear.

To investigate this potential trade-off, a *Nud* knockout population is being generated in the barley cultivar ‘Golden Promise’ using CRISPR/Cas9 technology and *Agrobacterium tumefaciens*-mediated transformation. A CRISPR/Cas9 system containing hygromycin resistance genes and *Nud*-specific guide RNAs was assembled and introduced into *A. tumefaciens*. Immature barley embryos were inoculated and cultured on callus induction and regeneration media, and putative transgenic tissues are currently undergoing plant regeneration.

Ongoing work includes plant regeneration and molecular verification of *Nud* gene inactivation by DNA sequencing. Confirmed knockout lines will be evaluated under abiotic stress conditions to assess the impact of *Nud* loss on plant growth and stress tolerance. This study aims to clarify the role of *Nud* in stress adaptation and to inform future barley improvement strategies under changing environmental conditions.

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A10

THE EFFECT OF THE *AP2L5* (*APETALA2-LIKE*) GENE ON INFLORESCENCE MORPHOLOGY IN BARLEY

Gabija Vėlavičiūtė, Vėjūnė Pukenytė, Bernadeta Kaminskaitė, Raimondas Šiukšta
Institute of Biosciences, Life Sciences Centre, Vilnius University, Saulėtekis Ave. 7, Vilnius, Lithuania.
gabija.velaviciute@gmc.stud.vu.lt

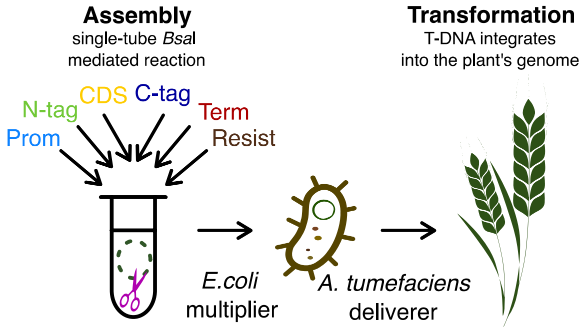
Barley (*Hordeum vulgare* L.) is a widely cultivated cereal crop and an important component of the human diet, particularly in developing countries. Due to its rich nutritional profile, including dietary fiber, complex carbohydrates, vitamins and minerals, barley makes a significant contribution to global food security. In addition, it is extensively used as livestock feed and as a primary raw material for malting and alcoholic beverage production [1]. Owing to its agronomic importance and well-characterized genome, barley serves as an attractive model for genetic and developmental studies [2]. One of the key traits influencing cereal yield is inflorescence architecture, however, the molecular mechanisms regulating inflorescence development are still not fully understood.

In barley, the *APETALA2-like* gene (*HvAP2L5*) plays a central role in the regulation of spike architecture and development [3]. However, *AP2L* genes have been more extensively characterized in wheat. Loss-of-function of both *AP2L2* and its paralog *AP2L5* converts lemmas into glume-like organs, eliminates or transforms lodicules. In contrast, mutations that make *AP2L2* resistant to miR172 produce shorter and more compact spikes [4]. The aim of this study is to investigate the effects of altered *AP2L5* expression on barley inflorescence morphology using the *GreenGate* vector system and to determine the expression pattern of *AP2L5* using molecular markers.

For this study, internal *BsaI* sites in the *AP2L5* promoter and coding sequence were removed by introducing silent mutations. This step ensures sequence compatibility with the *GreenGate* cloning system. Afterwards, twelve target plasmids were assembled with different combinations of markers, promoters and coding sequences. The assembled constructs were verified by restriction analysis and *Oxford Nanopore* sequencing, when transformed into *A. tumefaciens*.

Barley immature embryos were isolated and inoculated with *A. tumefaciens* containing assembled constructs. Following inoculation, callus induction and selection, transformed plants are currently regenerating. Subsequently, molecular and functional analyses of the regenerated plants will be conducted to evaluate the role of *AP2L5* in inflorescence architecture development.

Figure 1. A schematic overview of the complete experimental workflow.



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A11

LINE-1 CpG 328 METHYLATION AND ORF2 EXPRESSION AS POTENTIAL BIOMARKERS FOR COLORECTAL CANCER DIAGNOSIS AND PROGRESION

Ignas Dailidė[1], Rima Miknaitė[1], Inga Kildušienė[2,3], Rytė Rynkevičienė[4], Kęstutis Sužiedėlis[1,4], Giedrė Smailytė[2,5]

[1]Life Sciences Center, Vilnius University, Lithuania
[2]Laboratory of Cancer Epidemiology, National Cancer Institute, Lithuania
[3]Department of Abdominal and General Surgery and Oncology, National Cancer Institute, Lithuania
[4]Laboratory of Molecular Oncology, National Cancer Institute, Lithuania
[5]Department of Public Health, Institute of Health Sciences, Vilnius University, Lithuania
ignas.dailide@gmc.stud.vu.lt
rima.miknaite@gmc.stud.vu.lt

Colorectal cancer (CRC) is the third most common cancer worldwide, with more than 1.15 million new cases and 576,000 deaths annually [1]. CRC develops through a multistep process driven by genetic and epigenetic alterations, primarily via three pathways: chromosomal instability (CIN, ~85%), microsatellite instability (MSI, ~15%), and CpG island methylator phenotype (CIMP, ~25%) [2].

Long interspersed nucleotide element-1 (LINE-1), the most abundant repetitive element in the human genome, plays a crucial role in maintaining genomic stability. Aberrant LINE-1 activation, primarily regulated by DNA methylation, is a hallmark of cancer. LINE-1 retrotransposons are usually silenced by methylation; however, their hypomethylation is common in CRC and precancerous lesions, correlating with genomic instability and cancer risk. LINE-1 methylation thus serves as a marker for global methylation status [3].

The present study aimed to validate the methylation status at three CpG sites (328, 321, and 318) within the LINE-1 (X58075.1) promoter. Adenocarcinoma samples (n=20) and corresponding adjacent normal tissues were collected (NCI, Vilnius, Lithuania). Quantitative determination of the methylation status of the three LINE-1 promoter CpG sites in bisulfite-converted DNA was performed using pyrosequencing (Qiagen). The expression of LINE-1 was evaluated using RT-PCR for both open reading frames, ORF1 and ORF2. All data were analyzed using GraphPad Prism. The Spearman rank correlation coefficient was used to determine the strength of linear relationships. The Mann-Whitney U test was used to compare differences between groups. Findings were considered statistically significant at $p < 0.05$.

The findings of this study show that methylation levels at all three CpG sites differed significantly, allowing for the distinction of adenocarcinoma from healthy tissue. Notably, the methylation level of the CpG site at position 328 distinguished adenocarcinoma from healthy adjacent tissue with very high specificity and sensitivity, yielding an AUC (area under the curve) of 0.950.

LINE-1 ORF1 and ORF2 expression levels were strongly correlated (Spearman's $r = 0.79$, $p < 0.0001$). Significantly, ORF2 levels dropped during the T2 to T3 transition, reflecting increasing genomic instability. ROC analysis (AUC = 0.80, $p = 0.073$) indicates that absolute ORF2 expression could serve as a useful biomarker for this stage transition.

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FUNCTIONAL IMPLICATIONS OF *KDM5B* SILENCING IN PROSTATE CANCER CELLS

Aukse Versekenaite[1], Ruta Maleckaite[1], Kristina Daniunaite[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
aukse.versekenaite@gmc.stud.vu.lt

Prostate cancer (PCa) is the 4th most diagnosed cancer, with over 370,000 annual deaths globally [1]. Epigenetic mechanisms play a crucial role in prostate carcinogenesis, particularly post-translational histone modifications. *KDM5B* is one of the genes associated with histone methylation that has been identified as an oncogene in many cancers [2]. It encodes a lysine-specific histone demethylase that is often dysregulated in PCa cells, which correlates with poor patient survival [3]. Thus, modulation of *KDM5B* expression in cancer cells could be a potential target for PCa treatment.

Our aim was to modulate *KDM5B* gene expression in PCa cells by transfecting with small interfering RNA (siRNA) and to assess the resulting potential changes in cell behavior. In this study, PCa cell lines with differing invasive characteristics were employed, specifically the highly invasive PC-3 cell line and the less invasive LNCaP cell line. The relative expression of *KDM5B* was evaluated using quantitative PCR, and commercial assays were used to assess cell viability, proliferation, invasiveness, and migratory capacity.

KDM5B expression was successfully downregulated in both PC-3 and LNCaP cells after siRNA transfection, with a statistically significant decrease in LNCaP cells ($P < 0.01$). Functional analyses indicated reduced PC-3 cell viability ($P = 0.013$) and proliferation ($P = 0.019$) following *KDM5B* silencing, with no significant changes in apoptotic or necrotic cell populations ($P > 0.050$). Similar tendencies were also observed in the LNCaP cells. Additionally, decreased migratory capacity was observed in both cell lines, with a statistically significant difference in PC-3 cells at 12 h post-transfection ($P = 0.028$), suggesting a potential role of *KDM5B* in regulating PCa cell motility.

To conclude, our initial results demonstrate that siRNA-mediated downregulation of *KDM5B* expression in PCa cells reduces cell viability, proliferation, and migration, suggesting a potential role of *KDM5B* in PCa progression and its pertinence to drug development.

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A13

PROTEASOME GENE POLYMORPHISMS ARE ASSOCIATED WITH THERAPY-SPECIFIC NEDA OUTCOMES IN A LATVIAN MULTIPLE SCLEROSIS COHORT

Jegors Paramonovs[1], Ilva Trapina[1], Samanta Plavina[1], Daniela Malakovska[1], Jolanta Kalnina[2], Natalia Paramonova[1]

[1] Genomics and Bioinformatics Center, Department of Pharmaceutical Sciences, Faculty of Medicine and Life Sciences, University of Latvia, Jelgavas iela 1, Riga LV-1004, Latvia
[2] Multiple Sclerosis Center, Latvian Maritime Medicine Center, Patversmes iela 23, Riga LV-1005, Latvia
jegors.paramonovs@lu.lv

Background: No evidence of disease activity (NEDA) is widely used as a practical composite outcome to track treatment response in multiple sclerosis (MS), a chronic immune-mediated disease of the central nervous system. In most studies it is defined as NEDA-3-no relapses, no MRI activity, and no confirmed disability worsening [1]. Achieving NEDA early after treatment initiation is generally associated with better medium-term outcomes, yet it is also clear that NEDA does not fully capture the slower neurodegenerative component of MS. This gap has driven interest in biological predictors of sustained NEDA and in factors that might explain why NEDA rates differ both between therapies and between individuals receiving the same therapy [1-3]. In this context, the proteasome and immunoproteasome are attractive candidates because they shape antigen processing and inflammatory signalling, processes that many disease-modifying therapies directly or indirectly modulate, so genetic variants in proteasome-related genes could plausibly contribute to therapy-specific differences in disease activity control [4].

Aim: To test whether selected proteasome-gene polymorphisms are associated with NEDA during the first two years after treatment initiation and to explore therapy-specific genotype effects across interferon (IFN), glatiramer acetate (GA), and mitoxantrone (MX) groups.

Methods: MS patients from a Latvian cohort were evaluated for NEDA in year 1 and year 2 after treatment initiation and stratified by therapy (IFN, GA, MX). SNPs in proteasome-related genes (PSMB5 rs11543947; PSMA6 rs2277460 and rs1048990; PSMA3 rs2348071; PSMC6 rs2295826/rs2295827) were genotyped using PCR-based assays. Genotype distributions were compared by NEDA status within and between therapy groups. Odds ratios (OR) were computed for NEDA-negativity; OR<1 indicates a protective association and OR>1 increased risk. Effect sizes are reported as Cramér's V.

Results: The strongest therapy-dependent association over 2 years was observed for PSMC6 rs2295826/rs2295827. The combined genotype group (GG/TT+AG/CT) differed between medication groups ($P=2.20\times10^{-3}$; V=0.37). In IFN-treated patients, GG/TT+AG/CT showed a more favourable NEDA profile (NEDA+67.69%) and reduced odds of NEDA-negativity ($P=2.48\times10^{-3}$; OR=0.38). In contrast, in GA-treated patients the same genotype group was associated with low NEDA+ (16.67%) and increased odds of NEDA-negativity ($P=4.22\times10^{-2}$; OR=5.67). Within the IFN stratum, preparation-specific differences were observed for PSMA6 rs1048990 after 1 year (GG+CG distribution across IFN-A/IFN-R/IFN-B/E: $P=2.80\times10^{-2}$; V=0.45). In IFN-A, GG+CG was linked to higher odds of NEDA-negativity ($P=8.44\times10^{-3}$; OR=16.29). In year 2, an association was also observed for IFN-B/E ($P=4.31\times10^{-2}$; OR=4.24). After 2 years, PSMA6 rs2277460 differed across IFN preparations (CA genotype: $P=4.55\times10^{-2}$; V=0.49); in IFN-B/E, CA carriers were predominantly NEDA− (83.33%; $P=3.29\times10^{-2}$; OR=5.14).

Conclusions: Proteasome-related variants, particularly PSMC6 rs2295826/rs2295827 and PSMA6 polymorphisms, show therapy-specific associations with NEDA, consistent with gene-treatment interaction. Notably, PSMC6 (GG/TT+AG/CT) showed opposite directions across therapies (more favourable under IFN, less favourable under GA). These loci warrant validation in larger independent cohorts.

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QUANTIFICATION OF CIRCULAR RNA IN PROSTATE CANCER CELL LINES WITH EXPERIMENTAL MODULATION OF SELECTED MICRORNA

Gerda Bertulyte[1], Ruta Maleckaite[1], Aukse Versekenaite[1], Arnas Stucinskas[1], Marta Tamosiunaite[1], Kristina Daniunaite[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania

gerda.bertulyte@gmc.stud.vu.lt

Prostate cancer (PCa) is the second leading cause of cancer related mortality among men in 48 countries [1]. Scientific interest has shifted toward noncoding circular RNAs (circRNAs), given that their abnormal expression patterns are linked to various types of cancer and tumor development or progression [2]. Certain circRNAs are able to function as microRNA (miRNA) ‘sponges’ or interact with RNA-binding proteins, thereby influencing gene expression [3]. However, the regulatory interactions between different circRNAs and miRNAs in PCa cells remain insufficiently characterized.

This study aims to quantify selected circRNAs (circFOXO3, circFAT1, and others) in PCa cells with experimentally altered miR-155 and miR-137 expression. A miRNA mimetic approach was used to induce overexpression of selected miRNAs in PCa cells with low endogenous expression levels. CircRNA levels were assessed using real-time PCR using an intercalating dye. Divergent primers were specifically designed to amplify circular transcripts and to distinguish them from linear counterparts.

Our preliminary results demonstrated a trend toward upregulated circHIPK3 and circASXL-1 expression in the PC-3 cell line following transfection with synthetic miR-155; however, these changes did not reach statistical significance ($P > 0.05$). Although circFOXO3, circFAT1, and circCSNK1G3 expression levels did not differ significantly from the scrambled control, the corresponding linear transcripts exhibited decreased expression in PC-3 cells transfected with miR-137. Comparative analysis between PC-3 and LNCaP cell lines following miR-155 overexpression revealed differential circRNA expression patterns. Notably, circASXL-1 was upregulated in PC-3 cells relative to LNCaP.

In conclusion, these preliminary findings suggests that experimental modulation of specific miRNAs is associated with measurable alterations in circRNA expression in PCa cell lines. This potential regulatory interplay between circRNAs and miRNAs may contribute to the molecular mechanisms underlying PCa biology and warrants further investigation.

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THE ANALYSIS OF EPIGENETIC BIOMARKERS FOR NON-INVASIVE RENAL CANCER DETECTION AND PROGNOSIS

Reda Kybartaitė[1], Raimonda Kubiliūtė-Mikalauskienė[1], Austėja Lesnickaitė[1], Algirdas Žalimas[2], Sonata Jarmalaitė[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Saulėtekio Av. 7, Vilnius, Lithuania
[2]Lithuanian National Cancer Institute, P. Baublio str. 3B, Vilnius, Lithuania
reda.kybartaite@gmc.stud.vu.lt

Renal cancer is ranked 7th in Lithuania in terms of the number of incidence and mortality [1]. The most common form of kidney cancer is clear cell renal cell carcinoma (ccRCC), which accounts for about 75–85% of all kidney tumors [2]. Depending on the stage of the tumor, the 5-year survival rate for patients can range from 93% in the early stages to 19% in advanced cases [3]. Due to the frequent lack of early symptoms, diagnosis often occurs incidentally or at late stages, highlighting the need for molecular biomarkers to enable early detection and prognosis.

It is known that changes in DNA methylation play an important role in the development and progression of kidney cancer and are therefore considered potential biomarkers for early diagnosis and prognosis. Previously, our laboratory performed a genome-wide DNA methylation profile analysis using the microarray method, during which genes suitable for potential biomarker studies were selected. Follow-up DNA methylation analysis of the selected three genes related to the regulation of gene transcription revealed significant methylation changes linked to clinical features. To confirm the reliability of these results, this study aims to investigate the level of DNA methylation in the regulatory regions of the same genes in the urine samples collected from the independent cohort of patients having renal cancer and benign renal diseases and to evaluate their diagnostic and prognostic properties.

A total of 144 urine samples were examined, including 39 samples from ccRCC patients, 27 asymptomatic control samples, 18 samples from patients diagnosed with benign kidney tumors, and 30 samples from patients diagnosed with small renal masses. Samples were collected in accordance with Lithuanian Bioethics Committee permits No. 158200-18/12-1077-585 and No. 158200-17-952-457. DNA was extracted during a previous study using the Phenol/Chloroform/Isoamyl alcohol DNA extraction method. Then DNA was bisulfite converted using the EZ DNA Methylation^{TM} Kit (Zymo Research). To evaluate the levels of DNA methylation in the regulatory regions of the genes in the samples, Quantitative Methylation-Specific PCR (qMSP) was used.

The analysis revealed that the level of DNA methylation in the regulatory region of one out of three gene is statistically significantly higher in urine samples from ccRCC patients compared to benign kidney tumors ($P = 0.039$). The study also showed that the same gene has statistically significant diagnostic ability in ccRCC (sensitivity – 64%, specificity – 63%, $P = 0.045$). In addition, in the case of small renal tumors, the two out of three genes showed statistically significant differences in methylation levels compared to asymptomatic controls ($P = 0.001$; $P = 0.039$) and significant diagnostic values ($P < 0.005$).

These findings suggest that alterations in DNA methylation levels in the regulatory region of at least one selected gene in the urine samples could be a promising ccRCC biomarker, allowing for non-invasive and very early renal cancer detection. Further analysis of a larger sample size is needed to justify these findings.

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ANALYSIS OF EPIGENETIC BIOMARKERS FOR URINE-BASED KIDNEY CANCER DETECTION

Austėja Lesnickaitė[1], Raimonda Kubiliūtė-Mikalauskienė[1], Reda Kybartaitė[1], Algirdas Žalimas[2], Sonata Jarmalaitė[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]Lithuanian National Cancer Institute, Vilnius, Lithuania
austeja.lesnickaite@gmc.stud.vu.lt

Kidney cancer ranks as the 14th most common cancer worldwide, with over 400,000 new cases and approximately 155,000 documented deaths in 2022 [1]. Early diagnosis remains challenging due to the lack of symptoms in the beginning of the disease, resulting in higher rates of metastatic progression and a poorer patient prognosis. For instance, the 5-year survival rate for stage I kidney cancer reaches 88% compared to just 14% for stage IV disease [2]. Currently, there are no clinically approved molecular biomarkers for the diagnosis of kidney cancer. Therefore, a strong focus is centered on identifying new potential biomarkers that can help diagnose the disease at an early stage and enable effective treatment without invasive and expensive procedures. Alterations in DNA methylation are among the most significant epigenetic biomarkers linked to cancerogenesis, which may improve the prognosis of kidney cancer.

The aim of this study is to investigate the methylation levels of the three genes in urine samples collected from patients with renal clear cell carcinoma (RCCC), small renal tumors, and benign kidney tumors, and to evaluate their potential for disease diagnosis and prognosis.

In total, 115 samples were analyzed, including 39 RCCC cases, 31 small renal tumor cases, 18 benign cases, and 27 asymptomatic controls. DNA was extracted from the urine samples and subsequently modified using sodium bisulfite. Methylation levels of the selected genes were established by the quantitative methylation-specific PCR (QMSP).

The combination of the three genes showed statistically significant diagnostic characteristics with a sensitivity of 61.54 % and a specificity of 74.04 %. The methylation levels of two out of three genes were statistically significantly higher in urine samples from patients with higher-grade renal clear cell carcinoma ($p = 0.018$, $p = 0.022$, respectively). One gene methylation level was also statistically significantly associated with tumor spread beyond the kidney ($p = 0.046$). In addition, the methylation level of one gene was statistically significantly higher in urine samples from patients with small renal tumors compared to asymptomatic controls ($p = 0.008$) and demonstrated statistically significant diagnostic characteristics.

This analysis demonstrates that the methylation of the selected genes has the potential to be diagnostic biomarkers for renal cancer. However, further studies should be conducted to confirm the clinical usefulness of the investigated biomarkers.

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GENERATION OF TRANSGENIC ZEBRAFISH (*DANIO RERIO*) LINES FOR MONITORING INTRACELLULAR ACTIN DYNAMICS

Gabrielė Skruzdytė[1], Edita Bakūnaitė[1], Darius Balčiūnas[1,2]

[1]Department of Eukaryote Gene Engineering, Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]Department of Biology, Temple University, Philadelphia, JAV
gabriele.skruzdyte@gmc.vu.lt

Actin is the most abundant protein in most eukaryotic cells, and its structure and function are highly evolutionarily conserved. Its unique ability to transition between monomeric and filamentous forms makes it extremely important for many cellular processes, such as cell migration, polarity establishment, maintenance of cell shape, and forming the structural basis of sarcomeres in muscle cells [1]. To better understand these intracellular mechanisms, it is crucial to visualize actin filament formation and remodeling *in vivo*. The zebrafish (*Danio rerio*) is a well-established vertebrate model organism due to its rapid development, optical transparency during embryogenesis, and the ease and efficiency of precise genetic manipulation, making it particularly suitable for studying actin cytoskeleton dynamics [2]. Although transgenic zebrafish lines for actin imaging have been developed, they lack temporal regulation of biosensor expression, which complicates the investigation of actin dynamics at certain developmental or physiological stages.

Precise spatiotemporal control of transgene expression can be achieved using Cre-dependent genetic systems. The Cre-*loxP* recombination system is one of the most widely used genetic tools for this purpose. It mediates site-specific recombination between *loxP* sequences in the presence of Cre recombinase, resulting in excision of a floxed DNA cassette and subsequent activation of a downstream gene [3, 4]. In zebrafish studies, the inducible CreER^{T2} system is frequently used, as it allows precise temporal control of recombination. Recombinase remains inactive until 4-hydroxytamoxifen administration, which initiates enzyme nuclear translocation and subsequently induces recombination at *loxP* sites. Furthermore, *CreER*^{T2} expression driven by tissue-specific promoter restricts recombination to particular cell types, enabling spatial control of transgene activation [5].

In this study, two conditional expression constructs were generated to monitor intracellular actin dynamics *in vivo*, each containing ubiquitous *ubb*^{R} promoter, *mRFP* cassette flanked by *loxP* sites, followed by sequences encoding actin-binding fluorescent reporters. The first construct encoded *eGFP-UtrCH*, a probe based on the calponin homology domain of utrophin, while the second vector contained *LifeAct-eGFP*, a short actin-binding peptide fused to enhanced green fluorescent protein. The constructed expression cassettes in a plasmid vector were injected into zebrafish embryos, and embryos carrying transgenesis markers were selected and grown to sexual maturity. Founder individuals transmitting the transgene to a subset of their offspring were successfully identified. The resulting F1 generation was raised and crossed with the *vln13Tg* zebrafish driver line carrying the *ubb*^{R} promoter driven *CreER*^{T2} recombinase gene. Embryos obtained from this cross were treated with 4-hydroxytamoxifen at 6 hours post-fertilization, leading to Cre-mediated recombination and activation of the actin reporter. Following induction, strong ubiquitous green fluorescence was observed, confirming successful recombination and functional activation of the conditional expression cassette. This system provides a flexible platform for future tissue-specific and temporal control of intracellular actin dynamics *in vivo*.

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A18

THERAPY – DEPENDENT ASSOCIATIONS BETWEEN VITAMIN D PATHWAY GENETIC VARIANTS AND NEDA OUTCOMES IN A LATVIAN MULTIPLE SCLEROSIS COHORT

Daniela Malakovska[1], Ilva Tapina[1], Samanta Plavina[1], Jegors Paramonovs[1], Jolanta Kalnina[2], Natalia Paramonova[1]

[1] Genomics and Bioinformatics Center, Department of Pharmaceutical Sciences, Faculty of Medicine and Life Sciences, University of Latvia, Jelgavas iela 1, Riga, LV-1004, Latvia.
[2] Multiple Sclerosis Center, Latvian Maritime Medicine Center, Patversmes iela 23, LV-1005, Riga, Latvia
daniela.malakovska@lu.lv

Vitamin D regulates immune responses through its interaction with the vitamin D receptor (VDR), while its bioavailability and systemic transport are largely mediated by the vitamin D binding protein (VDBP). Genetic variation within these components of the vitamin D pathway has been implicated in multiple sclerosis (MS) susceptibility and may influence treatment response. Nevertheless, substantial inter-individual variability remains, potentially contributing to differences in achieving "no evidence of disease activity" (NEDA). This study aimed to evaluate whether polymorphisms in *VDR* and *GC* are associated with NEDA during the first two years after treatment initiation and to explore potential therapy – and interferon (IFN) preparation – specific effects.

Latvian MS patients were assessed for NEDA at years 1 and 2 after treatment initiation and stratified by therapy (IFN, GA, MX); IFN-treated patients were additionally stratified by IFN preparations (IFN-A, IFN-R, IFN-B/E). *VDR* (rs1544410, rs7975232, rs731236) and *GC* (rs7041, rs4588) variants were genotyped by PCR-based assays, and genotype distributions were compared by NEDA status within and between therapy groups. ORs were computed for NEDA- (OR < 1 protective; OR > 1 risk).

Overall, loci within the vitamin D pathway demonstrated therapy-dependent associations with NEDA, most prominently at year 2. Under IFN, *VDR* rs1544410 (G>A) was associated with NEDA ($P = 3.98\times10^{-2}$; V = 0.16), with AA+GA showing a more favourable NEDA profile compared with GG. A similar IFN-stratum association was observed for *VDR* rs731236 (T>C) ($P = 2.99\times10^{-2}$; V = 0.16).

Among IFN-treated patients, further stratification by IFN preparation revealed preparation-specific heterogeneity for *VDR* rs7975232 (IFN-R: $P = 1.90\times10^{-2}$; V = 0.40) and *VDR* rs731236 (IFN-R: $P = 1.68\times10^{-2}$; V = 0.30).

Regarding vitamin D transport, *GC* rs4588 (C>A) differed between medication groups at year 2 ($P = 2.04\times10^{-2}$; V = 0.22) and exhibited opposite effect directions across therapies: AA+CA tended toward higher NEDA+ odds under IFN (OR ≈ 1.40) but lower NEDA+ odds under GA (OR ≈ 0.83), consistent with a potential gene – treatment interaction.

Additionally, *GC* rs7041 (G>T) showed a between-therapy difference in year 1 under MX ($P = 2.57\times10^{-2}$; V = 0.37), supporting therapy dependence also at earlier follow – up.

In conclusion, variants in *VDR* and *GC*, particularly *VDR* rs7975232, rs1544410, and rs731236, as well as *GC* rs4588 and rs7041, demonstrated therapy – and IFN preparation – specific associations with NEDA in this Latvian MS cohort, supporting a potential gene – treatment interaction. These findings warrant validation in larger, independent cohorts and may contribute to the development of integrated prediction models incorporating genetic, clinical, and treatment-related variables.

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A19

ADAPTATIONS OF *ACHROMOBACTER* SPP. IN PATIENTS WITH PRIMARY CILIARY DYSKINESIA

Lukas Beržinis[1], Helle Krogh Johansen[2], Rasmus L. Marvig[2,,4], Gytis Dudas[1], Migle Gabrielaite[1]

[1]Institute of Biotechnology, Department of Eukaryote Gene Engineering, Vilnius University, Lithuania
[2]Department of Clinical Microbiology, Rigshospitalet, Denmark
[3]Department of Genomic Medicine, Rigshospitalet, Denmark
[4]Department of Health Technology, Technical University of Denmark, Denmark
lukas.berzinis@proton.me

Achromobacter spp. are opportunistic Gram-negative pathogens, increasingly associated with chronic airway infections in people with mucociliary clearance disorders, such as primary ciliary dyskinesia (PCD) [1]. The mechanisms behind adaptation and antibiotic resistance of *Achromobacter* spp. during infection remain poorly understood, posing significant challenges for clinical management. Here, we analysed 54 clinical *Achromobacter* spp. whole genome sequences from people with PCD using bioinformatics tools such as *skani* [2], *panaroo* [3] and *snp-dists* [4] for species identification, pangenome construction, and evaluation of genetic distances both within and between patients, respectively. Isolates were assigned to nine distinct species, with *A. xylosoxidans* being predominant, and two patients were found to have been infected with multiple *Achromobacter* species across sequential samples. Antibiotic resistance and virulence factors, including beta-lactamase and efflux pump genes were detected across isolates, and a replicative plasmid gene *IncQ1* was identified in all samples from one patient. Pangenome analysis revealed high genetic diversity between *Achromobacter* species with average species-specific gene counts ranging from 364 to 1403. Our findings highlight the genomic complexity and adaptive potential of *Achromobacter* spp. during chronic infection, underscoring the need for species-level resolution and genomic surveillance to better understand how *Achromobacter* spp. adapts to the host environment in different disorders ultimately leading to the development of novel treatment strategies.

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A20

GENETIC POLYMORPHISMS IN *LYZL2*, *MPPED2*, *MTR*, AND *MTRR* IN DENTAL PATIENTS WITH INTELLECTUAL DISABILITY

Ula Burbulyte[1], Karole Simona Motiejunaite[1,2], Neringa Meilute[1,2], Indre Stankeviciene[2], Kristina Daniunaite[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]Institute of Dentistry, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
ula.burbulyte@gmc.stud.vu.lt

Dental caries is a chronic disease and a major cause of tooth loss, affecting a significant proportion of the global population [1]. The condition arises from the accumulation of dental plaque (biofilm), with its development being influenced by multiple factors (biological, behavioral, socioeconomic, etc.) [2]. Individuals with intellectual disabilities are at particularly high risk due to poorer oral hygiene, dietary challenges, limited access to dental care, and greater dependency on caregivers. While such factors as oral hygiene practices are well known, the contribution of genetics remains less clearly defined. Studies suggest that genetic variants of various genes may be linked to an increased susceptibility to dental caries with some influencing neurodevelopmental disorders as well [3].

The objective of this study was to investigate single-nucleotide polymorphisms (SNPs) in four selected genes – *LYZL2*, *MPPED2*, *MTR*, and *MTRR* – in dental patients with intellectual disabilities.

Our preliminary study included 63 individuals who underwent dental treatment for oral diseases under general anesthesia at the Zalgiris Clinic, Vilnius University Hospital Santaros Clinics (Vilnius, Lithuania). Buccal swab samples were collected by a single trained clinician during dental examination. Genomic DNA was extracted using the MagMAX Nucleic Acid Isolation Kit and the KingFisher Flex automated extraction system. Four SNPs were selected for molecular analysis: rs399593 (*LYZL2*), rs11031093 (*MPPED2*), rs1805087 (*MTR*), and rs1801394 (*MTRR*). Genotyping was conducted using TaqMan-based commercial SNP genotyping assays on the QuantStudio Pro 7 Real-Time PCR system (Thermo Fisher Scientific).

Genotype distributions of the analyzed SNPs are shown in Fig. 1. Homozygous alternative genotypes were determined in 79.4% (50 of 63), 8.1% (5 of 62), 4.8% (3 of 63), and 28.6% (18 of 63) cases for *LYZL2*, *MPPED2*, *MTR*, and *MTRR* SNPs, respectively. The minor allele frequency estimates of *LYZL2*, *MPPED2*, *MTR*, and *MTRR* SNPs were 0.199, 0.242, 0.214, and 0.444 respectively. Allele and genotype frequencies were similar to those reported for the general population. Further analyses are ongoing to evaluate the possible associations between these genetic variants and clinical oral health parameters, including the tooth loss rate.

In conclusion, our preliminary findings provide a foundation for further investigation of genetic contributions to dental caries in individuals with intellectual disabilities. However, the complex nature of these genetic influences requires further research to confirm these findings and clarify the mechanisms by which the SNPs affect caries development.

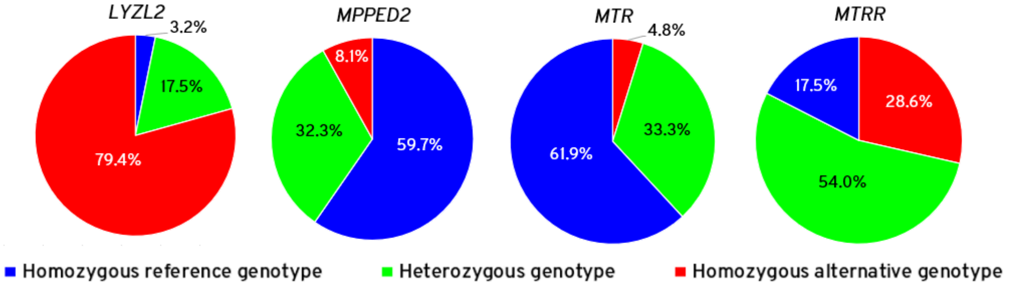

Figure 1. The genotype frequencies of *LYZL2*, *MPPED2*, *MTR*, and *MTRR* SNPs in dental patients with intellectual disabilities.

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A21

ENGINEERING TRANSGENIC ZEBRAFISH TO STUDY TIMP4.3 DEPENDENT MACROPHAGE FUNCTIONS DURING CARDIAC REPAIR

Paulina Lašaitė, Justas Lazutka

Department of Eukaryote Gene Engineering, Institute of Biotechnology, Life Sciences Center, Vilnius
University, Sauletekis av. 7, Vilnius, Lithuania
paulina.lasaite@chgf.stud.vu.lt

Cardiovascular diseases are the leading cause of death worldwide[1]. An adult heart after trauma in most cases leads to a scar formation and does not regenerate, therefore myocardial infarction can lead to irreversible heart damage and eventually heart failure or death. Unlike humans, zebrafish can effectively regenerate its heart after trauma. For this reason *danio rerio* is a suitable model for heart regeneration research.

timp4.3 (TIMP metallopeptidase inhibitor 4, tandem duplicate 3) is a zebrafish gene, which is predicted to enable metalloendopeptidase inhibitor activity. This gene is selectively expressed in a macrophage subpopulation associated with cardiac regeneration[2]. This subpopulation of macrophage is predicted to play an important role in extracellular matrix remodeling, cellular debris clearance and scar resolution [2].

To minimize *timp4.3* functions during cardiac repair are used genetic engineering methods, such as CRISPR/Cas9. These methods are used on zebrafish to knock out target gene, in this case 1-3 exons of *timp4.3*, therefore creating transgenic zebrafish lines for *timp4.3* functional characterization in cardiac regeneration.

To create *timp4.3* knockout transgenic fish lines, zebrafish zygotes were injected with Cas9 mRNA and two guide RNAs. The F1 generation fish were genotyped by amplifying *timp4.3* gene deletion's region via PCR and electrophoresis on a 1% agarose gel. Mutants were selected based on PCR product's length. Lastly, after Sanger sequencing, fish that had the expected deletion were selected for further research.

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INVESTIGATION OF THE GENOTOXICITY OF 70 NM POLYSTYRENE NANOPARTICLES IN HUMAN HEPATOCELLULAR CARCINOMA (HEPG2) AND LUNG ADENOCARCINOMA (A549) CELLS

Emilija Striogaitė[1], Vaiva Horbačauskaitė[1], Juozas Rimantas Lazutka[1]

[1]Department of Botany and Genetics, Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
emilija.striogaite@gmc.stud.vu.lt

The widespread use of plastic materials has raised serious environmental and health concerns due to their high persistence and tendency to fragment into nanoscale particles. Among plastic-derived nanomaterials, polystyrene nanoparticles (PS-NPs) are commonly detected, and their possible negative impacts on human health need thorough investigation [1].

This study aimed to evaluate the cytotoxic and genotoxic effects of 70 nm PS-NPs in human hepatocellular carcinoma (HepG2) and lung alveolar adenocarcinoma (A549) cell lines *in vitro*. The cellular uptake and levels of reactive oxygen species generated by PS-NPs were evaluated using the fluorescent, oxidant-sensitive dye 2,7-dichlorodihydrofluorescein diacetate (H_2DCFDA). Cytotoxicity was assessed using a mixture of acridine orange and ethidium bromide, based on cell integrity. Potential primary DNA damage was assessed using the alkaline comet assay, while oxidative DNA damage was evaluated with an enzyme-modified comet assay. Chromosomal damage was assessed using the cytokinesis-blocked micronucleus (CBMN) assay.

Flow cytometry analysis demonstrated effective uptake of PS-NPs by both HepG2 and A549 cells. In A549 cells, exposure to nanoparticles did not lead to significant changes in cell viability, primary and oxidative DNA damage, or chromosomal damage when compared to control cells.

In contrast, HepG2 cells showed a different response pattern. Although cell viability remained largely unaffected, nearly all tested nanoparticle concentrations induced significant primary DNA damage. Furthermore, oxidative DNA damage was significantly increased at concentrations of 10 and 20 µg/mL, as detected by the enzyme-modified comet assay. Higher concentrations (60, 80, and 100 µg/mL) led to a statistically significant increase in micronucleus frequency compared to the control, indicating chromosomal damage.

These findings demonstrate that, while PS-NPs are effectively internalized by both cell lines, their genotoxic effects are cell-type-dependent. A549 cells exhibited relative resistance to nanoparticle-induced genetic damage, whereas HepG2 cells were highly susceptible to primary, oxidative DNA and chromosomal damage. This highlights the potential risk of nanoscale polystyrene exposure and underscores the importance of assessing tissue-specific responses to nanoplastics.

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GENOTOXIC EFFECTS OF IONISING RADIATION AT DIFFERENT DOSE RATES ON HUMAN LYMPHOCYTES *IN VITRO*

Ula Balkutė[1], Lėja Žemantauskaitė[1], Gabija Rožėnaitė[1], Juozas Rimantas Lazutka[1], Akvilė Šlėktaitė-Kišonė[2,3], Marius Burkanas[3], Aleksandras Cicinas[3], Mindaugas Džiugelis[2,3], Jonas Venius[3]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
[2]National Cancer Institute, Biomedical physics laboratory, Vilnius, Lithuania
[3]National Cancer Center, Medical Physics Department, Vilnius, Lithuania
ula.balkute@gmc.stud.vu.lt

FLASH radiation therapy (FLASH-RT) in literature is described as a promising advancement in cancer treatment due to its selectivity – it results in sparing normal healthy tissues from damage while effectively targeting tumours [1]. Because using FLASH-RT radiation doses are delivered within miliseconds (dose rates exceeding 40 Gy/s), the same physical dose can produce a different biological response than when it is delivered slowly under conventional irradiation (where on average dose rates are < 0.1 Gy/s) [2]. Although FLASH effect has created considerable clinical interest, its genotoxic impact on radiosensitive non-target cells, such as peripheral blood lymphocytes (PBLs), is not yet fully explored.

The aim of this study was to evaluate the genotoxic effects of ionising radiation delivered at FLASH and conventional dose rates in human peripheral blood lymphocytes *in vitro*.

Peripheral blood samples were irradiated using a modified Varian TrueBeam (Varian Medical Systems, USA) linear accelerator with low-LET ionising radiation at two dose rates – conventional 0.17 Gy/s and FLASH 48 Gy/s. To assess genotoxicity, the micronucleus (MN) assay was applied. Biodose Tools software [3] was used to generate dose-response curves for MN frequency. At lower doses (2-4 Gy) genotoxic effects were similar between dose rates, while at higher doses the effects varied between experiments and the FLASH effect was not consistent. The replication index was used to assess cytotoxicity and showed decreased cell proliferation at higher doses, with higher proliferation observed at 0.17 Gy/s. Cell viability was slightly higher at 48 Gy/s.

A pilot flow cytometry analysis was performed during the first experiment to evaluate the effect of ionising radiation on cell cycle. Although too few cells were collected for a reliable statistical analysis, certain trends can still be observed – ionising radiation increased the number of lymphocytes in G1 phase and showed decreased numbers in S phase of cell cycle with all dose rates and doses. A less pronounced decrease of cells in G2 phase and mitosis was also observed, when compared with control group.

Ultimately, the results show that the tissue-sparing FLASH effect is inconsistent and varies between the experiments. These findings indicate that biological response to ultra-high dose rate (UHDR) ionising radiation is complex and needs further analysis.

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EVALUATION OF IN VITRO GENOTOXIC AND ANTIGENOTOXIC PROPERTIES OF MEDICINAL MUSHROOM EXTRACTS

Šašytė Miglė[1], Babonaitė Milda[1], Juozas Rimantas Lazutka[1]

[1]Department of Botany and Genetics, Institute of Biosciences, Vinius university, Vilnius Lithuania
migle.sasyte@gmc.stud.vu.lt

Since ancient times, various mushrooms have been valued by humans as a source of food. However, this is not the only reasons that makes mushrooms important. Medicinal mushrooms and fungi are believed to have about 130 healing properties, including anti-cancer and immunomodulatory effects.[1]. However, public opinion regarding their benefits is divided: some consider them beneficial, while others question their safety. Therefore, the aim of this study is to evaluate the biological effects of these extracts.

This study investigated the cytotoxic, genotoxic and antigenotoxic properties of four types of medicinal mushroom extracts: Auricularia, Cordyceps, Lion's Mane, and Reishi in human peripheral blood mononucelar cells. Cytotoxicity was assessed using the acridine orange/ethidium bromide staining test. To evaluate genotoxic and antigenotoxic properties, the alkaline comet assay, which detects primary DNA damage, such as DNA strand breaks and alkaline labile sites, was performed. Genoprotective potential was evaluated using two treatment regimens: pretreatment and posttreatment. In the pretreatment protocol, cells were incubated with the extracts, washed with phosphate-buffered saline (PBS), and subsequently exposed with H_2O_2. In the posttreatment protocol, cells were first exposed to H_2O_2, washed with PBS, and then incubated with the extracts.

The results of this study provide valuable insights into the genotoxic and antigenotoxic effects of medicinal mushrooms. They may also contribute to new recommendations for the use of their extracts.

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TOXIC AND GENOTOXIC EFFECTS OF ULTRA-HIGH DOSE RATE IONIZING RADIATION ON *DROSOPHILA MELANOGASTER IN VIVO*

Ugnė Tatarūnaitė[1], Danas Nikontas[1], Emilija Gruškaitė[1], Akvilė Šlėktaitė-Kišonė[2,3], Marius Burkanas[3], Aleksandras Cicinas[3], Mindaugas Džiugelis[2,3], Jonas Venius[3], Juozas Lazutka[1]
[1]Institute of Bioscience, Life Science Center, Vilnius University, Lithuania
[2]National Cancer Institute, Biomedical physics laboratory, Vilnius, Lithuania
[3]National Cancer Center, Medical Physics Department, Vilnius, Lithuania
ugne.tatarunaite@gmc.stud.vu.lt

Cancer is a leading global cause of death, and cancer-related mortality is estimated to nearly double by 2050 [1]. To effectively control disease course in cancer patients, radiation therapy (RT) is clinically prevalent, utilizing ionizing radiation to induce targeted genotoxic effects and arrest malignant cellular proliferation. Conventional RT is usually delivered at low dose rate (averagely ~0.03 Gy/s), whereas FLASH radiotherapy (FLASH-RT), which recently has emerged as a prominent area of clinical investigation, is characterized by ultra-high dose-rate delivery (≥40 Gy/s). FLASH-RT has been associated with reduced damage to healthy tissue while maintaining antitumor effectiveness [2]. Evidence for this so-called FLASH effect has been reported primarily in preclinical models, with early clinical observations also emerging.

The aim of this study is to evaluate DNA damage and survival responses after UHDR versus conventional (CONV) irradiation in vivo to investigate the biological response of FLASH effect.

In this study, Drosophila melanogaster lifespan assay was used to evaluate the effects of CONV and FLASH irradiation. Third-instar larvae were irradiated at 16, 24, 32, and 40 Gy and two dose rates (CONV - 0.17 Gy/s and FLASH - 48 Gy/s). Post-eclosion, adult flies' longevity was assessed over a 60-day period; survival curves were subsequently generated using Kaplan–Meier survival analysis with OASIS 2 software. The lifespan results revealed dose- and sex-dependent differences in irradiation response. Significant survival variation between CONV and FLASH irradiation was observed only at intermediate doses: at 16 Gy, FLASH improved survival in females but reduced it in males, whereas at 32 Gy, FLASH increased survival in both sexes. Moreover, survival curve divergence occurred primarily during early adulthood, indicating that dose rate effects were most pronounced in early mortality. Across all doses, females consistently survived longer than males, demonstrating a persistent sex-based difference in radiation response.

To assess dose-rate–dependent DNA genotoxicity in vivo, an alkaline comet assay was performed. Third-instar larvae were irradiated using the same dose rates and doses as those used in the lifespan assay. After exposure, haemocytes were isolated from the larvae and subjected to the alkaline comet assay. Genotoxicity was assessed based on the amount of DNA in the comet tail in percent. The results demonstrated a statistically significant difference between UHDR and CONV irradiation at 24 Gy, with a greater genotoxic effect observed at the CONV radiation. Moreover, at higher doses (24 and 32 Gy), CONV irradiation was associated with a higher number of damaged cells than UHDR radiation.

The genotoxic effect at the gene level was evaluated by the somatic mutation and recombination test (SMART). Third-instar larvae of D. melanogaster were divided into three groups and irradiated with three dose rates (0.2 Gy/s, 48 Gy/s, 98 Gy/s) at six doses ranging from 12 to 32 Gy. After eclosion, the viability of adult flies was assessed. However, no significant differences were found between the CONV and FLASH viability trends. SMART assay results showed that under FLASH irradiation, fewer mutations were detected compared to the conventionally irradiated test group, which may reflect a reduction in single-strand breaks (SSBs) and oxidative DNA damage. In contrast, FLASH test group showed an increased frequency of recombination events, possibly suggesting a higher number of double-strand breaks (DSBs). CONV and FLASH radiation displayed opposing dose-responses: with increasing dose, CONV conditions increased mutations and decreased recombination, whereas FLASH showed the reverse.

In conclusion, our preliminary findings display that UHDR irradiation leads to variable survival and genotoxic effects. These observations showcase the complexity of the FLASH effect and emphasize the importance of further investigation.

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CYTOTOXICITY AND GENOTOXICITY EVALUATION OF DIFFERENT SIZE AND SHAPE $GdPO_4:Eu^{3+}$ NANOPARTICLES

Adomas Mockus[1], Ilona Juodelytė[1], Milda Babonaitė[1], Eglė Ežerskytė[2], Vaidas Klimkevičius[2], Juozas Rimantas Lazutka[1]

[1]Department of Botany and Genetics, Institute of Biosciences, Vilnius university, Vilnius, Lithuania
[2]Department of Chemistry and Geosciences, Institute of Chemistry, Vilnius University, Vilnius, Lithuania
adomas.mockus@gmc.stud.vu.lt

Magnetic resonance imaging (MRI) is a widely used radiological diagnostic method in which contrast agents are commonly applied. Due to their paramagnetic properties, gadolinium-based contrast agents (GBCAs) are most frequently used, as they enhance MRI signal intensity [1]. However, their use raises concerns regarding the release of free Gd^{3+} ions and their potential toxicity.

Researchers at the Institute of Chemistry of Vilnius University have successfully synthesized a new generation of nanoparticles (NPs) - europium-doped gadolinium orthophosphate ($GdPO_4:Eu^{3+}$) nanoparticles - which are currently considered one of the most promising multimodal diagnostic nanomaterial platforms due to their unique combination of Gd^{3+} paramagnetism and Eu^{3+} luminescence. These properties enable the application of $GdPO_4:Eu^{3+}$ NPs in biomedicine, particularly as MRI contrast agents and fluorescent biomarkers [1]. In contrast to gadolinium chelates used in current clinical practice, $GdPO_4:Eu^{3+}$ NPs are structurally more stable. Their prolonged retention in the body may improve imaging quality while allowing a reduction in contrast agent dosage [2]. However, further studies are needed to establish their safety for biomedical use.

Therefore, this study aimed to assess the cytotoxic and genotoxic potential of differently sized $GdPO_4:Eu^{3+}$ NPs to human peripheral blood mononuclear cells (PBMCs) after 24-hour exposure. NP uptake was evaluated by flow cytometry using light scatter analysis. Reactive oxygen species (ROS) generation induced by $GdPO_4:Eu^{3+}$ NPs was assesed using oxidant-sensitive fluorescent dye H_2DCFDA. Cytotoxicity was evaluated using the dual acridine orange / ethidium bromide staining technique, while genotoxicity was assessed using two standard genotoxicity tests – alkaline comet assay for primary DNA damage analysis and cytokinesis-block micronucleus (CBMN) assay for chromosomal damage analysis.

The results of this study provide a comparison between cytotoxic and genotoxic potential of five different sizes and shapes of $GdPO_4:Eu^{3+}$ NPs, offering an important insight for their potential use in biomedicine.

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A27

PSMA3, PSMA6, AND PSMA7 PROTEASOME SUBUNIT EXPRESSION AS MOLECULAR INDICATORS OF MS DISABILITY AND TREATMENT STATUS: A LATVIAN COHORT STUDY

Samanta Plavina[1], Ilva Trapina[1], Daniela Malakovska[1], Jegors Paramonovs[1], Jolanta Kalnina[2], Natalia Paramonova[1]

[1]Genomics and Bioinformatics Center, Department of Pharmaceutical Sciences, Faculty of Medicine and Life Sciences, University of Latvia, Latvia
[2]Multiple Sclerosis Center, Latvian Maritime Medicine Center, Latvia
samanta.plavina@lu.lv

The Ubiquitin-Proteasome System (UPS) is essential for degrading misfolded proteins and mediating lymphocyte growth, activation, and differentiation. By regulating inflammatory factors and modulating CD4+, CD8+, and B-cell responses, the UPS plays a critical role in the pathogenesis of autoimmune diseases like MS [1].
In MS, inflammation and proteasomal dysfunction are closely linked. Although elevated enzymatic activities have been reported in the CNS of MS patients [2], this increased demand may eventually lead to a state of proteostatic insufficiency. The resulting accumulation of specific proteins due to such impaired proteasome efficiency can exacerbate inflammatory responses and neuronal damage [3]. Despite these mechanistic insights, the relationship between proteasome gene expression and the clinical severity and treatment context in MS cohorts remains poorly understood. This study aimed to assess associations between expression of selected proteasome genes and clinical parameters in Latvian MS patients, and to explore whether expression profiles differ across disease- and therapy-related strata.

Gene expression levels of key proteasome/UPS components (including PSMA3, PSMA6, PSMA7, and PSMC6) were quantified in MS patients from a Latvian cohort using PCR-based assays. Clinical variables included disability (EDSS), disease duration, age, and inflammatory/immunological markers (e.g., IgG). Group differences were tested using non-parametric methods (Mann-Whitney/Kruskal-Wallis or ANOVA where applicable), and associations with continuous clinical variables were evaluated by Spearman correlation. Therapy-related analyses compared patients on treatment versus not treated and/or across therapy types (e.g., IFN-β, GA, MX).

Across the MS group, expression medians did not show consistent differences by sex or by broad clinical subtype in the tested comparisons. In contrast, therapy context was associated with expression variability, with significant between-therapy differences detected for multiple genes, including PSMA3 (Kruskal–Wallis $P = 4.95\times10^{-2}$), PSMA7 ($P = 1.43\times10^{-2}$), and a stronger effect for PSMA6 ($P = 1.47\times10^{-3}$). Clinically, higher disability was linked to lower expression: EDSS showed negative correlations with PSMA3 ($\rho = -0.21$; $P = 2.04\times10^{-2}$), PSMA7 ($\rho = -0.20$; $P = 2.18\times10^{-2}$), and PSMA6 ($\rho = -0.20$; $P = 2.22\times10^{-2}$). Disease duration correlated with PSMA3 ($\rho = 0.18$; $P = 3.71\times10^{-2}$). Immunological status also related to UPS expression: IgG correlated negatively with PSMA3 ($\rho = -0.21$; $P = 2.06\times10^{-2}$) and PSMC6 ($\rho = -0.22$; $P = 1.65\times10^{-2}$). Age showed modest inverse associations with PSMA7 ($\rho = -0.20$; $P = 2.15\times10^{-2}$) and PSMA6 ($\rho = -0.15$; $P = 4.7\times10^{-2}$).

In conclusion, our results demonstrate a significant association between proteasome gene expression, EDSS, and immunological markers in MS, highlighting the UPS as a potential target for monitoring disease severity and therapeutic efficacy. Further studies are needed to evaluate the potential of these subunits as predictive biomarkers for disease progression and treatment efficacy.

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A28

INVESTIGATING CRITICAL PATHWAYS THAT ACT ALONGSIDE HISTONE INHERITANCE TO MAINTAIN EPIGENETIC CELL IDENTITY

Aina El Ravvas[1,2], Fion Jenkinson[1,2], Anja Groth[1,2]

[1]Danish Cancer Institute, Center for Epigenetic Cell Memory (EpiC), Copenhagen, Denmark
[2]University of Copenhagen, Novo Nordisk Foundation Center for Protein Research (CPR), Copenhagen, Denmark
aina.elrawas@gmail.com

Inheritance of cell identity depends on accurate propagation of epigenetic information during DNA replication. Parental histone recycling is a key mechanism underlying this process and is mediated in part by the replicative helicase subunit Mcm2. Disruption of symmetric parental histone recycling using a separation-of-function allele (*Mcm2-2A*) alters chromatin inheritance and impairs cell identity transitions in mouse embryonic stem cells (mESCs) [1], underscoring its importance in maintaining epigenetic cell identity. In addition to histone recycling, faithful epigenetic inheritance also relies on coordinated activity of DNA methylation and an array of chromatin-maturation pathways [2]. However, it is unclear whether all pathways that contribute to stable cell identity have been identified, and what their relative importance is in the contexts of cell division and differentiation.

To investigate this, this project aims to demonstrate and characterize synthetic genetic interactors of *Mcm2-2A* in self-renewing and differentiating mouse embryonic stem cells (mESCs). Inducible knockout wild-type and *Mcm2-2A* cell lines were generated of several gene candidates previously identified in a genome-wide CRISPR screen. A competition-based flow cytometry assay was established and confirmed to quantify cell fitness over time. This set up allowed relative mESC fitness to be assayed both in self-renewing conditions by cell culture in serum/LiF medium, and upon cell identity change by differentiation into Retinoic Acid (RA) medium.

Using this approach, a top-hit gene was validated as a genetic interactor of Mcm2-2A: surprisingly, knockout of this gene in wildtype cells conferred a fitness advantage in self-renewing conditions that was abrogated in the *Mcm2-2A* background. During differentiation in RA, the effects of these mutations became deleterious and are being further studied using complementary live-cell imaging, to temporally resolve synthetic lethal interactions during cell identity change. Ongoing experiments extend this strategy to additional candidate genes, including those with unannotated function or unestablished link to chromatin inheritance, as well as known players.

Through this framework, this project seeks to clarify how multiple epigenetic maintenance pathways cooperate with histone inheritance to appropriately preserve or alter cell identity. This may provide insight into diseases associated with epigenetic instability and cell identity aberrations, including cancer, and indicate vulnerabilities that could be therapeutically exploited.

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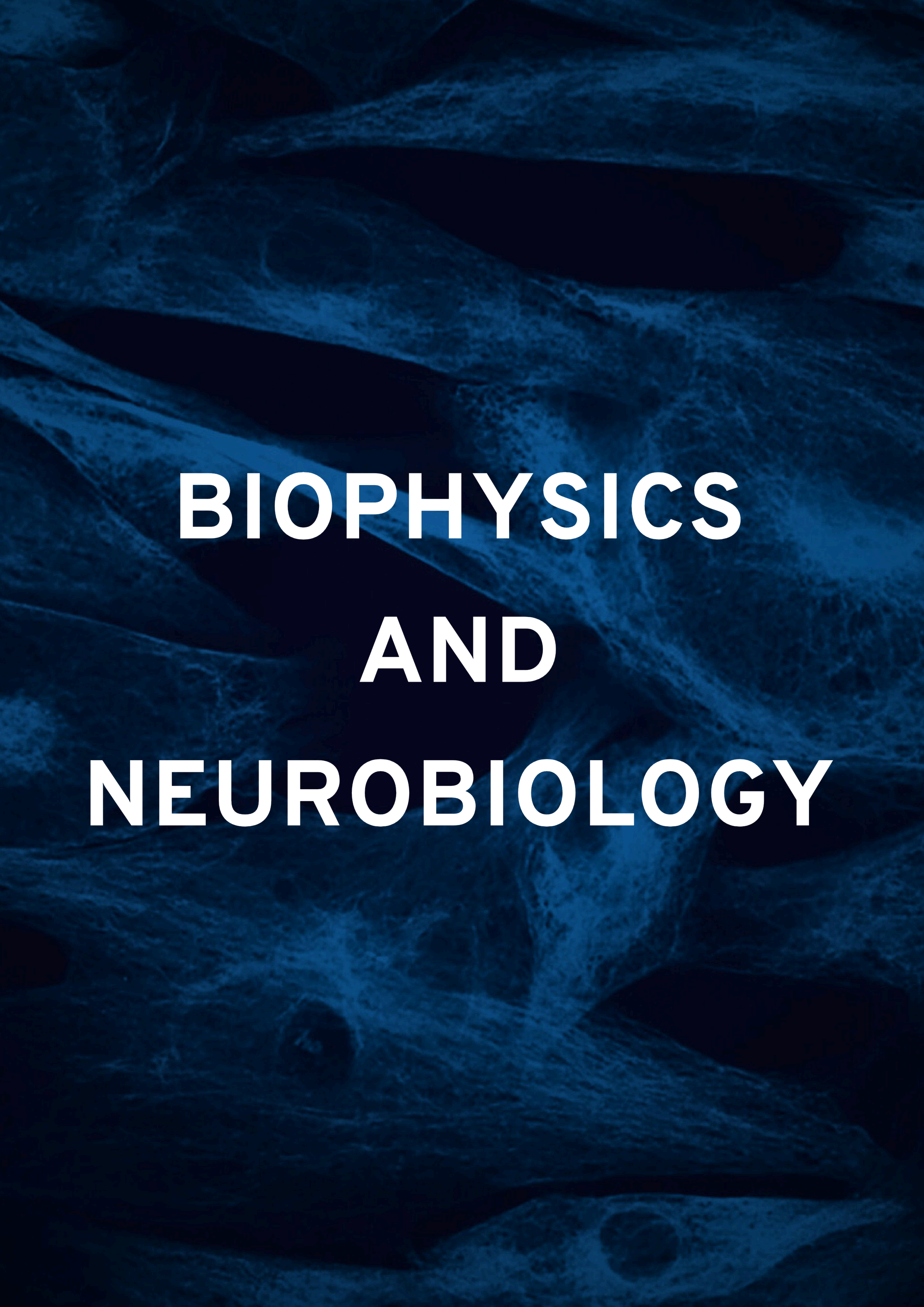
BIOPHYSICS AND NEUROBIOLOGY



ENGINEERING A CHIMERIC CARBONIC ANHYDRASE I TO REPROGRAM ISOFORM-SPECIFIC INHIBITOR BINDING

Ignas Glušnys, Aurelija Mickevičiūtė, Daumantas Matulis, Joana Smirnovienė

Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania
ignas.glusnys@gmc.stud.vu.lt

Carbonic anhydrases (CAs) are zinc-containing metalloenzymes essential for regulating physiological pH and bicarbonate homeostasis. Developing isoform-selective inhibitors remains a major pharmacological challenge due to the high structural conservation among the twelve catalytically active human CA isoforms [1]. Specifically, human CA I is highly abundant in erythrocytes and frequently acts as an unintended off-target for inhibitors designed for other therapeutic isoforms, resulting in systemic side effects. CA II is the most characterized isoform and a primary drug target for glaucoma, epilepsy, and edema.

This study investigates a genetically engineered chimeric protein, chCA I, based on the CA II scaffold but containing CA I-specific active site residues. The goal was to determine if these mutations alone are sufficient to switch the inhibitor recognition profile from CA II to CA I and analyze structural differences between CA I and CA II. The binding affinities of a focused library of sulfonamide-bearing inhibitors were analyzed against human CA I, CA II, and the engineered chCA I. The binding constants (K_b) were determined using the Fluorescent Thermal Shift Assay (FTSA, also known as Thermofluor®). This method allows for the precise quantification of ligand-induced thermal stabilization, which correlates directly with binding affinity [2]. Data analysis and parameter calculation were performed using the online Thermott software (Thermott.com, Figure 1) [3]. The introduction of CA I-specific active site mutations in CA II caused the chimeric protein to recognize sulfonamide inhibitors with affinities closely mirroring those of native CA I. As evidenced by the FTSA data, compounds that exhibited high affinity for CA I but low affinity for CA II bound to chCA I with high affinity (values approaching), effectively "switching" the enzyme's preference.

The results demonstrate that a set of active site residues is sufficient to dictate the isoform-selective binding profile of CA I, since the chimeric CA I successfully mimics the ligand recognition properties of CA I. The characterized chCA I protein could serve as a model for screening and designing highly CA-selective inhibitors, potentially reducing off-target interactions in future therapeutic developments.

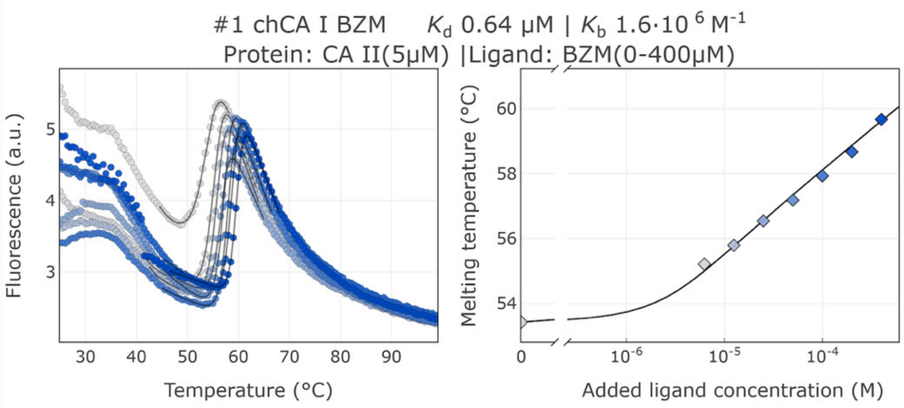


Figure 1. Fluorescent thermal shift assay (FTSA) results of chimeric carbonic anhydrase I binding to brinzolamide (BZM) – symbols correspond to experimental data while the line shows fitting using the K_d determination model. Data fitted and figure prepared using Thermott [3].

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B2

PSILOCYBIN INDUCES SYNAPTIC REMODELING BY MODULATING MICROGLIA

Milda Markutė[1], Ieva Šiugždaitė[1], Louise Ellen Schuberth[2], Maxime Veleanu[2], Stefan Vestring[2], Urtė Neniškytė[1]

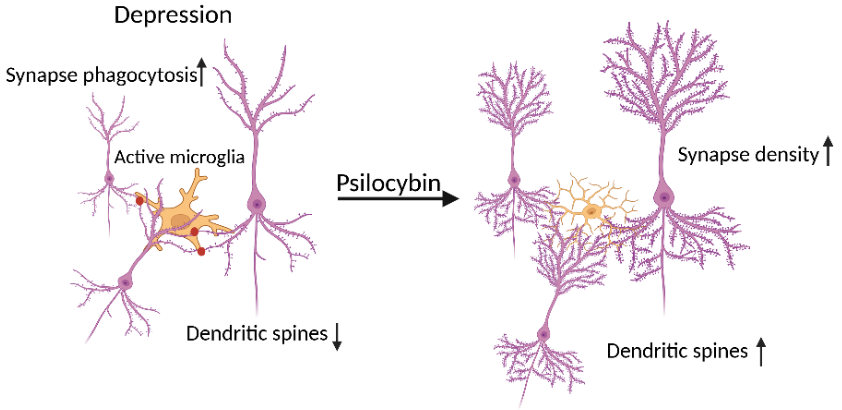
[1]Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]University Medical Center Freiburg, Dept. of Psychiatry and Psychotherapy, Germany
milda.markute@gmc.stud.vu.lt

According to the World Health Organization, Major Depressive Disorder (MDD) affects 5.7% of adults, of which 30% have treatment-resistant depression; therefore psychedelics are being investigated as a potential new therapy. MDD presents itself with a decrease in brain structure volume and plasticity, especially prefrontal cortex, hippocampus and amygdala [1]. Microglial cells are brain immune cells, that also prune inactive synapses. It is also known that in MDD, microglial phagocytosis of synapses increases, which leads to reduced synaptic plasticity. Psilocybin is now being researched as a possible treatment for MDD since it increases neuroplasticity and alleviates depression symptoms [2]; however, the mechanism is still not known. It is known that psilocybin lowers microglial phagocytic activity and production of ROS and NO [2], [3]. We hypothesize that psilocybin induces the number of synapses by modulating microglial activity (Fig. 1). This study aims to compare the number of synapses in stressed and psilocybin-treated animals and to identify microglial involvement in this process.

Experiment included six mice groups: control, 1 mg/kg psilocybin, 2 mg/kg psilocybin, held while others experienced CDM and saline, stressed and saline, stressed and 1mg/kg psilocybin. The brains were cryosectioned and stained using immunofluorescence. To identify synapses, we used presynapse (anti-VGAT) and postsynapse (anti-GEPHYRIN) antibodies and microglial (anti-IBA1) in the dorsal hippocampus. Brains were imaged using the SP8 confocal microscope. The differences in synaptic density between groups are presented in 3D reconstruction images obtained using IMARIS software.

Understanding the mechanism of psilocybin-induced neuroplasticity could help understand microglia cell physiology and extend MDD treatment options.

Figure 1. In depression, the number of synapses decreases, which could be caused by their increased phagocytosis by microglia cells. We hypothesize that psilocybin increases the number of synapses by modulating microglial phagocytic activity. Created by BioRender.



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Menstrual Cycle & Control: Impulsivity and Inhibition Across the Menstrual Cycle

Gabrielė Kukytė[1], Monika Palaimaitė[1], Ingrida Zelionkaitė[1], Rimantė Gaižauskaitė[1], Lauryna Filatovaitė-Vyčienė[1], Ramunė Grikšienė[1]

[1]Department of Neurobiology and Biophysics, Institute of Biosciences, Life Science Center, Vilnius University, Lithuania
gabriele.kukyte@gmc.stud.vu.lt

Fluctuations in ovarian hormones across the menstrual cycle have been proposed to modulate neural systems supporting cognitive control and response inhibition. However, menstrual cycle phase is often insufficiently reported in studies of inhibitory control and impulsivity. Given the role of GABAergic modulation in excitation–inhibition balance, precise cycle phase identification is critical for interpreting neurophysiological findings. This study examined menstrual cycle phase-dependent differences in impulsivity, well-being, and behavioral inhibition in healthy women.

To capture menstrual cycle dynamics with higher temporal resolution, data were collected six times during a cycle per participant. Assessments were conducted during the early and late follicular, at ovulation, and during the early, mid, and late luteal phases. The Barratt Impulsiveness Scale (BIS-11) and the Well-Being Questionnaire (WBQ) were administered twice - during the early follicular and late luteal phases, to assess impulsivity and well-being. Go/No-Go task was performed to measure response inhibition, omission, reaction times and behavioral response variability at all six phases.

Four healthy women (19–30 years) with regular menstrual cycles (24–35 days) completed the study by the time of abstract preparation.

Friedman tests were conducted to evaluate menstrual cycle phase-related differences of Go/No-Go task data, with Kendall's coefficient of concordance (W) reported as an estimate of effect size. Omission rates were zero across phases, indicating sustained attentional engagement. Inhibition accuracy showed no significant effect ($\chi^2(5) = 4.56$, $p = 0.47$), with a small to moderate effect size ($W = 0.23$). Median reaction time likewise remained stable ($\chi^2(5) = 7.24$, $p = 0.203$) despite a moderate effect ($W = 0.36$). Reaction time variability also showed no significant differences ($\chi^2(5) = 7.14$, $p = 0.21$), though a moderate effect size ($W = 0.36$) suggests subtle modulation of response stability. However, these findings should be interpreted cautiously given the small sample size and ongoing data collection.

No significant differences were found across phases of the menstrual cycle in Go/No-Go task performance in healthy women. However, small to moderate effect sizes suggest potential subtle effects on inhibitory control and response stability.

B4

DIETARY PREBIOTIC INTERVENTION CAN COUNTERACT HIGH-FAT DIET-ASSOCIATED MICROGLIAL ALTERATIONS IN THE AGED MICE

Urtė Minkevičiūtė[1], Arnas Kunevičius[1], Akshay Kumar Vijaya[1], Aurelijus Burokas[1]

[1]Department of Biological Models, Biochemistry Institute, Vilnius University, Lithuania
urte.minkeviciute@gmc.stud.vu.lt

Consumption of a chronic high-fat diet (HFD) and ageing are both associated with metabolic dysfunction and increased neuroinflammation. It contributes to the loss of gut microbiota diversity and the development of neurodegenerative diseases. Microglia, the resident immune cells of the central nervous system, are highly sensitive to metabolic and inflammatory signals, and their morphology is closely linked to their functional state. These metabolic and inflammatory molecular signals can be derived from the gut microbiota and influence microglial activation and disease progression. Gut microbiota-focused interventions have the potential to reduce chronic inflammation and have a protective effect on microglia. However, the impact of such interventions in the aged brain remains poorly understood, especially regarding their ability to reverse metabolically primed microglia phenotypes.

In this study, we examined how prebiotic supplementation with galactooligosaccharides and fructooligosaccharides (GOS+FOS) modulates microglial morphology in aged mice exposed to long-term HFD. The C57BL/6J mice were assigned to four dietary conditions for 18 months: control diet (CD), CD - GOS+FOS, HFD, and HFD - GOS+FOS. We utilised immunohistochemical staining to quantify three-dimensional branching architecture and two-dimensional shape descriptors.

Compared with CD, microglia in HFD-fed conditions exhibited quantifiable morphological change consistent with a metabolically primed microglial phenotype: increased total branch number and length, greater slab voxel count, triple-junction frequency, enlarged projected cell area and perimeter. HFD supplementation with GOS+FOS reduced several of these HFD-induced structural alterations: average branch length decreased towards CD levels, projected soma area and perimeter were significantly smaller than in HFD, indicating partial structural recovery. Importantly, in the CD - GOS+FOS group, microglial morphology was similar to that in the CD group, indicating that the effects of GOS+FOS are specific to reducing HFD-associated microglial alterations rather than modifying baseline microglial structure.

Collectively, these findings suggest that prebiotic supplementation with GOS+FOS can modulate microglial structural remodelling in the aged brain under metabolic challenge, highlighting a protective role for gut microbiota-targeted interventions in diet-induced neuroinflammatory conditions.

B5

BENZENESULFONAMIDES TARGETING CARBONIC ANHYDRASE ISOZYMES: A STRUCTURE-AFFINITY RELATIONSHIP STUDY

Ieva Šileikytė, Edita Čapkauskaitė, Audrius Zakšauskas, Asta Zubrienė

Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
ieva.sileikyte@gmc.stud.vu.lt

The quantification of the binding of low molecular weight chemical compounds to proteins is a field of great importance in biomedical sciences, especially in drug design [1]. Any drug-like chemical compound should bind the disease-related target protein with sufficiently high affinity. Furthermore, the compound must bind with high selectivity, avoiding strong interactions with nontarget proteins that could cause undesired side effects [2]. It has been proposed that the optimization of the thermodynamic and kinetic parameters of ligand binding to proteins should lead to compounds with improved interaction characteristics and greater potential as drugs.

Human carbonic anhydrases (CAs) are ubiquitous zinc enzymes that efficiently catalyze the reversible hydration of carbon dioxide and have many essential physiological functions. There are 12 catalytically active isozymes of CAs in the human body. The main functions of these enzymes are CO_2/bicarbonate transport, pH and electrolyte homeostasis and regulation. Several drugs targeting multiple CA isozymes are used to treat diseases like glaucoma, edema, obesity and epilepsy [3]. CA IX is recognized as a cancer-associated isozyme, as it is overexpressed in the majority of hypoxic tumors. Selective inhibition of CAIX is believed to potentially reduce the progression of the disease [4].

The predominant CA inhibition mechanism is binding to the catalytic zinc ion, and benzenesulfonamides are among the most investigated CA inhibitors that bind this way. Their amino group binds directly to the zinc ion in the enzyme's active site, forming a coordination bond that suppresses the activity of all CA isozymes. The binding affinity may vary and depends on the aromatic/heterocyclic scaffold of the sulfonamide and the functional groups linked to it [3].

In this study, we investigated a series of newly synthesized benzenesulfonamide derivatives containing different fragments at the 2-, 3-, and 4-positions as CA inhibitors. The binding affinities of these benzenesulfonamides with 12 catalytically active CA isozymes were measured using fluorescence-based thermal shift assay (FTSA). Structure-affinity relationship analysis of the compounds was performed to show how small changes in the substituents at various positions of the benzenesulfonamide ring affect binding affinities to CA isozymes. The bulky moiety-bearing compound was found to be the most potent and selective CAIX inhibitor, binding with a low nanomolar dissociation constant (Fig.1).

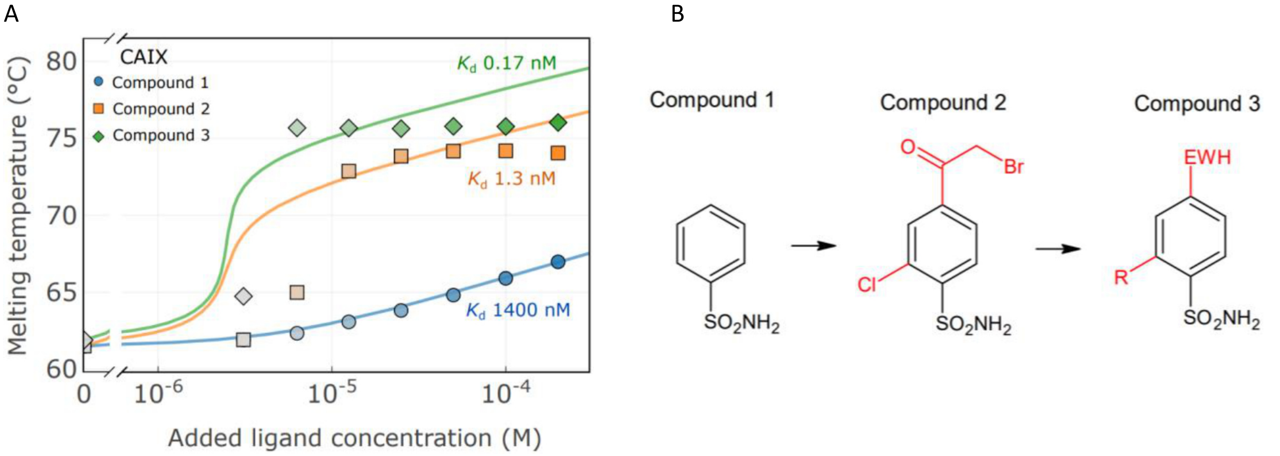

Figure 1. The dose-response curves illustrate the interaction of compounds 1, 2 and 3 with CAIX (A). Structural variations of benzenesulfonamides yielding an electrophilic warhead–containing CAIX ligand (B).

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PHOTOSTABILITY AND OPTICAL PROPERTIES OF BSA- GOLD NANOCLUSTERS FOR MULTIMODAL IMAGING

Skaistė Talanovaitė[1], Greta Tamoliūnaitė[1], Vilius Poderys[1], Vitalijus Karabanovas[1,2]

[1]Biomedical Physics Laboratory, National Cancer Institute, Vilnius, Lithuania
[2]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania
skaiste.talanovaite@gmc.stud.vu.lt

Multimodal imaging nanostructures show strong potential for improving clinical diagnostics, particularly detection of cancer. Technetium-99m labelled bovine serum albumin- gold nanoclusters (^{99m}Tc-BSA-AuNCs) complex is a promising multimodal imaging contrast agent that combines photoluminescence and SPECT/CT imaging. Previous studies have demonstrated that radiolabelling with 99mTc does not affect BSA-AuNCs optical properties [1]. However, their photostability and other optical characteristics have not yet been fully investigated. In this study, we measured absorption and fluorescence spectra, zeta potential and size distribution of BSA-AuNCs over several months. In addition, photobleaching lifetimes were experimentally determined and analysed.

BSA-AuNCs synthesis is based on Xie et al. [2]. Tetrachlorauric acid ($HAuCl_4$) was mixed with BSA and sodium hydroxide (NaOH). Synthesized nanoclusters were subsequently dialyzed and filtered. The resulting BSA-AuNCs had size ranging from 5 nm to 15 nm.

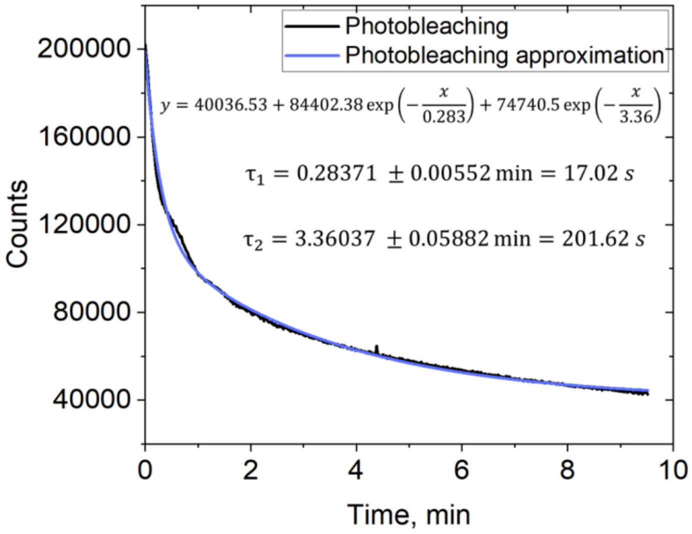
Two months after synthesis, fluorescence spectra exhibited a blueshift from 660 nm to 650 nm, indicating stability of nanoclusters and the absence of aggregation. Over the same period, the zeta potential of BSA-AuNC became more negative, which could potentially reduce interaction between BSA-AuNC and cancer cells. Nevertheless, the zeta potential remained around -25 mV, a value considered suitable for application in cancer treatment and diagnostics.

Photobleaching behaviour was analysed to assess suitability for clinical application. The calculated photobleaching lifetimes revealed two components: a short lifetime $\tau_1 = 17.02$ s and a long lifetime $\tau_2 = 201.62$ s. Moreover, when photobleaching and recovery times were equal (or recovery time exceeded photobleaching) the BSA-AuNCs fluorescence emission fully recovered.

Photobleaching behaviour was analysed to assess suitability for clinical application. The calculated photobleaching lifetimes revealed two components: a short lifetime $\tau_1 = 17.02$ s and a long lifetime $\tau_2 = 201.62$ s (Fig. 1). Moreover, when photobleaching and recovery times were equal (or recovery time exceeded photobleaching) the BSA-AuNCs fluorescence emission fully recovered.

In conclusion, our results demonstrate that BSA-AuNCs are photostable nanostructures with favourable optical properties, supporting their potential use as a multimodal agent for clinical imaging application.

Figure 1. Photobleaching of BSA-AuNCs with 405 nm and 30 mW laser and exponential approximation.



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B7

THE LINKS BETWEEN PUPIL PARAMETERS AND SEX STEROID HORMONES IN DIFFERENT PHASES OF THE MENSTRUAL CYCLE DURING AN EMOTION RECOGNITION TASK

Kamilė Gindulytė, Milda Brokevičiūtė, Dominyka Brūzdaitė, Rimantė Gaižauskaitė, Ramunė Grikšienė, Ingrida Zelionkaitė

Department of Neurobiology and Biophysics, Life Science Center, Vilnius University, Lithuania
kamile.gindulyte@gmc.stud.vu.lt

The menstrual cycle is characterised by fluctuations in estrogen and progesterone. These hormonal changes are associated with alterations in the autonomic nervous system activity and may be reflected by pupil dynamics. Previous research suggests that a rise in estrogen during the follicular phase may be associated with smaller pupils (increased parasympathetic activity), whereas elevated progesterone levels in the luteal phase may be linked to larger pupils (increased sympathetic activity) [1]. Although pupil size has been used as a marker of emotional processing [2], findings on its reactivity to emotional faces remain inconsistent, and research across menstrual cycle phases is limited. Therefore, this study aimed to investigate the association between hormonal fluctuations across the menstrual cycle and pupil parameters during an emotion recognition task.

32 naturally cycling women (19-35 years old) visited the laboratory during the early follicular, ovulation and mid-luteal phases of the menstrual cycle. During each visit the participants completed an emotion recognition task consisting of facial expressions portraying neutral faces and different emotions (anger, sadness, fear, happiness). Throughout the task, pupil size was recorded using a Tobii eye tracking device. Pupil parameters such as the amplitude of the light reflex, slope, average pupil diameter, and area under the curve were analysed. Saliva samples were collected before and after the task to determine progesterone and testosterone concentrations.

No effects of menstrual cycle phase were observed on emotion recognition accuracy or pupil parameters (all $p \geq .717$). Meanwhile, a significant effect of emotion on pupil size dynamics was found. Slope, area under the curve, and average pupil diameter were larger when participants looked at angry faces compared to all other emotions (all $p \leq 0.001$). The area under the curve was larger when looking at fearful faces compared to neutral ones ($p = 0.035$). Additionally, slope and average pupil diameter were smaller when observing happy facial expressions than fearful (all $p \leq 0.001$), but larger when looking at sad faces (all $p \leq 0.001$). All participants recognised neutral facial expressions more accurately than emotions (all $p \leq 0.001$). Correlation analysis revealed that higher progesterone levels were associated with increased amplitude of the light reflex in the luteal phase ($r = -0.45$, $p \leq 0.001$).

Findings suggest that pupils' reactivity to emotional facial expressions does not vary across the menstrual phases, but changes in pupil parameters occur when different emotions are being observed.

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B8

ANALYSIS OF COMPLEX FORMATION BETWEEN BLOOD PLASMA PROTEIN GOLD NANOCLUSTERS AND CE6 USING FLUORESCENCE ANISOTROPY

Greta Tamoliunaite[1], Skaiste Talanovaite[1], Vilius Poderys[1], Vitalijus Karabanovas[1,2]

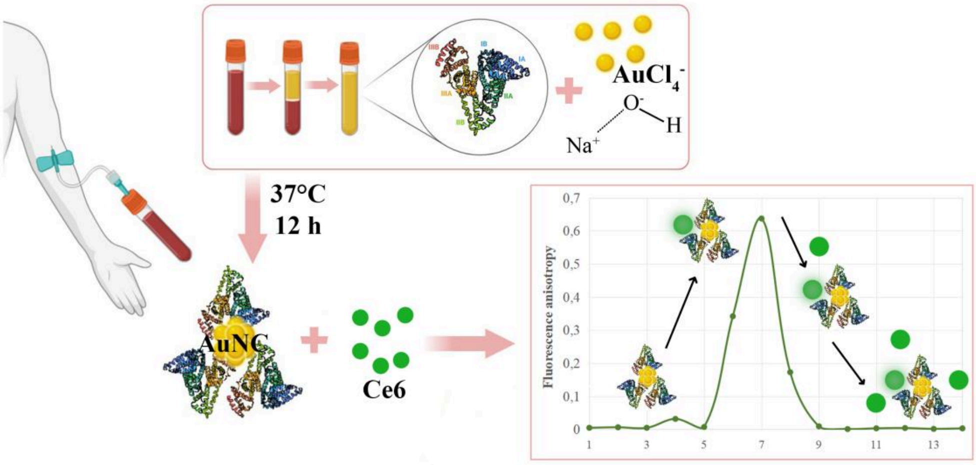
[1]Biomedical Physics Laboratory, National Cancer Institute, P. Baublio ST. 3b, Vilnius, Lithuania
[2]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania
greta.tamoliunaite@gmc.stud.vu.lt

Many current cancer treatment strategies rely on evading immune system activation or actively work against it, which can be remedied with multimodal personalized medicine [1]. Personalized biological compounds enriched with photosensitizer properties are key complexes to develop, but evaluation of their interaction is complicated. Fluorescence anisotropy, which evaluates the degree by which the polarized light changes in the sample, can be used as a quantitative analysis method for the complex formation [2].

The main objective of this study is to synthesize personalized gold nanoclusters using blood plasma proteins (HSA-AuNC) and combine this structure with photosensitizer Chlorin e6 (Ce6) to enable singlet oxygen generation. Following BSA-based AuNC synthesis and purification method [3], human blood plasma was used to decorate AuNC (Fig. 1). Resulting compounds were characterized by optical analysis methods and combined with different volumes of Ce6, initiating complex formation. Resulting HSA-AuNC-Ce6 interactions were characterized via fluorescence emission and fluorescence anisotropy measurements.

HSA-AuNC synthesis products were optically characterized as suitable and stable. Red-shift of the complex fluorescence emission spectrum confirms HSA-AuNC interaction with photosensitizer Ce6. Fluorescence anisotropy values are based on the changes of polarized light and indicate the rotational potential of Ce6. The complex interacts with polarized light differently than free Ce6, so anisotropy values increase with increasing Ce6 concentration as its rotational potential is highly restricted in the complex. Anisotropy saturation does not occur, but rather anisotropy values start rapidly decreasing when Ce6 starts dominating (Fig. 1).

Figure 1. Schematic representation of HSA-AuNC synthesis and FL anisotropy of its interaction with Ce6.



Our synthesized HSA-AuNC are decorated with blood plasma proteins, making them fit for personalized medicine route. A readily formed complex of HSA-AuNC and Ce6 would enrich the compound with photosensitizer properties and can be evaluated via fluorescence anisotropy.

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EEG INVESTIGATION OF EMOTION REGULATION ACROSS THE MENSTRUAL CYCLE: A PILOT STUDY IN HEALTHY WOMEN

Monika Palaimaitė[1], Gabrielė Kukytė[1], Ingrida Zelionkaitė[1], Rimantė Gaižauskaitė[1], Lauryna Filatovaitė-Vyčienė[1], Ramunė Grikšienė[1]

[1]Department of Neurobiology and Biophysics, Institute of Biosciences, Life Science Center, Vilnius University, Lithuania
monika.palaimaite@gmc.stud.vu.lt

Emotion regulation capacity in healthy has been suggested to vary across the menstrual cycle and is linked with changes in gonadal hormone levels and associated cognitive processes. However, most existing studies assess menstrual cycle effects using broad phase categorizations. Data capturing emotional regulation across multiple menstrual cycle phases using repeated within-subject remain limited. This pilot study aims to develop and evaluate the feasibility of an experimental paradigm for assessing emotional regulation across multiple phases of the menstrual cycle in healthy women, using repeated behavioral assessments and concurrent neural measures to explore preliminary phase-related trends.

Nine healthy women participated in the pilot study, and four completed the experiment by the date of abstract preparation. Therefore, data of four participants (25.5 ± 2.5 years) are presented here. Participants completed experimental sessions across early follicular (EF), ovulation (O), and late luteal (LL) phases of the menstrual cycle. During each session, women performed an emotion regulation (ER) task involving cognitive reappraisal, provided subjective valence and arousal ratings, and completed the Difficulties in Emotion Regulation Scale (DERS) and Emotion Regulation Questionnaire (ERQ). ERQ contains two subscales: cognitive reappraisal and expressive suppression. Electroencephalograms (EEG) were recorded to derive event-related potential (ERPs).

Emotion regulation success was quantified as changes in subjective valence and arousal ratings following regulation, with higher valence and lower arousal reflecting more effective emotion regulation. Mean arousal regulation effects were 21.0% (EF), 18.5% (O), and 17.8% (LL), valence increases were largest during ovulation (46.2%) and lowest during the late luteal phase (14.1%). These values suggest the smallest regulation effect during the late luteal (LL) phase relative to early follicular (EF) and ovulation (O) phases, a pattern consistent with existing menstrual cycle research. ERQ analysis indicated that participants in this pilot sample reported greater use of cognitive reappraisal than expressive suppression as an emotion regulation strategy. Mean reappraisal scores were 5.13 ± 0.44 (EF) and 4.96 ± 0.91 (LL), and suppression scores were 4.75 ± 0.89 (EF) and 4.31 ± 0.66 (LL). Self-reported emotion regulation difficulties (DERS) indicated generally low-to-moderate difficulties. Mean DERS total scores were similar across early follicular (M = 110.3) and late luteal (M = 113.0) assessments.

Although based on a small pilot sample, the descriptively lower regulation success during the late luteal phase is consistent with previous menstrual cycle research supporting the feasibility of the paradigm and its use for future biomedical research.

B10

ASSESING THE EFFECT OF H_2O_2 INDUCED OXIDATIVE STRESS ON *NITTELOPSIS OBTUSA* PHOTOSYNTHESIS

Viltė Mickytė[1], Aušrinė Navickaitė[1], Vilma Kisnierienė[1]

[1]Department of Neurobiology and Biophysics, Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
vilte.mickyte@gmc.stud.vu.lt

Excessive oxidative stress has a negative impact on plant and algae photosynthetic processes. It can result from adverse light conditions, including intense light exposure, UV radiation, along with nutrient shortages, exposure to various chemical agents. Since light emission and photochemical reactions compete directly for excitation energy, measuring the fluorescence emitted by chlorophyll molecules is a frequently used method to investigate how stressors affect photosynthetic performance in algae and plants. Increase in the fluorescence intensity is an indicator of disruption in the photosynthetic process (1).

Photosystem II (PSII) activity can be evaluated by monitoring photosynthetic parameters (2). To assess the effects of H_2O_2 induced oxidative stress to macroalgae *Nittelopsis obtusa*, we utilised bright-field microscopy, kinetic pulse-amplitude-modulation (PAM) fluorimetry and spectroscopic analysis of photosynthetic pigments to evaluate photosynthetic performance and changes of pigment composition after exposure to H_2O_2. During the experiments selected internodal *N. obtusa* cells were submerged in APW (artificial pond water) solution and kept for 12 h in low-intensity illumination. Afterwards, half of the cells were kept in APW solution, while other half were submerged in 250 µmol H_2O_2 solution and incubated for 6 to 24 h, depending on the experiment protocol.

Bright-field imaging revealed that after H_2O_2 exposure chloroplast layer within *N. obtusa* cells became less dense, as shown in Figure 1A-B. PAM fluorimetry analysis revealed a decline in the quantum yield (Y(II)) of PSII after cells were exposed to 250 µmol H_2O_2 solution. Spectroscopic pigment analysis following H_2O_2 treatment revealed a decrease in carotenoid content which functions as antioxidant in cells. The results indicate that H_2O_2 induced oxidative stress negatively affects structural organization of chloroplast layer within internodal cells, reduces photosynthetic efficiency of PSII and decrease in carotenoid levels.

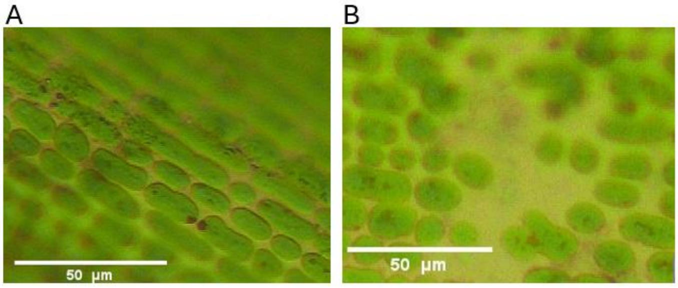

Figure 1. Brigth-field microscopy imaging under control conditions (A) and after incubation of cells in 250 µmol H_2O_2 solution (B).

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B11

EFFECTS OF VIRAL RNA-MIMETIC ON MICROGLIA CELL CULTURES, MITOCHONDRIAL FUNCTIONS AND NETWORK FORMATION

M. Merkelis[1,2,3], K. Pampuscenko[3], D. Umbrasas[3], V. Borutaite[3]

[1] Faculty of Pharmacy, Lithuanian University of Health Sciences, Kaunas, Lithuania
[2] Faculty of Chemical Technology, Kaunas University of Technology, Kaunas, Lithuania
[3] Neuroscience Institute, Lithuanian University of Health Sciences, Kaunas, Lithuania
e-mail of contact person: matas.merkelis@lsmu.lt

Neuroinflammation is one of the main processes linked to the development and progression of neurodegenerative diseases such as Parkinson's and Alzheimer's disease. These illnesses are caused by neuronal damage and loss in different brain areas. There are numerous pathogens that can cause neuroinflammation, including bacterial and viral infections. Infectious agents and their components activate immune response and cells, including specialized brain tissue macrophages - microglia, which play a crucial role in the development of neurodegenerative diseases and can have a negative effect on neuronal functions and viability. Viral components interact, activate and modulate resident immune cells' functions and responses via pattern recognition receptors (PPR) and different PPR family types such as Toll-like receptors (TLR). Several studies suggest that mitochondrial metabolism and dynamics can modulate neuroinflammatory responses in microglia, however the molecular mechanisms underlying this effect remain poorly characterized.

In this study, we investigated the effects of viral RNA-mimetic loxoribine, acting as a TLR7 activator, on microglial BV-2 cells and their mitochondrial dynamics and metabolic changes. The study was carried out using various methods, including fluorescence microscopy, BrdU assay, polymerase chain reaction (PCR) and Oroboros O2k oxygraphic system.

Our experimental data indicate that loxoribine exerts a concentration-dependent effect: lower concentrations of loxoribine increase complex I and II substrate-stimulated ADP phosphorylation rate, while substantially higher concentrations of loxoribine inhibit mitochondrial respiration rate at the same respiratory states. Also, at lower concentrations, loxoribine tends to increase significantly the expression of the mitochondrial biogenesis regulatory gene *PGC-1α* and, at higher concentrations, - expression of the mitochondrial fission factor gene *Mff*. Gene expression of other mitochondrial dynamics markers, such as fusion marker mitofusin 1 (*Mfn1*), mitofusin 2 (*Mfn2*), Optic Atrophy 1 (*Opa1*) or Dynamin-related protein 1 (*Drp1*), Mitochondrial fission 1 protein (*Fis1*) fission markers were not affected by loxoribine in BV-2 cell cultures. Moreover, loxoribine exerted no effect on microglial viability, proliferation or total mitochondrial level.

The present results suggest that viral RNA-mimetic loxoribine has concentration-dependent effects on mitochondria in microglial cells through increased expression of fission protein (Mff) and mitochondrial biogenesis regulatory factor (PGC-1α) as well as altered oxidative phosphorylation.

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B12

FUNCTIONALIZATION OF UPCONVERTING NANOPARTICLES WITH TUNABLE OEG-BASED COPOLYMERS: *IN VITRO* AND *IN VIVO* EVALUATION

Aleja Marija Daugelaite[1,2], Egle Ezerskyte[1,2], Danute Bulotiene[2], Kristina Bolgova[1], Arturas Katelnikovas[1], Vitalijus Karabanovas[2,3], Greta Butkiene[2], Vaidas Klimkevicius[1,2]

[1]Institute of Chemistry, Faculty of Chemistry and Geosciences, Vilnius University, Vilnius, Lithuania
[2]Biomedical Physics Laboratory, National Cancer Institute, Vilnius, Lithuania
[3]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania.
aleja.daugelaite@nvi.lt

Nanoparticles are promising platforms for drug delivery, bioimaging, and diagnostics due to their tunable size and surface chemistry. However, in biological environments, a protein corona rapidly forms around nanoparticles, which alters their physicochemical properties and biological behavior, thereby influencing circulation time, biodistribution, and cellular uptake [1]. Consequently, surface functionalization with hydrophilic polymers is widely employed to minimize nonspecific protein adsorption and improve colloidal stability. Poly(ethylene glycol) (PEG) has long been regarded as the gold standard for nanoparticle functionalization; however, its clinical limitations, including immunogenicity and accelerated blood clearance upon repeated administration, have motivated the development of alternative antifouling polymers such as poly(oligoethylene glycol) methacrylate, poly(2-oxazolines), etc. [2]. Despite advances in surface functionalization, the influence of polymer brush architecture, particularly side-chain length and grafting density, on nanoparticle–biological interactions remains insufficiently understood.

In this study, we designed and synthesized brush-type copolymers, p(DMAm-co-OEG$_x$MA), containing catechol anchoring groups and oligoethylene glycol (OEG) side chains of tunable length (x = 5, 9, 19). These copolymers were used to functionalize core–shell NaGdF$_4$:Yb^{3+},Er^{3+}@NaGdF$_4$:Yb^{3+},Nd^{3+} upconverting nanoparticles (UCNPs), which were synthesized according to the previously reported procedure with minor modifications [3]. UCNPs were selected for their unique near-infrared excitation, deep-tissue imaging capability, and high photostability.

This study aimed to investigate how the length of OEG side chains influences the cellular uptake of UCNPs *in vitro*, as well as circulation in the bloodstream and biodistribution *in vivo*. Cellular internalization was evaluated using laser scanning confocal microscopy and spectrometric analyses in breast and prostate cancer cell lines, as well as skin-derived mesenchymal stem cells (S-MSCs). All polymer-modified UCNPs demonstrated comparable cellular uptake across the tested cell types. Cytotoxicity, assessed using the lactate dehydrogenase assay, confirmed that the functionalized UCNPs were biocompatible and did not induce detectable membrane damage or cytotoxic effects. A pilot *in vivo* study in Albino Wistar rats demonstrated that PEGylation significantly prolonged nanoparticle circulation in the bloodstream. PEGylated UCNPs remained detectable in serum after 24 h post-administration, whereas bare UCNPs were rapidly cleared. Tissue imaging showed predominant accumulation of both bare and PEGylated UCNPs in the liver and spleen, with minor accumulation in the lungs, suggesting preferential uptake by organs associated with mononuclear phagocyte system.

These findings demonstrate that polymer surface engineering enables stable functionalization of UCNPs without altering their intrinsic biocompatibility and plays a key role in modulating *in vivo* circulation and biological fate. This study advances understanding of how polymer brush design influences nanoparticle behavior and supports the development of UCNP-based nanoplatforms for biomedical imaging and future theranostic applications.

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DISSECTING CORTICAL CONTRIBUTIONS TO GAMMA-RANGE ENTRAINMENT AND KETAMINE-INDUCED DEFICITS

Arnas Rakauskas[1], Urtė Jašinskytė[1], Robertas Guzulaitis[1]

[1] Life Sciences Center, Vilnius University, Lithuania

arnas.rakauskas@gmc.vu.lt

Introduction. Cortical circuits exhibit both spontaneous rhythmic activity and robust entrainment to periodic sensory stimulation. In the auditory system, rhythmic sounds elicit the Auditory Steady-State Response (ASSR), which is typically strongest in the gamma range, around 40 Hz [1,2]. Although this preferred entrainment frequency is well described in humans and rodents, it remains unclear whether it emerges from cortical network dynamics or is already predetermined in subcortical auditory structures. ASSR deficits are a promising biomarker for neuropsychiatric disorders, particularly schizophrenia, where 40-Hz entrainment is markedly reduced in patients and high-risk individuals [3–5]. Comparable impairments in cortical entrainment are observed in rodent schizophrenia-like models where NMDA receptors are systemically suppressed [6,7], yet the primary neural targets of NMDA antagonists remain unresolved. Here, we aimed to determine (1) whether optimal entrainment is set by cortical networks or inherited from subcortical inputs, and (2) whether NMDA antagonists directly disrupt cortical network dynamics underlying neural entrainment.

Methods. Wild-type C57BL/6 mice were implanted with Teflon-coated stainless-steel electrodes in primary auditory cortex (A1) to record local field potentials (LFPs). To directly activate thalamocortical inputs, medial geniculate body (MGB) neurons were transduced with ChR2 (pAAV-CaMKIIa-hChR2(H134R)-mCherry, Addgene). An optic fibre (FT400EMT, 400 µm, 0.39 NA; Thorlabs) positioned above A1 delivered light pulses to evoke direct steady-state responses (dSSRs). ASSRs and dSSRs were elicited using chirp trains (5–100 Hz, 3-s duration) of either 2-ms white-noise clicks or 2-ms light pulses, respectively, with 2-s inter-chirp intervals. LFPs were recorded before and 5 min after administration of sub-anesthetic ketamine (20 mg/kg). Time–frequency decomposition (Morlet wavelets) quantified evoked and induced power and inter-trial coherence (ITC).

Results. Under baseline conditions, cortical entrainment to auditory chirps showed reduced responses in the high-frequency range, whereas direct optogenetic activation of thalamocortical projections preserved high-frequency entrainment. Ketamine administration impaired gamma-range entrainment during both auditory and optogenetic stimulation, reducing power and ITC across high frequencies.

Conclusions. These findings indicate that (1) the optimal entrainment to gamma frequency is imposed by subcortical auditory processing before reaching auditory cortex, and (2) ketamine directly disrupts cortical network dynamics governing entrainment. These results provide mechanistic insight into ASSR generation and its disruption in NMDA antagonist-based models of schizophrenia.

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IMMUNOLOGY

C1

CHARACTERISATION OF MONOCLONAL ANTIBODIES AGAINST STREPTOLYSIN O AND EPITOPE DETERMINATION

Adrija Buturlakina, Martynas Simanavičius, Indrė Kučinskaitė-Kodzė

Department of Immunology, Institute of Biotechnology, Life Scieces Center, Vilnius University, Lithuania
adrija.buturlakina@gmc.stud.vu.lt

Streptolysin O (SLO) is a cholesterol-dependent cytolysin (CDC) produced by *Streptococcus pyogenes*, also known as group A *Streptococcus* (GAS). It is a main virulence factor of *S. pyogenes* that causes mild human infections such as pharyngitis, scarlet fever or severe infections such as necrotizing fasciitis and streptococcal toxic shock syndrome [1]. In addition, recurrent GAS infections can lead to autoimmune diseases, including acute rheumatic fever and rheumatic heart disease (RHD) [2]. The pathogenic effect of SLO is based on its ability to form large pores approximately 25 to 30 nm in the cholesterol-containing cell membrane and induce host cell lysis [3]. Therefore, characterized SLO neutralizing antibodies may provide valuable tools for reducing pathogenic effects.

In this study, a 37 clones hybridoma collection producing monoclonal antibodies (MAbs) against SLO were characterized, evaluating their affinity, specificity to the antigen and potential applications in research. The MAbs were purified from a hybridoma growth medium using affinity chromatography. The purified MAbs were further characterized for specificity using enzyme-linked immunosorbent assay (ELISA), western blot (WB) and neutralizing activity determination methods. Among 37 MAbs, 16 were exclusively reactive with SLO, 21 MAbs were cross-reactive with perfringolysin O (PFO) and 22 MAbs were able to neutralize the cytolytic activity of SLO on a lung epithelial cell line. The results revealed four putative groups of the MAbs directed to different immunogenic regions of SLO. Furthermore, to determine the MAbs interaction site (epitopes), 8 overlapping SLO fragments were expressed in *E. coli*. WB revealed 20 out of 37 MAbs epitopes, other 17 MAbs recognition sites weren't determined.

In conclusion, the characterized MAbs in this study may be useful for functional and structural studies of SLO and for the development of therapeutic or detection tools.

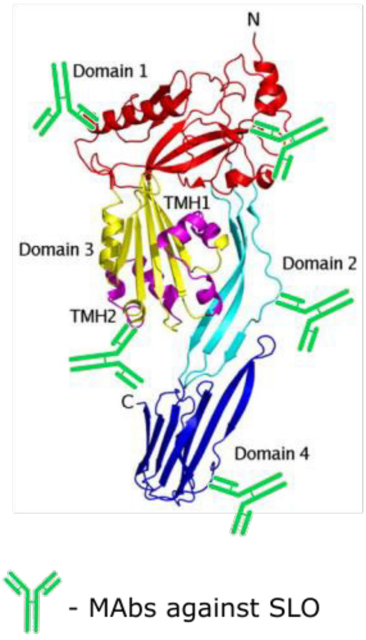

Figure 1. SLO crystal structure [4].

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C2

Effect of Cell Culture Additives on Phase Separation of SARS-CoV-2 Nucleocapsid Protein With RNA of Various Origin.

Arnas Treimakas[1], Indrė Kučinskaitė-Kodzė[1]
[1]Department of Immunology, Vilnius University, Lithuania
arnas.treimakas@gmc.stud.vu.lt

The Nucleocapsid (N) protein is one of four structural proteins that make up the viral particle of SARS-CoV-2 and is responsible for the packaging and protection of the virus' genomic RNA during its life cycle. Due to the heavily conservative amino acid sequence, strong immunogenicity and key role during viral replication of the N protein in comparison to other SARS-CoV-2 structural proteins, it is an attractive target for the development of vaccines, anti-viral medications and for use in COVID-19 diagnostics [1] . Although the replication of the SARS-CoV-2 virus occurs inside the cell, the N protein can be found exposed on the cell surface and in the extracellular space during active infection [2]. The concentration of N protein in blood serum has been shown to correlate with severity of symptoms and patient outcomes [3].

The N protein, upon binding to viral genomic RNA and/or oligomerization, goes through liquid-liquid phase separation and forms biomolecular condensates, which can be observed by optical microscopy [4]. In mammals, RNA found in the extracellular space is hypothesized to be used by cells for distant communication [5]. The N protein has also been shown to be able to bind host mRNA and other nucleic acids that are not SARS-CoV-2 genomic RNA [6]. By interacting with extracellular RNA, N protein may alter the availability of RNA ligands for pattern recognition receptors, thereby modulating immune sensing and downstream inflammatory signaling.

To evaluate the cellular response to these N protein-RNA condensates in cell culture, it is vital to ensure that phase separation occurs consistently and in a controlled manner during experiments within the cell culture medium. We present our findings on how common cell culture media and additives affect the ability of N protein to undergo phase separation with various types of RNA, utilizing differential interference contrast microscopy imaging of resulting biomolecular condensates.

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C3

Paracrine Signaling from Brain Endothelial Cells Reprograms Microglia Towards an Anti-inflammatory Phenotype

Kotryna Milovanovaitė[1], Agnė Pociūtė[1], Dr. Augustas Pivoriūnas[1]
[1]Department of Stem Cell Biology, Centre for Innovative Medicine
kotryna.milovanovaite@gmc.stud.vu.lt

Background. The blood-brain barrier (BBB) separates the vascular system from the central neural system (CNS), maintaining brain homeostasis [1]. Capillary brain endothelial cell (BEC) monolayer, basement membrane, astrocyte endfeet and pericytes form the BBB [2]. Together with neurons, vascular smooth muscle cells and microglia they function as a neurogliovascular unit [2]. Microglial cells are immunocompetent CNS cells that protect the brain by scanning their environment and phagocytosing pathogens, cellular debris and apoptotic bodies [3, 4]. Alongside their protective role, microglial cells regulate the BBB. Microglia can directly interact with BECs as well as modulate the barrier by releasing paracrine factors [5, 6]. While research mostly focuses on the effect of activated microglia towards the BBB, little is known about how BECs affect microglial cell functions.

Methods. In this study, a non-contact co-culture model was used to investigate how induced pluripotent stem cell-derived BECs affect microglial phagocytic activity and activation under inflammatory and physiological conditions (Fig. 1 A). First, microglial cells were activated with 20 ng/mL tumor necrosis factor alpha (TNFα). Co-culture with BECs was initiated 48 hours later and maintained for 5 days. In order to assess microglial phagocytic activity, 6 hours before cell fixation, fluorescently labeled latex beads were added into the basolateral chamber.

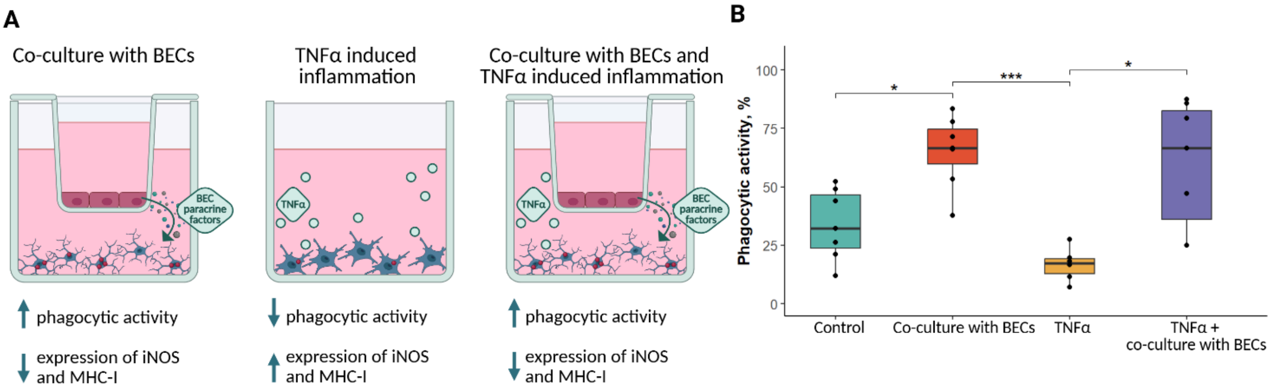

Figure 1. A) Scheme of the experiment. Created with BioRender.com; B) BECs effect on microglial phagocytic activity under physiological and inflammatory conditions. Welch ANOVA analysis and a Games-Howell post-hoc test were performed. *p < 0,05, **p < 0,01, ***p < 0,001, ****p < 0,0001

Results. Our results showed that TNFα suppressed microglial phagocytosis (Fig. 1 B). In contrast, co-culture with BECs statistically significantly increased microglial phagocytic activity under both physiological (by 31 % ($\text{CI}_{95\%}$[-55.55, -7.2], p = 0.011, n = 7)) and inflammatory (by 43 % ($\text{CI}_{95\%}$[7.22, 78.19], p = 0.022, n = 7)) conditions.

The same BBB model was used to investigate the expression of proteins critical to the inflammatory response in microglia. After TNFα treatment, expression of both inducible nitric oxide synthase (iNOS) and major histocompatibility complex class I (MHC-I) increased. Cocultivation with BECs reduced iNOS and MHC-I expression levels in microglia by half ($\text{CI}_{95\%}$[-1.17, -0.09], p = 0.022, n = 4 and $\text{CI}_{95\%}$[-0.72, 0.16], p = 0.157, n = 4 respectively) under inflammatory conditions.

Conclusion. Paracrine signaling from BECs reprograms microglia towards an anti-inflammatory state by increasing microglial phagocytosis and reducing iNOS and MHC-I expression under both physiological and inflammatory conditions.

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THE ROLE OF THE CB2 RECEPTOR IN REGULATING THE INFLAMMATORY RESPONSE OF MOUSE MICROGLIA

Kristupas Čeika[1], Liutauras Usonis[1], Egidijus Šimoliūnas[1], Daiva Baltriukienė[1, *] Monika Iešmantaitė[1,*]

[1]Department of Biological Models, Vilnius University, Lithuania
[*]These authors have contributed equally
kristupas.ceika@gmc.stud.vu.lt

Neuroinflammation is the inflammatory response of the CNS to various stimuli, such as infections, trauma, toxins, or autoimmune processes [1]. Neuroinflammation can be beneficial in acute injuries, but complications arise in chronic inflammation and neurodegenerative disorders such as Parkinson's or Alzheimer's disease [2]. Microglia, the innate immune cells of the CNS, are the main cells responsible for these processes. It is believed that modulating the functionality of microglia can alleviate the symptoms associated with neuroinflammation [3]. Recent studies suggest that the endocannabinoid system, particularly cannabinoid receptor type 2 (CB2R), may be one of the targets through which this modulation could be achieved. Activation of CB2R is known to induce anti-inflammatory effects, making it a promising target for modulating microglial function in neurodegenerative diseases. However, the exact mechanisms by which CB2R regulates microglial activity are not yet understood [4].

This study investigated the role of CB2R in the response of microglia to the potent pro-inflammatory stimulus lipopolysaccharide (LPS) by examining changes in CB2R expression, microglial functionality, the expression of pro-inflammatory and anti-inflammatory factors, and NF-κB signaling pathway activation. We showed that after short LPS stimulation (3 h), representing acute neuroinflammation, the amount of CB2R protein remains unchanged, but the amount of *CNR2* mRNA decreases, while during long LPS stimulation (24 h), representing chronic neuroinflammation, a decrease in both CB2R protein and mRNA is observed, indicating that CB2R is modulated under chronic inflammatory conditions. In this study, LPS significantly increased the phagocytic activity of microglia, consistent with the overactive phagocytosis of microglia described in the literature, which is toxic to normal neurons [5]. This effect was reversed by CB2R activation with the selective agonist JWH-133, which returned microglia to a resting phenotype. Additionally, the influence of CB2R on reactive oxygen species (ROS), which is produced in cells during stress, was evaluated. It was observed that LPS induced the production of ROS and that this increase was reversed when CB2R was activated. These findings suggest that the ability of microglia to reduce phagocytosis and oxidative stress depends on functional CB2R signaling. Next, we demonstrated that LPS significantly increases the expression of pro-inflammatory genes (*TNF-α*, *IL-6*, *IL-1β*) mRNA in microglia cells. CB2R agonist counteracted the harmful effects of LPS by reducing the expression of these factors. However, we observed that CB2R activation reduces the mRNA expression of an anti-inflammatory gene *Arg-1*. Together, these results indicate that CB2R activation has anti-inflammatory properties, but further experiments are needed to understand the changes in *Arg-1* expression. Furthermore, studies show that cannabinoids diminish pro-inflammatory cytokine production in LPS-stimulated microglia that is accompanied by decreased transcription factor NF-κB activity and translocation into the nucleus [6]. However, the present study found no statistically significant effect of CB2R on the activation of the NF-κB signalling pathway. This may suggest different signalling pathways through which CB2R decreases neuroinflammation.

These results provide new insight into the molecular mechanisms responsible for the anti-inflammatory effects of CB2R activation and offer additional insight needed for further research into the use of CB2R agonists as a potential compound for treating neurodegenerative diseases.

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EVALUATING MURINE MICROGLIAL MODELS FOR CB2 RECEPTOR FUNCTIONAL STUDIES

Liutauras Usonis[1], Egidijus Šimoliūnas[1], Kristupas Čeika[1], Daiva Baltriukienė[1]*, Monika Iešmantaitė[1]*

[1]Department of Biological Models, Vilnius University, Life Sciences Center, Lithuania
*These authors contributed equally to this work.
liutauras.usonis@gmc.stud.vu.lt

Neuroinflammation is a biological response within CNS that has gained recognition as a pivotal contributor to the pathogenesis of wide range neurodegenerative diseases, psychiatric conditions and cerebral injuries. Once seen as a transient protective response to acute stimuli, neuroinflammation is now understood to have dual functions – either maintaining CNS homeostasis or driving neurodegeneration. Multiple types of cells are responsible for neuroinflammatory process development, however, resident immune cells of the CNS – microglia, are the main players. Microglial cells primarily exist in a resting phenotype, scanning the brain for potential threats, but when they are detected, microglial cells enter a reactive state. Microglial reactivity is crucial for proper pathogen neutralization, cellular debris clearance, synaptic pruning and many other functions in the CNS. However, prolonged microglial activation in the case of neurodegenerative diseases, poor diet, stress and trauma can lead to long-term structural damage to several brain regions. Due to their diverse membrane receptor ensemble microglia can detect pathogens, cellular damage and determine the intensity of the inflammatory response and react accordingly.

The cannabinoid receptor 2 (CB2), mainly expressed by immune cells, including microglia, plays a pivotal role in microglial-derived neuroinflammation, since it modulates microglia proliferation, migration, and differentiation and immune response. This receptor is emerging as a novel target for the development of therapeutic approaches to treat chronic CNS inflammation as it is considered to be an anti-inflammatory mediator. However, some studies challenge such views. To get a concise answer to question whether CB2R activation can be used as a way to target neuroinflammation, it is paramount to compare the data from many validated methods based on protein loss-of-function principles.

To investigate the functions of the CB2R, we developed an immortalized mouse microglia (IMM) cell line derived from neonatal primary microglial cells, that was validated by comparing it to primary cells. Functional assays, such as phagocytic activity and reactive oxygen species (ROS) production, indicated similar patterns between IMM and primary cells: in the LPS treatment group ROS levels were elevated, while the number of phagocytised beads per cell decreased. Similarly, cytokine gene expression has also shown similar levels of Tnf, Tgfb1, Il1b, Il6 and Il10 mRNA before and after LPS stimulation in both IMM and primary cells. Overall, IMM proved to be a reliable and reproducible model that reflects the characteristics of primary cells.

We employed CRISPR–Cas9 nuclease-mediated gene editing, RNA interference, and CB2 protein antagonist treatment in both IMM and primary cells to generate a Cnr2 loss-of-function phenotype, which enables for a functional investigation of the CB2R. Using Cas9 nucleases we introduced a single-base deletion in the coding exon of the IMM Cnr2 gene resulting in a premature STOP codon. This new Cnr2$^{-/-}$ model will be further evaluated and compared to the siRNA-transfected and CB2 antagonist-treated primary cells. To evaluate functional differences between wild-type and gene-silenced cells, assays measuring migration, phagocytic activity and the production of both reactive oxygen species and nitric (II) oxide (NO) will be performed under both homeostatic and inflammatory (LPS-induced) conditions.

Our research provides detailed comparison of different microglial models and offers scientists the opportunity to select an appropriate model based on available resources for future CB2 receptor and neuroscience research.

C6

Plasma IgG Levels as a Candidate Biomarker of Immune Status in Baltic Grey Seal Pups

Tomas Vainauskas[1], Vaida Survilienė[2], Mart Jüssi[3], Indrė Kučinskaitė-Kodzė[1]

[1]Department of Immunology, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]Department of Neurobiology and Biophysics, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[3]Independent researcher, Pro Mare, Estonia
tomas.vainauskas@gmc.vu.lt

The Baltic Sea represents one of the most contaminated marine ecosystems worldwide, characterized by limited water exchange and prolonged pollutant retention [1]. Persistent contaminants bioaccumulate along the food chain and may reach high concentrations in top predators. Such long-term exposure raises concerns regarding potential effects on immune competence and overall health [2], [3]. Baltic grey seals (*Halichoerus grypus grypus*), as top predators, are particularly susceptible to bioaccumulating pollutants. Assessing their immune status is therefore essential for ecological health monitoring. In this study, we evaluated whether total plasma immunoglobulin G (IgG) concentration could serve as a complementary indicator of immune status in grey seal pups.

We analysed 51 collected blood samples from grey seal pups. Routine haematological parameters were assessed using a blood analyser, peripheral blood mononuclear cells (PBMCs) were isolated for parallel immunological studies and plasma samples were obtained for antibody quantification. Ammonium sulfate ($(NH_4)_2SO_4$) precipitation was applied to concentrate plasma immunoglobulins. The resulting fractions, enriched primarily in IgG, were used for IgG quantification. We further examined whether variation in IgG levels could enhance the interpretative value of conventional immune and inflammatory markers, including C-reactive protein (CRP) and blood cell profiles. IgG concentrations were also evaluated in relation to externally assessed clinical parameters, including visible signs of infection, skin lesions, and traumatic injuries. Given that the study focused on seal pups, comparisons were also performed between sexes and across nutritional status categories.

Total IgG concentration in plasma did not show significant correlations (Pearson r ranging from –0.1 to 0.4, $p > 0.05$) with routine haematological parameters, including lymphocyte, monocyte, granulocyte, total white blood cell counts, haemoglobin concentration, red blood cell count, platelet count, or haematocrit percentage.

However, considerable inter-individual variability in IgG concentrations was evident and was further supported by SDS-PAGE analysis of the immunoglobulin-enriched fractions. Although no significant correlations with routine haematological markers were identified, the pronounced differences observed among samples warrant further investigation. Future studies will focus on exploring potential associations with additional clinical and physiological parameters to better define the significance of IgG variation.

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C7

DEVELOPMENT OF HYPOALLERGENIC VARIANTS OF ART V 3 AND THEIR EXPOSURE ON THE vB_EcoS_NBD2 BACTERIOPHAGE-ORIGINATED NANOTUBES

Eva Kupetytė, Laima Čepulytė, Rasa Petraitytė–Burneikienė

Department of Eukaryote Gene Engineering, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
eva.kupetyte@gmc.stud.vu.lt

Allergic diseases are chronic inflammatory disorders whose prevalence has increased worldwide over recent decades due to lifestyle changes, reduced contact with nature, air pollution, climate change, dietary changes, and increased stress [1]. Allergic reactions can be triggered by various allergens, including food, pollen, dust mites, insects, animals, and pharmaceuticals. The human body produces antigen-specific immunoglobulin E (sIgE) in response to allergens, leading to allergy symptoms such as sneezing, coughing, skin rashes, and runny noses. Seasonal pollen allergies commonly appear as allergic rhinitis, conjunctivitis, asthma, urticaria, and dermatitis [2]. *Artemisia vulgaris* (common mugwort) is one of the major sources of airborne pollen allergens in Europe, affecting approximately 10–15% of patients with pollinosis during the late summer and autumn months [3]. One of its allergenic components, the non-specific lipid transfer protein Art v 3, has not yet had its IgE epitopes identified. The reactivity of this protein with serum sIgE from patients allergic to *Artemisia vulgaris* varies between 22% and 70%, although specific IgE epitopes remain unidentified [4].

Allergen-specific immunotherapy (ASIT) is the only approved disease-modifying treatment for IgE-mediated allergic disorders, capable of inducing long-term immune tolerance [5]. Recent ASIT approaches include recombinant hypoallergens and allergen delivery systems such as virus-like particles (VLPs), which act as carriers for allergens [6]. VLPs are self-assembling virus-based nanostructures that mimic the form and size of native viruses, but do not contain their genetic material, so they cannot cause infection [7]. Recombinant hypoallergens are engineered to minimize IgE binding while maintaining immunogenicity, enabling the induction of regulatory T cells and allergen-specific IgG antibodies while reducing the risk of IgE-mediated inflammatory reactions [8]. Next-generation ASIT vaccine candidates use nanoparticle-based platforms, including VLPs, to display allergens in high amounts, enhancing their adjuvant properties and promoting immune tolerance [9].

The aim of this study was to display native-like Art v 3 and its mutant variants with reduced IgE reactivity on the surface of bacteriophage vB_EcoS_NBD2 (NBD2) nanotubes. During this study, native-like Art v 3 and mutant Art v 3 variants, containing amino acid substitutions at specific target sites, potentially involved in IgE-binding epitope formation, were genetically fused with the gene encoding NBD2 nanotubes. The chimeric polytubes were expressed in the *Saccharomyces cerevisiae* yeast expression system.

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C8

EVALUATION OF SOLUBLE IMMUNE CHECKPOINT PROTEINS IN PLASMA OF BLADDER CANCER PATIENTS

Ugnė Rimkutė[1], Agnė Šeštokaitė[2], Agata Mlynska[2], Rasa Sabaliauskaitė[1,2]

[1]Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]National Cancer Institute, Vilnius, Lithuania
ugne.rimkute@gmc.stud.vu.lt

Bladder cancer (BCa) is one of the most frequently diagnosed cancers worldwide. High rates of recurrence, progression, and unsatisfactory survival outcomes remain major problems despite improvements in surgery, chemotherapy, and intravesical treatments [1]. These challenges have invoked the development of more effective and personalized therapeutic strategies, including immunotherapy. Recent studies in tumor immunology have demonstrated the critical role of immune checkpoint proteins in cancer development. By manipulating immune checkpoint proteins, such as PD-1, PD-L1, and CTLA-4, tumors can effectively evade immune detection through inhibition of T cell activity. Targeting these molecules with immune checkpoint inhibitors can restore antitumor immunity. Although these regulatory strategies have gained considerable attention in the context of therapy, they also play a fundamental role in the underlying biology of tumor evolution and progression [2]. Therefore, deeper insight into the interactions between cancer cells and the immune system is required for clarifying mechanisms of BCa tumor development and progression.

The objective of this research was to quantify the most frequently detected immune checkpoints in the plasma of BCa patients, determine differences in protein concentrations from those in individuals without BCa, and analyze the correlation to clinical features.

Plasma samples were obtained from 89 BCa patients and 15 control subjects. 10 most common soluble immune checkpoints (ICs) and their ligands (sCD25 (IL-2Ra), 4-1BB, B7.2 (CD86), Free Active TGF-β1, CTLA-4, PD-L1, PD-1, Tim-3, LAG-3, Galectin-9) were quantified using the LEGENDplex™ HU Immune Checkpoint Panel (BioLegend, USA) and analyzed B flow cytometry. Data normality was assessed using the Shapiro-Wilk test, and as the distribution was non-normal, group differences were evaluated with the Mann-Whitney U test. Correlations were assessed with Spearman's Rho test. Results were considered statistically significant at $p < 0.05$.

Compared with controls, BCa patients‘ plasma showed markedly higher concentrations of Tim-3, Galectin-9, sCD25, and B7.2. Further comparison of clinical parameters such as age, sex, stage, and tumor grade indicated that there might be considerable correlations between some of the groups. Galectin-9 showed a statistically significant moderate correlation with tumor grade and a statistically significant weak association with cancer stage. Age showed weak positive correlations with Tim-3, Galectin-9, and sCD25. Median differences between sexes (male and female) were statistically significant for Free Active TGF-β1 and LAG-3.

In conclusion, patients with bladder cancer exhibited increased plasma concentrations of several immune checkpoint proteins, with Tim-3, Galectin-9, sCD25, and B7.2 emerging as the most evident findings. Associations with clinical parameters suggest that immune checkpoints may reflect disease characteristics; however, the observed relationships were generally weak. Accordingly, further studies are needed to better understand the biological role of immune checkpoints and to determine their potential value for improving immunotherapy.

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BIOMEDICINE

D1

EMULSION BASED PCL-BG 45S5 MICROPARTICLES WITH TUNABLE SIZE AND SURFACE MORPHOLOGY FOR BONE REGENERATION

Dinas Tverijonas[1], Mantas Liudvinaitis[1], Ieva Šimoliūnė[1], Milda Alksnė[1], Aleksej Žarkov[2], Egidijus Šimoliūnas[1]

[1]Department of Biological Models, Vilnius University, Lithuania
[2]Institute of Chemistry, Vilnius University, Lithuania
dinas.tverijonas@chgf.stud.vu.lt

Bone regeneration remains a significant clinical challenge due to the complexity of restoring both the structural and functional integrity of damaged tissue. Current treatment options provide no consistently satisfactory solution: autografts, regarded as the gold standard, are limited by tissue availability and donor site morbidity, while the use of exogenous bone carries a risk of pathogen transmission and rejection by the recipient's body [1]. Synthetic alternatives often fail to support adequate regeneration or possess poor mechanical properties, while rigid, monolithic scaffolds do not conform to irregularly shaped defects and fail to provide sufficient porosity needed for neovascularization and appropriate metabolite exchange [2]. Consequently, the creation of a mechanically competent and shape-adaptive synthetic alternative that could effectively facilitate the healing of damaged bone tissue is warranted.

In this study, we developed a novel porous, free-packed system based on poly(ε-caprolactone) (PCL) and bioactive glass (BG) 45S5 composite (mass ratio of 4:1) microparticles (MP) with tunable size and surface porosity. MPs were produced via the emulsion solvent evaporation method (Fig. 1), varying composite concentration in dichloromethane (DCM) (20–30 % w/v), stirring speed (300–900 RPM), and fabrication temperature (20–40 °C). Emulsification was carried out in an aqueous phase supplemented with sodium dodecyl sulphate (SDS) and hydroxypropyl cellulose (HPC). All MPs were thoroughly washed before characterization. Samples intended for cellular experiments were additionally filtered, sterilized, and coated with gelatin. Characterization of the produced MPs included optical microscopy for size distribution and Scanning Electron Microscopy (SEM) for morphometric analysis. Inductively Coupled Plasma Optical Emission Spectroscopy (ICP-OES) was used to evaluate ion release. Cytocompatibility was assessed via fluorescent microscopy of EGFP-expressing dental pulp stem cells.

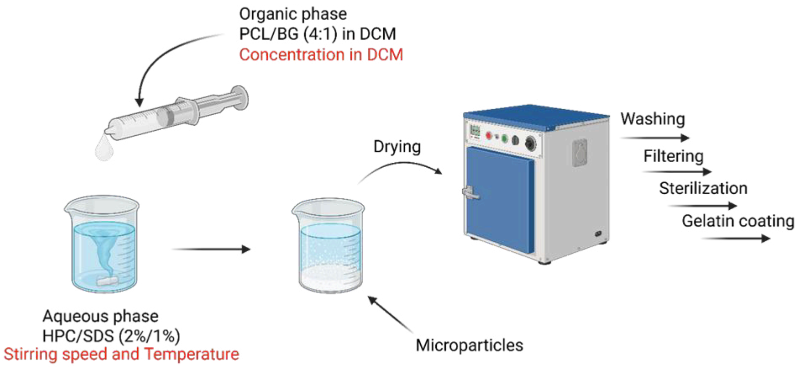

Figure 1. Schematic illustration of the MP fabrication process. Processing parameters investigated in this study are highlighted in red.

Size distribution and morphometric analysis identified that stirring speed was the primary determinant of MP size and surface morphology; increasing speed from 300 to 900 RPM resulted in, on average, 1.84 times smaller MPs, while surface porosity changed in a non-linear manner. An inverse relationship was also observed between MP diameter and both temperature of the aqueous phase and, interestingly, composite concentration. ICP-OES results indicate that the embedded BG particles retained their ion-release capability. Fluorescent microscopy of EGFP-expressing dental pulp stem cells confirmed the system's cytocompatibility, demonstrating sustained cell attachment and migration for over 32 days. While further studies are needed to evaluate the effect of MPs on cell differentiation, the unique combination of injectability, tunable size, surface characteristics, and cytocompatibility make this system a promising candidate for minimally invasive bone tissue engineering applications.

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SCREENING CARBONIC ANHYDRASE IX SELECTIVE INHIBITORS AS POTENTIAL ANTICANCER THERAPEUTIC AGENTS

Justinas Malevskis, Joana Smirnovienė, Mantas Žvirblis, Virginija Dudutienė, Daumantas Matulis, Vytautas Petrauskas

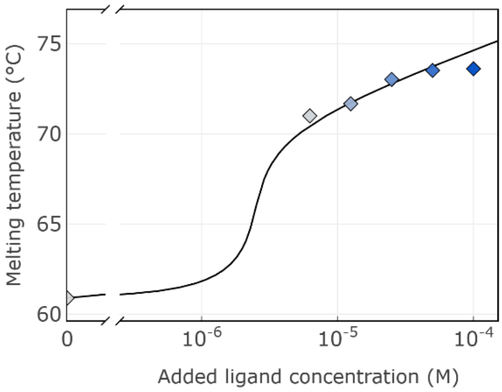
Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
justinas.malevskis@chgf.stud.vu.lt

Carbonic anhydrases (CA) are naturally occurring enzymes in living organisms; specifically, α-CAs are found in humans. These enzymes catalyze the reversible hydration of carbon dioxide, producing bicarbonate ion and a proton. Thus, the function of CA is to regulate the pH of the cell medium. CA IX is one of the twelve catalytically active human isoforms. Abnormal biosynthesis of CA IX is observed in hypoxic tumor cells, leading to metastasis. Therefore, CA IX is an attractive anticancer target. Primary sulfonamides bearing various substituents on a benzene ring are attractive CA inhibitors due to their high affinity and relatively simple synthesis. Nevertheless, it is necessary to synthesize such drugs that specifically inhibit CA IX, while maintaining the other 11 CA isoforms' physiological function [1].

The aim of our research is to design a selective inhibitor, evaluating affinities of newly synthesized compounds bearing fluorine and aliphatic side-chain substituents on primary benzenesulfonamides. By employing high-throughput fluorescent thermal shift assay (FTSA), multiple ligands with varying concentrations are screened at once. FTSA determines the ligand's stabilizing effect on a protein by its thermal shift in melting temperature ΔT_m, which subsequently is recalculated to a binding constant K_b by a sophisticated thermodynamic equation [2]. CA-ligand interactions were determined by running the FTSA experiment with CA I, CA II, chimeric CA VI, CA VII, CA IX, CA XII, and CA XIII. Additionally, the stopped-flow CO_2 hydration assay was employed to confirm inhibition of CA catalytic activity. All experimentally measured binding and inhibition data have been deposited into the Protein–Ligand Binding Database (PLBD, plbd.org), creating a curated dataset that will be directly leveraged for AI-driven drug discovery and predictive modeling of CA IX inhibitor selectivity and potency.

Screening a group of compounds allowed us to identify lead compound MZ24-11 that exhibited nanomolar affinity (K_d –1.4 nM) and selectivity towards CA IX (Fig. 1) over CA I, CA II, chimeric CA VI, CA VII, CA IX, CA XII, and CA XIII. Despite the lead inhibitor exhibited high potency, further structural development of the molecule is needed to develop it as an anticancer drug candidate.

Figure 1. A dose-response curve obtained by varying concentrations of the lead inhibitor MZ24-11 and registering CA IX melting temperatures T_m using a fluorescent dye by FTSA. The data was fitted, and a figure was generated using Thermott (thermott.com, [3]).



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Effect of Hydrazone Derivatives on Pancreatic Cancer Cell viability and migration

D. Narkevičiūtė[1], J. Kiltinavičius[2], I. Tumosienė[2], K. Kantminienė[2], V. Petrikaitė[1,3]

[1] Laboratory of Drug Targets Histopathology, Lithuanian University of Health Sciences

[2] Faculty of Chemical Technology, Kaunas University of Technology

[3] Institute of Biotechnology, Life Sciences Centre, Vilnius University

narkeviciutedigna@gmail.com

Pancreatic adenocarcinoma is one of the deadliest gastrointestinal cancers, accounting for over 90% of pancreatic malignancies and being frequently diagnosed at advanced stages due to late-onset symptoms and the lack of specific biomarkers [1,2]. Hypoxia within the tumour microenvironment plays a crucial role in pancreatic cancer progression by promoting aggressiveness and resistance to therapy [3].

This study aimed to evaluate the cytotoxic activity and effects on single-cell migration of hydrazone derivatives synthesized at Kaunas University of Technology in human pancreatic cancer cells in vitro. Cytotoxicity of 22 compounds was assessed in MIA PaCa-2 and PANC-1 cell lines using the MTT assay. Compound effect on cell migration was assessed by single-cell migration assay at their EC50 concentrations.

Several compounds significantly reduced pancreatic cancer cell viability, and IT199, IT206, IT207, and IT227 were selected for further analysis. Their EC50 values were determined in cancer cells and human fibroblasts, with dasatinib (DST) used as a reference compound. Compared to DST, the investigated hydrazone derivatives exhibited 3–146-fold lower activity, depending on the cell line and oxygen conditions. Under normoxic conditions, IT227 was the most active compound in MIA PaCa-2 (EC50 = 1.7 µM) and PANC-1 (EC50 = 4.3 µM) cells, whereas under hypoxia, IT207 (EC50 = 27.3 µM) and IT199 (EC50 = 21.7 µM) demonstrated the highest cytotoxicity. Single-cell migration assays revealed that under normoxic conditions, IT199 increased cell migration compared with control, with the strongest effect observed after 1 hour (1.33-fold increase). Elevated migratory activity persisted at 3 and 5 hours, indicating that IT199 modulates cellular motility. Previous studies have shown that tyrosine kinase inhibitors may affect cancer cell migration. These effects have been associated with tumour cell phenotypic plasticity, characterised by loss of epithelial features, increased motility, enhanced invasiveness, and higher metastatic potential [4], which may explain the IT199-induced increase in single-cell migration observed in this study.

In conclusion, the hydrazone derivatives exhibited differential cytotoxic effects against pancreatic cance cell lines, with compounds containing a double hydrazone fragment showing higher activity. The most active compounds (IT199, IT206, IT207, and IT227) were 3–146-fold less potent than DST. Single-cell migration analysis demonstrated that IT199 increased cell migration by 1.33-fold under normoxic conditions compared with the control group. Further studies are required to elucidate the mechanisms of action and therapeutic potential of the investigated compounds.

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DETERMINATION OF ERYTHROCYTE MEMBRANE FATTY ACIDS AND THEIR ASSOCIATIONS WITH CARDIOVASCULAR DISEASE RISK FACTORS

Justas Dijokas[1], Linas Černiauskas[2], Inga Bikulčienė[2]

[1]Faculty of Medicine, Vilnius University, Vilnius, Lithuania
[2]Biomarker Research Laboratory, Translational Health Research Institute, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
justas.dijokas@mf.stud.vu.lt

Introduction. Cardiovascular disease (CVD) is the leading cause of death worldwide, accounting for 32% of all deaths [1]. In Europe, CVD results in the loss of over 60 million potential life-years annually, predominantly in men [2]. In Lithuania, CVD is responsible for 50.7% of deaths, with a mortality rate of 658.7 per 100,000 [3]. The fatty acid composition of erythrocyte membrane phospholipids has emerged as a promising biomarker of cardiovascular risk [4]. Profiling erythrocyte membrane fatty acids in individuals at high risk of CVD may provide insights into the mechanisms of cardiovascular pathogenesis and improve risk stratification. Identifying fatty acids associated with lower risk could guide the development of targeted preventive and therapeutic strategies.

Methods. An analytical cross-sectional study was conducted with approval from the Vilnius Regional Biomedical Research Ethics Committee (No. 2023/9-1518-998). Participants were recruited at the Cardiology and Angiology Centre of Vilnius University Hospital Santaros Klinikos, according to the following inclusion criteria: aged 40–65 years, enrolled in a cardiovascular disease prevention program and willing to participate in a biomedical study. Blood samples from the selected participants were processed to isolate membranes, extract lipids, and prepare fatty acid methyl esters (Christie, 1993) [5]. Erythrocyte membrane fatty acids were analysed using gas chromatography–mass spectrometry (GC–MS). Anonymised sociodemographic data, lipid metabolism markers, cardiovascular risk indicators and erythrocyte membrane phospholipid fatty acid data were analysed. Statistical analysis was conducted using Microsoft Excel 2024 and IBM SPSS Statistics 30.0.0. Statistical significance was set at $p < 0.05$.

Results. The study included 48 patients; 31 (64.6%) were male. Median age was 51 years (IQR = 44.3–56.0). Metabolic syndrome criteria were met by 26 (54.2%) patients. The mean SCORE2 cardiovascular risk estimate was 11.81 ± 6.24%. Saturated fatty acids (SFAs) constituted the largest proportion of erythrocyte membrane phospholipids (36.38 ± 2.23%), whereas omega-3 polyunsaturated fatty acids (n-3 PUFAs) were the lowest (13.29 ± 2.02%). C18:0 correlated positively with atherogenic lipid ratios: total cholesterol/high-density lipoprotein cholesterol (TC/HDL-C; $p = 0.049$, $r = 0.285$) and low-density/high-density lipoprotein cholesterol (LDL-C/HDL-C; $p = 0.040$, $r = 0.297$). Men had a significantly higher proportion of C18:0 in erythrocyte membrane phospholipids than women (18.18% vs. 17.58%, $p = 0.027$; 95% CI [0.072–1.138]). C18:2n-6 showed positive correlations with atherogenic lipid metabolism parameters, including TC ($p = 0.005$, $r = 0.402$), LDL-C ($p = 0.009$, $r = 0.376$), non-HDL-C ($p = 0.034$, $r = 0.307$), and apolipoprotein B ($p = 0.037$, $r = 0.308$). Similar associations were identified for C18:3n-6 ($p = 0.027$, $r = 0.320$; $p = 0.035$, $r = 0.306$; $p = 0.035$, $r = 0.305$; $p = 0.023$, $r = 0.328$, respectively). C20:3n-6 in erythrocyte membrane phospholipids was significantly higher in participants with metabolic syndrome (2.91% vs. 2.65%, $p = 0.030$; 95% CI [0.03–0.50]), and correlated positively with serum triglycerides ($p = 0.008$, $r = 0.380$), suggesting n-6 PUFAs' involvement in increased atherogenic risk. C20:5n-3 was inversely associated with LDL-C concentration ($p = 0.045$, $r = -0.291$), indicating potential anti-atherogenic effects.

In the multivariable logistic regression model ($\chi^2(9) = 17.183$, $p = 0.046$, Nagelkerke $R^2 = 0.418$), four fatty acids emerged as significant predictors of metabolic syndrome (odds ratio (OR), 95% CI): C16:1n-7 (46,498.968; [3.574–604,885,115.04]; $p = 0.026$), C18:0 (31.550; [1.642–606.252]; $p = 0.022$), C18:3n-6 (4,956,666.107; [2.854–8.641 × 10^{12}]; $p = 0.035$), and C22:5n-6 (13.221; [1.190–146.828]; $p = 0.036$). In the multiple linear regression model ($F(10, 37) = 2.527$, $p = 0.020$, adjusted $R^2 = 0.245$), the SCORE2 estimate was significantly influenced by the following fatty acids (B, 95% CI): C16:1n-7 (13.582; [4.194–22.971]; $p = 0.006$), C18:0 (3.970; [0.429–7.511]; $p = 0.029$), C20:1n-9 (13.693; [4.077–23.308]; $p = 0.006$), and C22:5n-3 (−11.814; [−21.373–−2.255]; $p = 0.017$).

Conclusions. Erythrocyte membrane fatty acid composition may serve as an early marker of metabolic and cardiovascular imbalance, with potential value for risk assessment and the development of preventive strategies. n-3 PUFAs may exert cardioprotective effects, whereas certain SFAs, monounsaturated fatty acids, and n-6 PUFAs are associated with indicators predisposing to an increased risk of cardiovascular events.

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MICROGLIAL DYSFUNCTION IN CSF1R-RELATED LEUKOENCEPHALOPATHY: MOLECULAR MECHANISMS AND THERAPEUTIC PERSPECTIVES

Ramojus Balevičius[1,2], Lidija Marija Smertinaite[2], Virginija Danylaite Karrenbauer[2,3]

[1]Department of Biological Models, Institute of Biochemistry, Life Sciences Center, Vilnius, Lithuania
[2]Department of Clinical Neuroscience, Karolinska Institutet, Stockholm, Sweden
[3]Department of Neurology, Karolinska University Hospital, Stockholm, Sweden
ramojusbalevicius@gmail.com

CSF1R-related leukoencephalopathy (CRL) is a rare, progressive neurodegenerative disorder caused by mutations in the CSF1R gene, leading to microglial dysfunction [1]. CSF1R encodes a receptor tyrosine kinase critical for microglial survival and homeostasis. Ligand-induced CSF1R activation triggers STAT3 signaling, which regulates transcriptional programs essential for microglial survival, proliferation, and immune regulation. In CRL, disruption of CSF1R–STAT3 signaling leads to microglial loss, impaired clearance of apoptotic cells, and chronic neuroinflammation, driving demyelination and axonal injury [2].

Neuroinflammation caused by microglial dysfunction attracts peripheral immune cells into the CNS. IL-6–secreting B cells infiltrate the CNS, amplifying STAT3-mediated pro-inflammatory signaling and disrupting oligodendrocyte homeostasis. This dynamic CNS–periphery crosstalk establishes a self-perpetuating inflammatory environment that contributes to progressive white matter degeneration [3-4].

Rituximab (RTX), a B cell–depleting therapy, targets IL-6–producing B cells, potentially interrupting this inflammatory cascade. By reducing IL-6–driven STAT3 activation, RTX may restore microglial homeostasis, promote oligodendrocyte survival, and support remyelination [5-6].

We report longitudinal outcomes in two genetically confirmed CRL patients treated with RTX. Clinical and functional assessments included the Unified Parkinson's Disease Rating Scale (UPDRS), Amyotrophic Lateral Sclerosis Functional Rating Scale (ALSFRS-R), Expanded Disability Status Scale (EDSS), and Hospital Anxiety and Depression Scale (HADS). Cognitive evaluation comprised the Mini-Mental State Examination (MMSE), Montreal Cognitive Assessment (MoCA), and Symbol Digit Modalities Test (SDMT). Quality of life was assessed using the Multiple Sclerosis Impact Scale (MSIS-29) and the EuroQol-dimension/Visual Analogue Scale (EQ-5D/VAS). Serum neurofilament light chain (sNfL) levels in cerebrospinal fluid (CSF) and plasma were measured as biomarkers of axonal injury. Brain magnetic resonance imaging (MRI) was performed to evaluate white matter changes. CSF1R variants were confirmed by Sanger sequencing and classified according to American College of Medical Genetics and Genomics (ACMG) criteria. RTX was administered intravenously at 500 mg every six months for two years.

These cases provide preliminary evidence that modulation of peripheral IL-6–producing B cells can mitigate microglial dysfunction and preserve white matter integrity. Our findings underscore the central role of STAT3 signaling in CRL pathogenesis and highlight the translational potential of immunomodulatory strategies as disease-modifying interventions.

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Profiles of circulating bacterial DNA in patients recovering from severe SARS-CoV-2 infection

Kamile Milkintaite[1,2], Gabrielė Žebrauskaitė-Keblikienė[1,3], Diana Žaliaduonytė[3], Ugnė Meškauskaitė[1] Vilius Skipskis[1], Vacis Tatarūnas[1]

[1]Institute of Cardiology, Laboratory of Molecular Cardiology, Lithuanian University of Health Sciences, Kaunas, Lithuania
[2]Faculty of Medicine, Lithuanian University of Health Sciences, Kaunas, Lithuania
[3]Department of Cardiology, Kaunas Hospital of the Lithuanian University of Health Sciences, Kaunas, Lithuania
kamile.milkintaite@stud.lsmu.lt

Introduction: Severe manifestations of COVID-19 are frequently characterized by acute systemic inflammation, endothelial dysfunction, and a heightened predisposition toward cardiovascular and thrombotic events (1). Many patients who developed severe COVID-19 have pre-existing chronic conditions, such as cardiovascular or metabolic disorders, which are commonly associated with impaired or dysregulated immune response. SARS-CoV-2-induced hypoxia, cytokine-driven inflammation, and direct injury to epithelial and endothelial tissues can disrupt vital biological barriers, such as the intestinal epithelium and the alveolar-capillary junction, thereby facilitating the movement of microbial components into the bloodstream (1-3). The presence of bacterial components may further augment immune activation and inflammatory cascades. Emerging data suggest that bacterial DNA fragments in the bloodstream may trigger inflammatory pathways leading to vascular injury and endothelial activation (4,5). This interaction among viral infection, bacterial translocation, and impaired host immunity may underlie increased susceptibility to complications. Nevertheless, the occurrence, composition, and taxonomic diversity of circulating bacterial DNA in complicated COVID-19 patients have not been thoroughly characterized.

Aim: To use next-generation sequencing (NGS) to detect circulating bacterial DNA and characterize its taxonomic diversity and inter-individual heterogeneity in patients recovering from severe SARS-CoV-2 infection.

Methods: The study was conducted at the Laboratory of Molecular Cardiology, Institute of Cardiology, Lithuanian University of Health Sciences. Venous blood samples were obtained after completion of inpatient treatment in patients with a prior episode of severe COVID-19, following acute hospitalization (n=101). Bacterial DNA was amplified by PCR targeting the 16S rRNA gene. Samples yielding detectable 16S rRNA products underwent sequencing using Oxford Nanopore Technologies (ONT, The Oxford Science Park, UK). Taxonomic classification of both 16S rRNA amplicon data and shotgun metagenomic reads was performed using the EPI2ME platform (ONT). Reads were classified at the class level. Patients from the same clinical cohort in whom bacterial DNA was not detected served as an internal comparator group. Additionally, clinical and laboratory parameters related to cardiac injury, systemic inflammation, and coagulation were assessed to provide a clinical context.

Results: Circulating bacterial DNA was detected in most of the patients (76% of the total cohort, n=101). The analysis highlighted significant variation among individual microbial profiles. Taxonomic profiling demonstrated that Gammaproteobacteria and Alphaproteobacteria were the most dominant taxa, occurring in 95% of the bacterial DNA-positive samples. In comparison, Betaproteobacteria were identified in 43% of cases, while Bacilli and Actinomycetes were less frequent, appearing in 33% and 29% of the analyzed samples, respectively.

Conclusions: Bacterial DNA is detectable in the blood of patients recovering from severe COVID-19 and exhibits marked interindividual diversity. The recurrent detection of Gammaproteobacteria may indicate a non-random microbial pattern; however, further studies with rigorous contamination controls are required. These results underscore the need for further research into how bacterial components might exacerbate systemic inflammation in viral infections.

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D7

GENETIC POLYMORPHISMS OF β-DEFENSIN 1 GENE AND CARIES EXPERIENCE IN ADULTS WITH INTELLECTUAL DISABILITIES

Karole Simona Motiejunaite[1,2], Indre Stankeviciene[1], Neringa Meilute[1,2], Ula Burbulyte[2], Kristina Daniunaite[2]

[1]Faculty of Medicine, Institute of Odontology, Vilnius University, Vilnius, Lithuania
[2]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
karole.motiejunaite@mf.stud.vu.lt

Dental caries is a multifactorial disease shaped by behavioral, environmental and genetic factors [1]. Adults with intellectual disabilities represent a vulnerable group with increased risk of poor oral health and higher treatment needs [2]. Genetic variation in *DEFB1* (encoding human β-defensin-1) – notably *DEFB1* rs11362 single-nucleotide polymorphism (SNP) – has been linked to caries susceptibility [3].

The aim of the study was to investigate the association of *DEFB1* rs11362 (C>T) with oral health in adults with intellectual disabilities. Patient samples and clinical data were collected between 2023-2025 from adults with intellectual disabilities treated under general anesthesia at Vilnius University Hospital Santaros Klinikos Žalgiris Clinic. Clinical examinations were conducted by a single calibrated examiner and caries experience was recorded using the DMFS index (the sum of decayed (D), missing (M), and filled (F) tooth surfaces). Sociodemographic data were obtained from caregivers using the WHO (2013) Adult Oral Health Questionnaire [4]. Genomic DNA was extracted from oral mucosal swab samples using a magnetic bead–based automated system. Genotyping was performed by real-time qPCR with commercial, hydrolysis probe-based primer assays.

The study included 63 adults with intellectual disability, with the mean DMFS index (±SD) of 39.4±26.5. The distribution of *DEFB1* genotypes was as follows: CC 30.2% (n = 19), CT 44.4% (n = 28), and TT 25.4% (n = 16), and did not differ significantly from global population frequencies (CC 34.2%, CT 47.7%, and TT 18.0%, respectively; $P > 0.050$) [5]. Allele frequencies for the reference (C) and alternative (T) alleles were 52.4% and 47.6%, respectively, and were likewise comparable to global estimates (58.1% and 41.9%, respectively; $P > 0.050$) [5]. DMFS was not significantly associated with *DEFB1* SNP genotypes (Fig. 1), however, a tendency toward a slightly higher median DMFS value was observed in individuals with the C/C homozygous genotype compared to other genotypic groups, while T/T homozygotes demonstrated a wider dispersion of DMFS values, indicating greater variability of this quantitative indicator within this subgroup.

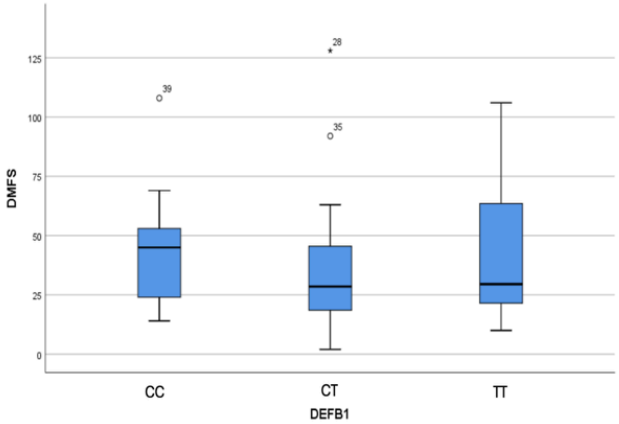

Figure 1. Distribution of DMFS index according to *DEFB1* rs11362 genotypes.

In conclusion, *DEFB1* rs11362 was not associated with DMFS in dental patients with intellectual disabilities, although minor genotype-related differences in central tendency and variability were observed. Larger studies are needed to clarify its role in caries susceptibility in this vulnerable population.

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D8

EVALUATION OF ANTICANCER ACTIVITY OF NOVEL TRIAZOLE DERIVATIVES IN HUMAN LUNG, TRIPLE-NEGATIVE BREAST CANCER AND MELANOMA 3D CULTURES

Dainius Lukauskas[1], Aida Šermukšnytė[2], Ingrida Tumosienė[2], Kristina Kantminienė[3], Vilma Petrikaitė[1,4]

[1]Department of Drug Targets Histopathology, Lithuanian University of Health Sciences, Lithuania
[2]Department of Organic Chemistry, Kaunas University of Technology, Lithuania
[3]Department of Physical and Inorganic Chemistry, Kaunas University of Technology, Lithuania
[4]Life Sciences Center, Vilnius University, Lithuania
dainius.lukauskas@lsmu.lt

Oncological diseases remain a leading cause of mortality worldwide, with lung, breast, and skin malignancies displaying particularly high incidence rates [1]. Lung cancer is the foremost cause of cancer-related deaths, accounting for 18.7% of all such fatalities globally [1]. Triple-negative breast cancer, representing 12–17% of all breast cancer diagnoses, is characterized by an aggressive clinical trajectory and poor patient prognosis [2]. Furthermore, while melanoma constitutes only 1% of skin cancer cases, it drives over 80% of skin cancer mortality [3,4]. Although targeted therapies have become a cornerstone of modern oncological treatment [5], conventional two-dimensional (2D) cell cultures used in early-stage in vitro screening fail to replicate the complex in vivo tumor microenvironment [6]. Three-dimensional (3D) cell cultures, or spheroids, provide a more accurate architectural representation, encompassing cell-cell interactions, hypoxic cores, and realistic drug penetration barriers, making them a crucial and more reliable model for evaluating the true efficacy of novel anticancer compounds [6].

In this study, we evaluated the anticancer activity of newly synthesized triazole derivatives against 3D spheroid models of human lung adenocarcinoma (A549), triple-negative breast cancer (MDA-MB-231), and melanoma (IGR39) cell lines. The evaluated compounds were previously selected based on their inhibition of cell viability in earlier tests, for which EC_{50} values had already been established. Spheroids were generated using magnetic 3D Bioprinting method. Following formation, the spheroids were treated with 10 µM of the selected triazole derivatives and comparative compounds sunitinib and dasatinib for 10 days. Spheroid growth was monitored by measuring changes in diameter over time, and end-point cell viability was quantified using the MTT assay.

The results demonstrated that compound IT168 significantly hindered spheroid growth, reducing the diameter of MDA-MB-231 and IGR39 spheroids by approximately 7% and 30% (comparable to both sunitinib and dasatinib), respectively, relative to untreated controls. While IT168 reduced IGR39 spheroid viability to 78%, the derivatives IT164 and IT165 exerted a stronger cytotoxic effect on both MDA-MB-231 and IGR39 spheroids, reducing cell viability to 55–56% (an effect comparable to sunitinib) without significantly affecting overall spheroid size. None of the evaluated triazole derivatives significantly inhibited the growth or viability of A549 spheroids.

In conclusion, the novel triazole derivatives IT164, IT165 and IT168 exhibited notable anticancer activity in 3D cultures, suggesting potential applications in future therapeutic development.

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D9

VALIDATION OF GREENB1 APTAMER SPECIFICITY IN PANCREATIC DUCTAL ADENOCARCINOMA MODELS UNDER STATIC AND MICROFLUIDIC CONDITIONS

Sunteiks Kristaps[1], Goluba Karina[1], Parfejevs Vadims[1], Riekstina Una[1], Kunrade Liga[1]

[1]Department of Pharmaceutical Sciences, University of Latvia, Faculty of Medicine and Life Sciences, Latvia
kristaps.sunteiks@lu.lv

Although in recent years increased attention has been paid to expanding the possibilities for diagnosis and therapy of malignant tumours, pancreatic ductal adenocarcinoma (PDAC) remains one of the most aggressive types of cancer, ranking highly on the list of the deadliest malignant tumours [1]. The peculiarities of the pancreatic environment and the tumour itself make it particularly resistant to chemotherapy, requiring an effective and innovative targeted therapy approach [2]. One of the strategies for targeted therapy is aptamers and their conjugates. Aptamers are single-stranded oligonucleotides that, due to their unique three-dimensional structure, can bind to target molecules with high affinity and selectivity. Although they can serve as active substances on their own, conjugating them with auxiliary and pharmaceutical molecules allows for the development of targeted drug delivery systems. GreenB1 is an integrin β1 (ITGB1) specific aptamer [3, 4], and, since ITGB1 is often overexpressed in tumour cells, its characterisation in various model systems and conditions would enable understanding of integrin expression and GreenB1's potential application as a vector for targeted therapy. Organoid culture systems have emerged as a robust complement to studies performed in immortalised cell lines and animal models. These self-organising 3D cellular structures more accurately recapitulate the structural and functional properties of the original tissue compared to conventional 2D cultures [5]. Subsequently, these structures can be deployed within microfluidic organ-on-a-chip (OoC) systems to evaluate cellular responses under dynamic physiological conditions. The use of various PDAC cell lines, organoids, and OoC model systems would provide in-depth information about the GreenB1 aptamer's ability to bind to tumour cells, depending on environmental conditions.

The aim of the current study was to characterise the affinity of GreenB1 in PDAC cell lines and organoids obtained from patient biopsies under static and microfluidic conditions, for possible development of a targeted drug delivery approach.

Experimental condition optimisation and GreenB1 affinity testing for established cancer cell lines (CAPAN, MIA-PaCa), primary patient-derived PDAC and normal pancreatic organoid cultures were done using ImagestreamX MarkII (Cytek Biosciences) imaging flow cytometer (FC). OoC model systems with and without endothelial channels were established using 4-channel CellBox Labs microfluidic chips. GreenB1 binding validation in microfluidic conditions was assessed using immunofluorescence analysis (IF). Fluorescence signal of GreenB1 was amplified using Alexa Fluor^{TM} 488 Tyramide SuperBoost^{TM} kit.

FC data indicate that Capan cells have a superior affinity for GreenB1; thus, further optimisation was performed using the Capan cell line, where we found that one hour of 500nM staining yields the best results. Capan cells were further used in an OoC system in which the binding of GreenB1-FAM could not be detected by IF due to low signal intensity. Signal amplification enhanced the detection of GreenB1 binding in static conditions and enabled the visualisation of aptamer binding to Capan cells in a PDAC OoC model.

GreenB1 binding conditions were optimised for the Capan cell line. Preliminary data demonstrate that GreenB1 binding is detectable in an OoC system. These findings support the feasibility of developing a further aptamer-based targeted delivery approach in physiologically relevant conditions. The next objective is to conjugate GreenB1 to extracellular vesicles to assess PDAC targeted drug delivery.

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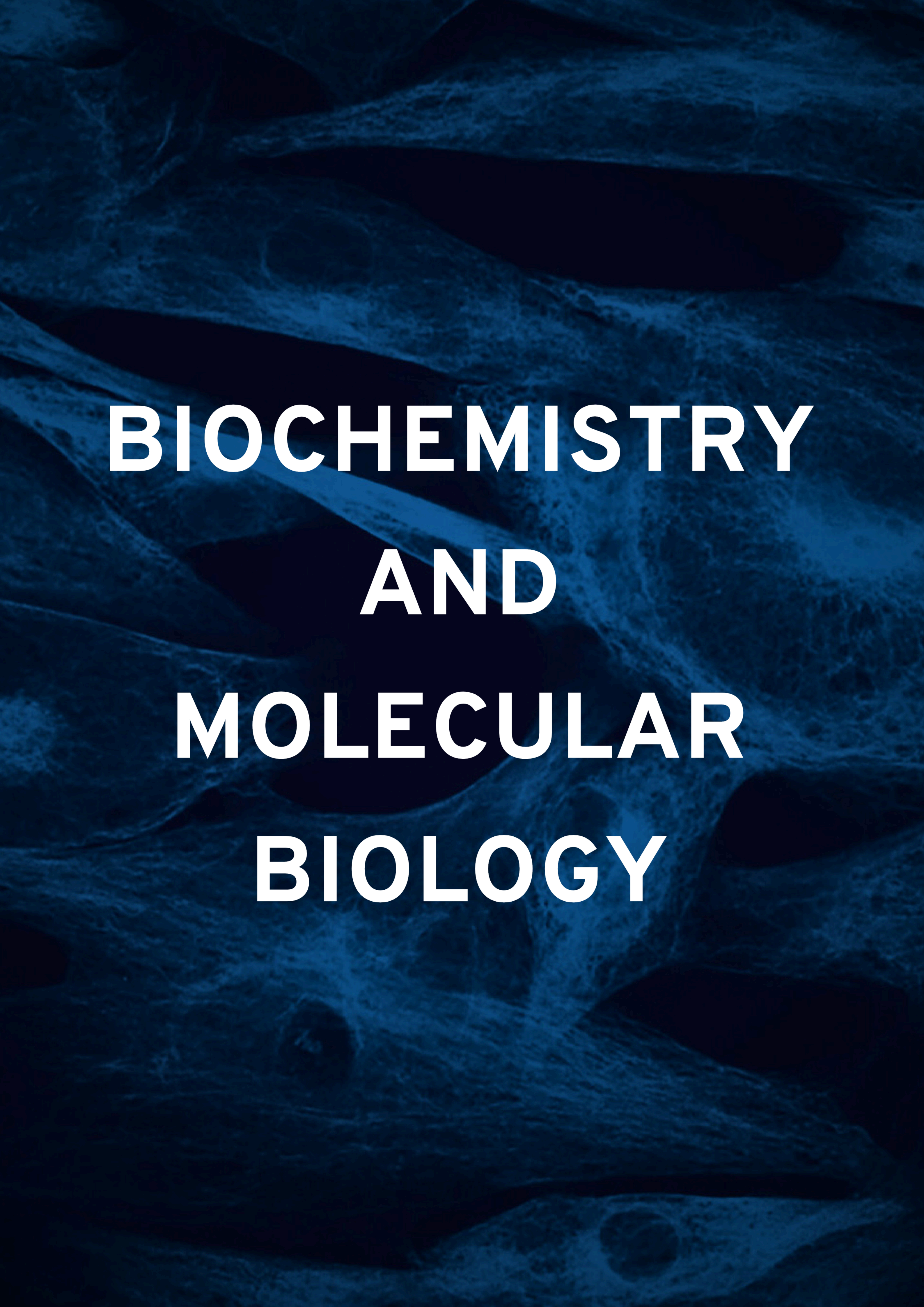

BIOCHEMISTRY AND MOLECULAR BIOLOGY

SYNTHESIS, STRUCTURAL CHARACTERIZATION, AND APPLICATION OF NITROGEN- AND BORON-DOPED REDUCED GRAPHENE OXIDE FOR ELECTROCHEMICAL DOPAMINE SENSING

Amelia Hendik[1], Julius Smaliukas[1], Gintare Rimkute[1], Justina Gaidukevic[1]

[1]Faculty of Chemistry and Geosciences, Vilnius University, Lithuania
amelia.hendik@chgf.stud.vu.lt

Recently, attention has shifted to developing sensitive, selective dopamine (DA) sensors due to its key role as a hormone and neurotransmitter in regulating motor function, mood, and cognition. A proper DA balance in the body is vital not only for physical but also for psychological well-being, as low DA concentrations have been strongly linked to Parkinson's disease, depression, and schizophrenia [1]. Conversely, elevated DA levels can lead to cardiotoxicity, tachycardia, hypertension, and even heart failure [2]. Thus, the development of accurate, sensitive, and selective DA sensors is of paramount importance for timely detection, early intervention, and management of these conditions.
Electrochemical sensors offer a rapid, cost-effective, and sensitive method for DA detection, with growing research focused on improving their performance for clinical diagnostics [3-5]. However, despite substantial progress, several critical challenges remain. Many sensors still struggle to differentiate DA from structurally similar interferents such as ascorbic acid (AA), uric acid (UA), and other neurotransmitters (e.g., serotonin, norepinephrine), which exhibit overlapping redox potentials. Achieving high sensitivity at nM concentrations, essential for early diagnosis, also remains difficult [3]. To address these challenges, it is essential to develop scalable and cost-effective synthesis and surface modification strategies for electrode materials used in electrochemical neurotransmitter sensors, focusing on improving selectivity, reducing LOD, and achieving stable, reproducible, and environmentally sustainable sensor designs.

The aim of this study is to synthesize and characterize nitrogen- and boron-doped reduced graphene oxide and to evaluate its electrochemical activity toward dopamine detection. Graphene oxide was prepared by graphite oxidation using the Hummers method, followed by hydrothermal reduction and heteroatom doping employing indole and boric acid as dopant sources. Structural, chemical, and textural properties of the synthesized materials were investigated by Raman spectroscopy, X-ray photoelectron spectroscopy, nitrogen adsorption-desorption analysis, and scanning electron microscopy. XPS results confirmed the successful incorporation of nitrogen and boron into the graphene framework through the formation of C-N and C-B bonds, while Raman analysis indicated disruption of the π-conjugated system due to defect formation. All samples exhibited slit-like porous structures characterized by H3-type hysteresis loops. Electrochemical evaluation demonstrated that nitrogen and boron co-doped reduced graphene oxide exhibits enhanced electrochemical performance and represents the most promising material for dopamine sensing applications.

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IN VITRO CHARACTERIZATION OF THE MOKOSH TYPE 1 ANTI-PHAGE DEFENSE SYSTEM

Matas Račickas[1], Edvinas Jurgelaitis[1], Arūnas Šilanskas[1], Mindaugas Zaremba[1]

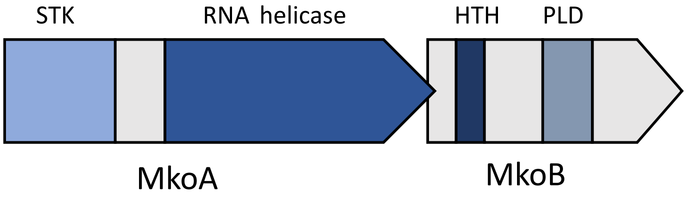
[1]Department of Protein-DNA Interactions, Institute of Biotechnology, Life Sciences Center, Vilnius University
matas.racickas@gmail.com

During the constant battle between bacteria and bacteriophages, bacteria have evolved a plethora of anti-phage defense systems. While restriction-modification, and CRISPR-Cas systems have been characterized to various extents, there remain completely uncharacterized and novel anti-phage defense systems. One of these systems, named after the Slavic goddess, protector of women's destiny, Mokosh, is a family of defense systems that is evolutionarily linked to eukaryotic immune system proteins, more specifically anti-transposon piwi-interacting RNA pathway proteins [1]. The Mokosh type I system is one of two in the Mokosh family and contains two proteins MkoA and MkoB, which contain four predicted domains (STK, RNA helicase, HTH, PLD, fig. 1) [2].

The primary aim of this study is to examine the specifics of each domain and its function. Here we present our findings on the Mokosh type I defense system, focusing mostly on *in vitro* experiments. Firstly, we optimized the expression of the proteins MkoA and MkoB in *E. coli* using different strains. Additionally, after successfully purifying both proteins together, two mutants were selected and purified as well. Subsequently, we tested different nucleic acid substrates using denaturing urea polyacrylamide gel electrophoresis, possible inhibitory mechanisms and nucleotide hydrolysis with microplate assays, as well as identifying possible oligomerization states using mass photometry and SEC-MALS.

This research unveils a new potential tool for biotechnology and could provide new insights into the evolution of eukaryotic immune system proteins. Our gathered data serves as a valuable first step in understanding the Mokosh type I anti-phage system and its mechanisms of action.

Figure 1. Schematic representation of Mokosh type I predicted domain organization.



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3-(3-AMINO-3-CARBOXYPROPYL) URIDINE CATABOLIC PATHWAY ANALYSIS IN SOIL BACTERIA

Domantas Barkauskas[1], Rokas Statkevičius[1], Rolandas Meškys[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center,
Vilnius University, Saulėtekio av. 7, Vilnius, Lithuania
domantas.barkauskas@chgf.stud.vu.lt

Currently there are over 200 known natural RNA modifications, 80% of them are found on tRNA molecules, which maintain their stability and functionality [1]. One of these tRNA post-transcriptional modifications is 3-(3-amino-3-carboxypropyl) uridine (acp3Ud), it is important for cell growth in prokaryotes and eukaryotes alike. This modification likely inhibits Watson-Crick base pairing between N3 atom of uracil, in turn increasing the melting point of tRNA. It is known that Escherichia coli encodes tapT (tRNA-uridine aminocarboxypropyltransferase), which modifies U47 in the variable loop of tRNA using S-adenosylmethionine (SAM) as a 3-amino-3-carboxypropyl donor, creating acp3U47. Meanwhile in humans, mice and Drosophila melanogaster this modification occurs in positions U20 and U20a [2]. However, nothing is known about the fate of this modification when tRNA is no longer needed and enzymes which cleave the N-glycosidic bond between the modified uracil and ribose. Therefore a thorough search for acp3Ud metabolizing enzymes in soil bacteria was conducted.

Soil and water sample screening revealed a total of 5 samples, which exhibited degradation of acp3Ud. After successfully isolating several microorganisms, sequencing results unveiled that they belong to the Aminobacter genus, which hydrolyze the N-glycosidic bond in acp3Ud. Three genes, two of which are ribonucleoside hydrolases (reaction scheme shown in Figure 1), were selected as being responsible for enzymatic degradation of acp3Ud and their expression was successfully induced. Current research efforts are focused on purifying these enzymes, capable of metabolising acp3Ud.

In summary, this study aims to identify microorganisms responsible for catabolising acp3Ud and uncovering enzymes cleaving the carbon-nitrogen bond between the nitrogenous base and its modification.

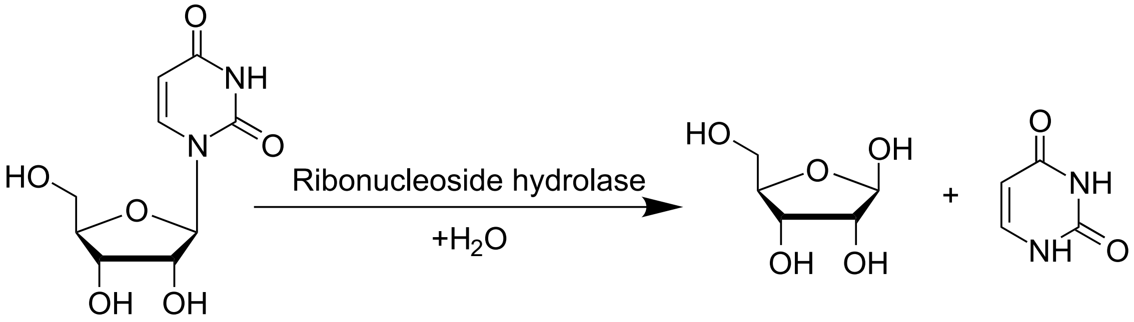

Figure 1. Hydrolysis of uridine catalysed by ribonucleoside hydrolases.

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INTERACTION BETWEEN PHAGE UNCHARACTERIZED PROTEIN AND BIFUNCTIONAL POLYMYXIN RESISTANCE PROTEIN ArnA

Agnė Vyšniauskaitė[1], Lidija Truncaitė[1]

[1] Department of Molecular microbiology, Institute of Biochemistry, Life Sciences Center, Vilnius University
agne.vysniauskaite@chgf.stud.vu.lt

Bacteriophages are the most abundant and ubiquitous biological entities on Earth. Recent large-scale genomic studies have identified more than 100,000 lytic phage genomes found in the cells beside bacterial hosts DNA, further expanding the known diversity of bacteriophages [1]. Due to their extensive genetic variability and high bioavailability, bacteriophages represent a major potential resource for biotechnological and medical applications. Especially as risks in medical and agriculture fields rise due to antibiotic resistance.

Phages are cellular parasites proficient in their host targeting strategy. Their sole purpose is to successfully replicate by overriding host internal systems. To facilitate this process, phages encode specialized proteins that interfere with host cellular systems, mainly essential ones, such as nucleotide synthesis [2]. These proteins play a crucial role in modulating host physiology during infection and are of particular interest for applied research, as they may reveal phage-specific mechanisms that determine infection efficiency, host specificity, or susceptibility

ArnA is a bifunctional polymyxin resistance protein that is encoded in the genome of *Escherichia coli* K-12 [3]. This enzyme causes resistance to antibiotic polymyxin B, which specifically targets the outer Gram – negative bacteria membrane [4]. The potential spread of resistance genes raises risks in treating Gram-negative bacterial infections and ensuring the safety of food supply. Polymyxin class antibiotics are considered as last resort.

Here we propose strategy and results from investigating interaction between the small uncharacterized phage protein and the bifunctional *Escherichia coli* protein ArnA. The analysis consists of information gathered from both *in vitro* and *in vivo* experiments, such as pull-down assay, protein–protein interaction analysis using the bacterial adenylate cyclase two-hybrid (BACTH) system, as well as evaluation of protein interaction effect on antibiotic resistance using disk diffusion assay.

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E5

A NEW ADDITION TO THE TYPE III CRISPR-CAS DEFENSE REPERTOIRE

Marija Duchovskyte[1], Dalia Smalakyte[1], Gintautas Tamulaitis[1]

[1]Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
marija.duchovskyte@gmc.stud.vu.lt

Clustered regularly interspaced short palindromic repeats (CRISPR), together with CRISPR-associated (Cas) proteins, defend prokaryotes against viral infections by specifically recognizing and cleaving invading nucleic acids. Based on the architecture of the interference complexes and signature proteins, CRISPR-Cas systems are classified into seven types [1]. In type III CRISPR-Cas systems, the recognition of viral transcripts by the interference complex initiates the synthesis of cyclic oligoadenylates (cOAs). These second messengers bind to the sensory domains of downstream effector proteins, thereby activating their effector domains. Activated effectors disrupt the bacteriophage infection cycle through diverse mechanisms, including DNA or RNA cleavage [2], inhibition of transcription [3] or translation [4], ATP deamination [5], or degradation of NAD^+ [6].

The diversity of type III CRISPR–Cas auxiliary effectors continues to grow, with recent systematic analyses of previously unannotated genes within CRISPR–Cas identifying novel effector proteins [7]. These novel effectors are predicted to display biochemical activities that have not previously been associated with CRISPR-Cas immunity, thus their part in type III CRISPR–Cas-mediated defense remains largely unknown.

In this study, we present the first insights into novel type III CRISPR–Cas effector proteins. The effects of activation of putative effectors on bacterial viability were evaluated in a heterologous *Escherichia coli* host using a plasmid-based system for cOA production, as described in [4]. Effector proteins exhibiting functional activity were subsequently purified by affinity chromatography and submitted for further biochemical characterization.

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α-HEMOLYSIN EFFECT ON MODEL MEMBRANES OF VARYING COMPOSITIONS

Agnė Liuterytė[1], Tomas Sabirovas[1]

[1]Department of Bioelectrochemistry and Biospectroscopy, Institute of Biochemistry, Life Sciences Center, Vilnius University, Lithuania.
agne.liuteryte@chgf.stud.vu.lt

The antibiotic resistance of *Staphylococcus aureus* bacteria (e.g., the spread of MRSA strains) remains a significant clinical and public health issue; consequently, there is an increasing focus on alternative antimicrobial strategies, including the neutralization of virulence factors and their understanding [1]. One of the most critical virulence factors is α-hemolysin (Hla), a pore-forming toxin that inserts into host cell membranes and oligomerizes into heptameric transmembrane pores, thereby compromising their barrier integrity and inducing cell lysis or death [2]. Since Hla pore formation is modulated by the lipid composition of the membrane, this study examined three tethered bilayer lipid membrane (tBLM) models with different compositions: phosphatidylcholine:cholesterol (PC:Chol), red blood cell (RBC) mimicking, and brain capillary endothelial cell (BCEC) mimicking membranes.

In this study, tBLMs were formed on functionalized gold electrodes via vesicle fusion. Vesicle solutions of varying compositions were utilized: a PC:Chol mixture (60:40 mol. %), an RBC composition (Chol:SM:PC:PE:PS, 45:15:15:15:10 mol. %), and a BCEC composition (Chol:SM:PC:PE:PS, 30:20:20:20:10 mol. %). The formation of the tBLMs and the interaction of α-hemolysin with these phospholipid membranes were monitored via electrochemical impedance spectroscopy (EIS). Spectra were recorded in complex impedance coordinates across a frequency range of 0.1 Hz to 100 kHz, with the resulting data presented in complex capacitance and Bode phase plots. Experimental EIS data were modeled using custom *Python*-based software and a physical tBLM impedance model, which enabled the estimation of defect (pore) probability density functions $P(N_{\mathrm{def}})$ and the calculation of the average defect density $\langle N_{\mathrm{def}} \rangle$ [3].

Based on the EIS data and subsequent modeling, three distinct tBLM compositions were formed on gold electrodes: PC:Chol (60:40 mol. %), RBC-type, and BCEC-type lipid compositions. Their baseline (natural) defect densities, estimated from EIS modelling were 0.111; (SD = 0.059), 0.119; (SD = 0.165) and 0.281; (SD = 0.322) μm^{-2}, respectively. Further investigation into the interaction with Hla revealed that Hla-induced defects (pores) exhibit a heterogeneous distribution across all tested membrane compositions [4], independent of the Hla concentration (range 1–150 nM). Nevertheless, based on the average defect density $\langle N_{\mathrm{def}} \rangle$, the lowest detectable Hla concentration was observed in BCEC-type membranes (1 nM), whereas for RBC-type and PC:Chol tBLMs, the detection threshold was higher (5 nM). The RBC and BCEC mimicking tBLMs demonstrated significantly higher sensitivity than in the PC:Chol tBLM: once the critical concentration was reached, $\langle N_{\mathrm{def}} \rangle$ increased more steeply. An upper-limit plateau in average defect densities was observed at 50–150 nM across all tBLM compositions. The maximum defect density (saturation) was highest in the RBC model, reaching approximately 40 defects/μm^{-2}, whereas the BCEC mimicking tBLM and the control PC:Chol model plateaued at 10–20 defects/μm^{-2}.

These results demonstrate that the lipid composition of the membrane critically modulates the extent of Hla-induced damage, while biomimetic tBLM models provide a robust platform for comparing toxin activity across various biologically relevant membrane compositions.

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MUTAGENESIS OF THE PmlABCDEF MONOOXYGENASE TO ENHANCE EPOXIDATION STEREOSELECTIVITY

Kristupas Bendikas[1], Greta Mačiuitytė[1], Rolandas Meškys[1], Vytautas Petkevičius[1]

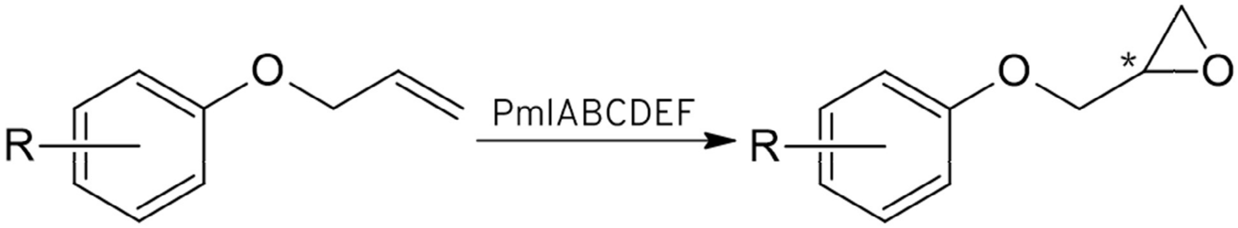
[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center,
Vilnius University, Lithuania
kristupas.bendikas@chgf.stud.vu.lt

Epoxides have a wide range of uses across different industries. They are desirable intermediates in drug synthesis due to versatile ring-opening reactions. Chiral epoxides are attractive intermediates in pharmaceutical synthesis because, depending on their (S) or (R) configuration, active pharmaceutical ingredients (APIs) can have vastly different effectiveness [1]. This research studies PmlABCDEF (PML for short) monooxygenase, which can oxidize a wide range of substrates, including ones bearing C=C double bond, resulting in a chiral epoxide. PML exhibits regio- and stereoselective epoxidation, but for some targeted substrates, these properties are suboptimal [2]. Our objective is to create a PML monooxygenase mutant that can oxidize selected alkene substrates with higher enantioselectivity than wild type enzyme.

Using site-directed mutagenesis with our designed primers in PCR, we created PML monooxygenase mutant library. These mutants were screened with four selected alkene substrates, to determine whether epoxidation activity was present, we performed a color reaction with 4-(4-nitrobenzyl)pyridine. For mutants that showed epoxidation activity with selected substrates, biocatalytic reaction products were extracted with ethyl acetate and analyzed by thin-layer chromatography and high-performance liquid chromatography (HPLC) coupled with MS/UV-VIS spectrometers.

Of the 19 created PML monooxygenase mutants, 11 retained the ability to oxidize the C=C double bond to an epoxide. Further investigation is needed to determine how these mutations affected regio and stereoselectivity.

Figure 1. Schematic representation of the biocatalytic oxidation performed using PmlABCDEF monooxygenase.



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CHARACTERIZATION OF A NON-CANONICAL TYPE IV-A1 CRISPR-CAS SYSTEM

Gintarė Žvejytė, Rimvydė Čepaitė, Aistė Skorupskaitė, Patrick Pausch
Life Sciences Centre - European Molecular Biology Laboratory (LSC-EMBL) Partnership Institute for Genome Editing Technologies, Vilnius University, Vilnius, Lithuania
gintare.zvejyte@gmc.stud.vu.lt

CRISPR-Cas systems provide adaptive immunity against mobile genetic elements in bacteria and archaea. Among them, type IV-A1 CRISPR-Cas systems are distinct from other CRISPR types in that they lack canonical DNA or RNA nuclease activity. Instead, these multisubunit complexes recruit a DinG helicase, which unwinds target DNA and induces transcriptional interference rather than cleavage, stopping the infection [1][2].

Structural and biochemical studies have primarily focused on canonical type IV-A1 systems, which employ a Cas8 homolog in combination with a Cas5 to mediate protospacer adjacent motif (PAM) recognition [3][4]. In contrast, a recently reclassified non-canonical type IV-A1 system – formerly designated type IV-A2 – remains largely unexplored [1]. For a long time, it was accepted that these systems lack a Cas8 homolog, suggesting an alternative mechanism of target recognition [5]. However, it was shown that non-canonical type IV-A1 systems consistently co-occur with a small, conserved, *cas8-like* gene located adjacent to the *cas* operon and transcribed in the opposite orientation. Elucidating the function of the protein encoded by this gene may reveal a previously unrecognized component of type IV-A1 interference and expand our understanding of CRISPR-Cas systems diversity.

The aim of this study is to elucidate the structural basis and mechanism of action of a non-canonical type IV-A1 CRISPR-Cas system. Here, we present early structural and mechanistic characterization of a representative system using cryo-electron microscopy, complemented with functional analyses to probe target recognition and interference.

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INFLUENCE OF BACTERIAL DEFENSE SYSTEM COMPONENTS ON CELL VIABILITY

Austėja Narkevičiūtė[1], Tomas Šinkūnas[1]

[1]Department of Protein–DNA Interactions, Institute of Biotechnology, Life Sciences Center, Vilnius University
austeja.narkeviciute@gmc.stud.vu.lt

The ongoing arms race between bacteria and bacteriophages has led to the development of various defense strategies in bacteria. Prokaryotes have evolved mechanisms to terminate viral infection at multiple stages of the phage life cycle. Modification or masking of cell surface receptors prevents phage recognition, while superinfection exclusion systems (Sie) block the injection of bacteriophage nucleic acids if recognition occurs. Restriction-modification and the adaptive CRISPR-Cas systems target foreign nucleic acids that have entered the cell, and secondary metabolites can inhibit viral replication or transcription. Finally, abortive infection (Abi) systems halt bacteriophage propagation by inducing cell dormancy or programmed cell death [1].

It is now known that genes encoding these antiviral defense systems tend to cluster in regions called defense islands within the bacterial genome [2]. This observation has facilitated the discovery of previously unknown defense systems around already characterized defense genes. A four-gene operon, QatABCD, is one of the novel defense systems identified this way [3]. However, its precise mechanism of action remains unclear.

In this study, we investigate the effects of QatABCD components on *Escherichia coli* cell viability to determine a potential effector of the system. The growth of bacterial cells carrying plasmids with different deletion variants of the operon was monitored by measuring optical density in a plate reader under uninfected conditions. Additionally, phage infection dynamics were evaluated at varied multiplicities of infection (MOIs) using cells expressing the full QatABCD system. We find that specific QatABCD constructs differentially affect cell viability, pointing to candidate effector components of the system.

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Gene Editing and Delivery Techniques for Aceruloplasminemia

Neringa Daugelaviciene[1,2], Simonas Kutanovas[1,2], Eimina Dirvelyte-Valauske[1], Rusne Uzielaite[1], Olgerd Babkin[1], Modestas Mazerimas[3], Rima Budvytyte[3], Urte Neniskyte[1,2]

[1]Institute of Bioscience, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]VU LSC-EMBL Partnership for Genome Editing Technologies, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[3]Institute of Biochemistry, Life Sciences Center, Vilnius University, Vilnius, Lithuania
neringa.daugelaviciene@gmc.vu.lt

Aceruloplasminemia (ACP) is a rare, autosomal recessive neurodegenerative disorder caused by mutations in the ceruloplasmin (CP) gene, leading to iron buildup in the brain, liver, and pancreas. Current therapies, including iron chelation and enzyme replacement, offer limited clinical benefits. To tackle these challenges, we aim to develop a comprehensive gene therapy approach tailored for ACP.

Our strategy employs patient-derived induced pluripotent stem cells (iPSCs) and mouse organotypic brain slices to investigate and correct CP deficiency. We aim to restore CP function using two complementary delivery platforms: neurotropic, non-replicating HSV-1 vectors for targeted delivery of genome-editing tools to neural tissue, and lipid nanoparticles (LNPs) for delivering CRISPR prime editing components. Prime editors enable precise correction of point mutations in the CP gene, overcoming limitations of earlier CRISPR technologies. Lipid compositions for LNP formulations will be optimized to enhance delivery efficiency and tissue targeting, with a focus on the brain.

Our study evaluates HSV-mediated delivery of gene editing components into patient-derived iPSCs and explores LNP-mediated delivery in mouse organotypic brain slices. By comparing these delivery platforms, we aim to determine the best strategy that combines the most effective genome-editing method with the most efficient delivery system, enabling assessment of therapeutic potential at both molecular and functional levels.

E11

INHIBITION OF THE CRISPR-CAS SYSTEM BY AN ANTI-CRISPR PROTEIN

Darija Andrušaitytė[1], Tomas Šinkūnas[1]

[1]Department of Protein-DNA Interactions, Institute of Biotechnology, Vilnius University, Lithuania
darija.andrusaityte@gmc.stud.vu.lt

The ongoing co-evolutionary arms race between bacteria and bacteriophages has led to the development of various defense and attack strategies. One of the bacterial defense systems against phages is CRISPR-Cas. This system detects and integrates invading DNA into the CRISPR array. The transcript of the CRISPR array is processed into small crRNA molecules, which together with Cas proteins form ribonucleoprotein effector complexes. Guided by crRNAs, these complexes recognize and cleave complementary phage DNA, thereby providing adaptive immunity [1]. To overcome CRISPR-Cas–mediated defense, bacteriophages have evolved anti-CRISPR (Acr) proteins that inhibit CRISPR-Cas activity, most commonly by preventing target DNA binding or nuclease function [2]. Beyond their biological relevance, Acr proteins have attracted considerable interest as regulators of CRISPR-Cas–based genome editing technologies [3,4]. Despite the rapid expansion in the number of identified Acr proteins, detailed molecular mechanisms of inhibition have been elucidated for only a small subset [4].

In this study, we analyse the inhibition mechanism of a previously uncharacterized AcrIF protein targeting the type I-F CRISPR-Cas system. *In vivo* activity assays demonstrate that this AcrIF protein inhibits the CRISPR ribonucleoprotein effector complex. The protein was purified using liquid chromatography, and its inhibitory activity was assessed through a series of biochemical assays. Our current data indicates a unique mode of action for this AcrIF protein, the molecular basis of which we aim to elucidate.

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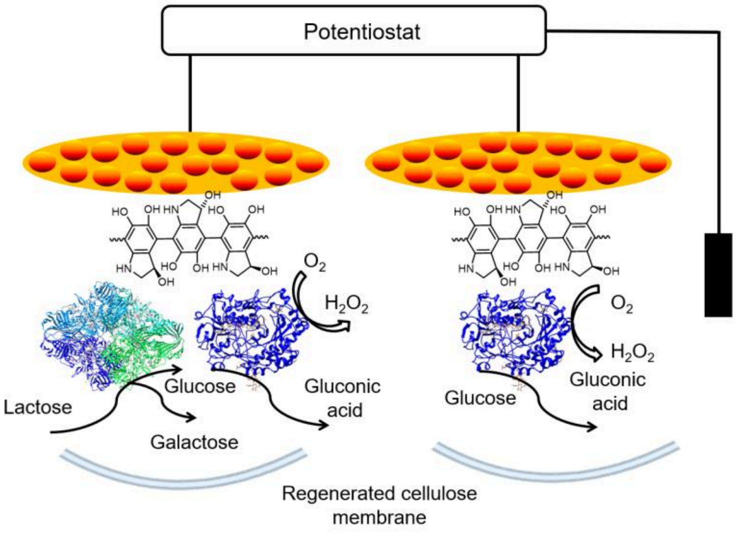
DUAL-CHANNEL AMPEROMETRIC BIOSENSOR FOR PARALLEL DETECTION OF LACTOSE AND GLUCOSE IN MILK PRODUCTS

Daina Slavinskaitė[1], Mykolas Stankevičius[1], Gintautas Bagdžiūnas[1]

[1] Group of Supramolecular Analysis and Bioelectronics at Institute of Biochemistry, Life Sciences Center, Vilnius University, Lithuania
Email: daina.slavinskaite@gmc.vu.lt

Lactose, a disaccharide composed of glucose and galactose residues, is a primary component of milk and a significant energy source in the human diet. However, as lactose intolerance affects a significant portion of the global population, the dairy industry is increasingly prioritizing the production of lactose-reduced and lactose-free alternatives. [1] Consequently, the development of methods for the rapid and accurate detection of lactose levels is essential for industrial quality control and consumer safety. [2]

Figure 1. The schematic presentation of the dual-channel electrochemical biosensor



In this work, we developed a dual-channel biosensor capable of simultaneously quantifying lactose and glucose concentrations. The sensor architecture is composed of two gold working electrodes modified with gold nanoparticles and a polynoradrenaline layer sensitive to molecular oxygen. To achieve this selectivity, the first electrode was functionalized with a bi-enzymatic system (β-galactosidase and glucose oxidase), while the second electrode was functionalized solely with glucose oxidase. Additionally, both electrodes were covered with a regenerated cellulose membrane. This configuration enables the parallel detection of both analytes by monitoring the enzymatic depletion of molecular oxygen. The biosensor was characterized, and its performance was validated using real milk samples, demonstrating high selectivity, sufficient sensitivity and practical feasibility for lactose detection in milk products.

Acknowledgement: This work was funded by the “Nanostructured Bioconjugates for Quantum Bioelectronics” project, which has received funding from the Research Council of Lithuania (LMTLT), agreement no. S-ITP-25-11.

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E13

TRANSCRIPTOMIC ANALYSIS OF THE EFFECTS OF INDIRUBIN DERIVATIVES ON CANCER CELLS

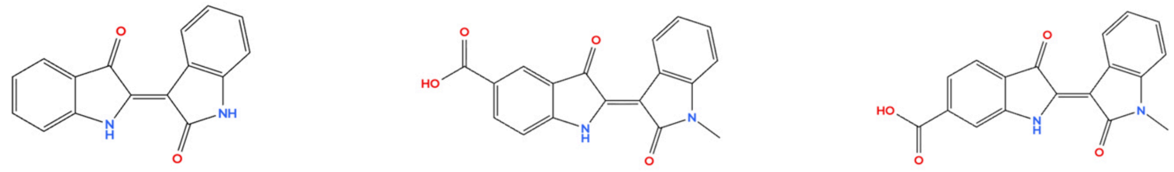
Mija Hakaitė[1], Viktorija Preitakaitė[1] , Mikas Sadauskas[1], Rolandas Meškys[1]

[1]Institute of Biochemistry, Department of Molecular Microbiology and Biotechnology, Life Sciences Center, Vilnius University
mija.hakaite@gmc.stud.vu.lt

Indirubin is the main bioactive component of Indigo naturalis, a traditional Chinese medicine, with anti-inflammatory properties, and it also exhibits anticancer activity. In recent years, modified indirubin derivatives have been shown to display even stronger anti-inflammatory and anticancer effects [1]. Although indirubin derivatives are well known as cyclin-dependent kinase inhibitors, accumulating evidence suggests that they may act through additional molecular mechanisms, many of which remain poorly understood. In this study, we aimed to investigate and compare the effects of 5 indirubin derivatives (Fig. 1) on human cancer cells using transcriptomic analysis.

Human breast cancer MCF-7 cells were treated with five indirubin derivatives (1, 2, 2a, 2b and 2c). Three biological replicates were performed for each condition. Total RNA was isolated from treated cells and RNA sequencing was performed (Seqvision, Vilnius, Lithuania). Differential gene expression was calculated using the EPI2ME analysis platform provided by Oxford Nanopore Technologies. Functional analyses were performed using bioinformatics tools including Metascape, GOrilla, STRING, and NDEx IQ.

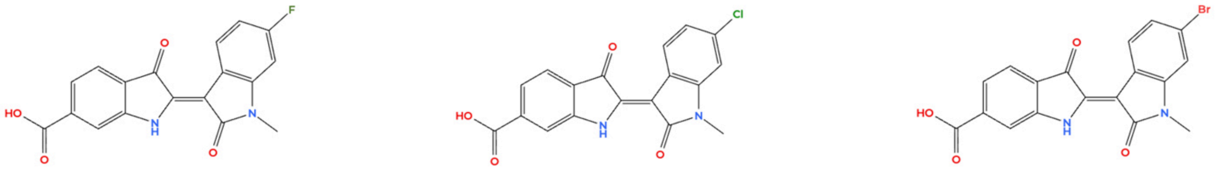
Figure 1. Chemical structures of indirubin and its derivatives (1, 2, 2a, 2b and 2c).



Indirubin

1-methylindirubin-5'-carboxylic acid
1

1-methylindirubin-6'-carboxylic acid
2



6-fluoro-1-methylindirubin-6'-carboxylic acid
2a

6-chloro-1-methylindirubin-6'-carboxylic acid
2b

6-bromo-1-methylindirubin-6'-carboxylic acid
2c

We expect that RNA sequencing will reveal clear differences in gene expression between treated and control samples. Functional enrichment analyses are expected to highlight pathways associated with mitochondrial function and cellular stress responses. These changes are consistent with published data showing that water-soluble indirubin derivatives negatively affect mitochondria, leading to their disruption, increased production of reactive oxygen species (ROS), and activation of the intrinsic apoptotic pathway. [2, 3]

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E14

EVALUATING THE SUITABILITY OF TNPB NUCLEASES FOR PROTEIN ENGINEERING USING A BACTERIAL SCREENING PLATFORM

Justė Dautaraitė[1], Gytis Druteika[1], Tautvydas Karvelis[1]

[1]Department of Protein-DNA Interactions, Life Sciences Center, Vilnius University, Lithuania
juste.dautaraite@chgf.stud.vu.lt

Insertion sequences (IS) are a class of mobile genetic elements found in bacteria that encode only the genes required for their propagation and maintenance. IS elements belonging to the IS200/IS605 and IS607 families represent some of the simplest known transposable elements and contain genes encoding the TnpA and TnpB proteins. While TnpA has long been known to be indispensable for transposon excision and integration, TnpB proteins function in transposition has remained elusive, although sequence analyses have indicated a possible evolutionary connection to CRISPR-Cas nucleases [1, 2].

At present, the most widely used CRISPR-Cas nucleases for genome editing are Cas9 and Cas12a, which serve as RNA-guided DNA endonucleases that cleave DNA in a PAM (Protospacer Adjacent Motif)-dependent manner. Although highly efficient, these nucleases exhibit several limitations, including off-target activity, PAM sequence requirements, and delivery challenges associated with their large protein size. Consequently, there is a growing demand for the development of alternative genome-editing tools.

Recent studies suggest that TnpB nucleases share a minimal structural and functional core with the Cas12 protein family, showing the greatest similarity to Cas12f. Like Cas12 nucleases, TnpB is guided by the transposon right-end element-derived reRNA and requires a TAM (Transposon-Associated Motif) adjacent to the reRNA-complementary target site. TnpB-mediated DNA cleavage has been demonstrated in vitro as well as in bacterial and eukaryotic cells [3, 4]. However, natural TnpB variants generally exhibit lower editing efficiencies than larger Cas editors, and only a subset of TnpB proteins are active in human cells. To improve TnpB-based tools, mutagenesis and screening can be performed directly in cells providing functional confirmation of nuclease activity.

This study aimed to identify TnpB proteins that are active in *E. coli* cells. A set of TnpB proteins were selected for evaluation using a survival-based assay. In this system, nuclease activity enabled cell survival, whereas inactive proteins resulted in cell death.

The results indicate that TnpB nucleases with differing TAM sequences are active in *E. coli*. Owing to their compact size, simplified architecture, and programmable nuclease activity, these TnpB proteins represent promising candidates for protein-engineering efforts aimed at improving performance and expanding targeting range.

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SUBSTRATE-POWERED MOLECULAR SWITCHING IN THE BIOCONJUGATED GLUCOSE OXIDASE MONOLAYERS

Simonas Grybas, Ieva Grigonytė, Kamilė Ratkevičiūtė, Marija Jankunec, Lidija Tetianec, Gintautas Bagdžiūnas

Supramolecular Analysis and Bioelectronics group at the Institute of Biochemistry, Life Sciences Center, Vilnius
simonas.grybas@gmc.stud.vu.lt
ieva.grigonyte@gmc.stud.vu.lt

Modern semiconductor manufacturing has approached a critical bottleneck due to the limits of extreme ultraviolet (EUV) lithography, which uses 13.5 nm wavelength light to print nanometer-scale circuit patterns onto silicon wafers. [1] Consequently, achieving smaller nanostructures on chips has become increasingly complex. To advance the future of nanoelectronics, the objective is to develop single-molecule transistors with a total footprint below 10 nm [2]. Building on our previous theoretical [3] and experimental [4] findings that proteins can function as semiconductors, we explore a new frontier in bioelectronics: using individual protein molecules as functional transistors or molecular switches.

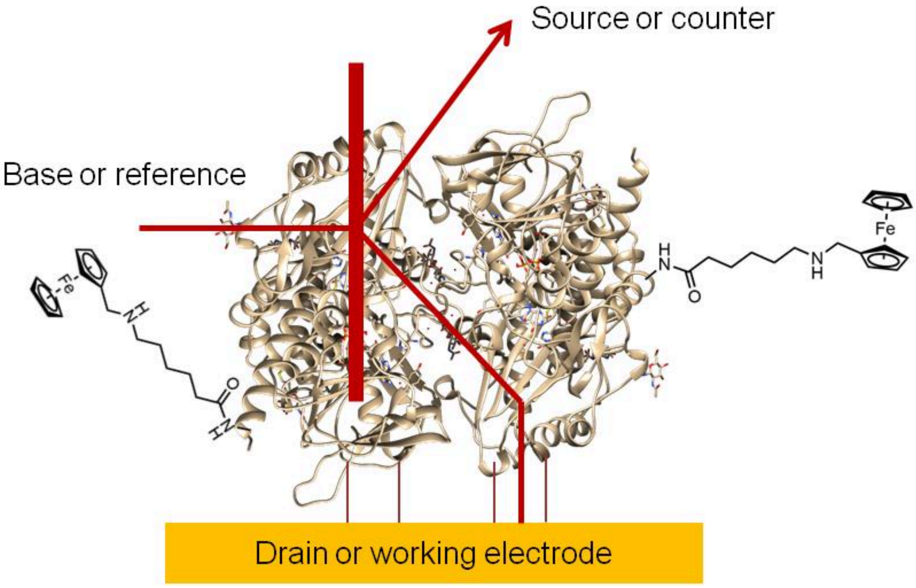

Figure 1. A schematic presentation of a molecular switch based on SAM of the bioconjugated glucose oxidase

A primary challenge in molecular electronics is the reproducible attachment of single molecules to electrodes to create stable molecular junctions. In this study, we prepared a self-assembled monolayer (SAM) of glucose oxidase bioconjugated with *N*-(ferrocenylmethyl)-6-aminohexanoic acid via an amide bond (Figure 1). The topographic distribution of individual enzymes within the monolayer was verified using atomic force microscopy (AFM).

Furthermore, we demonstrated that direct charge transfer occurs across this bioconjugated enzyme monolayer in the presence of glucose. We established that this charge transport can be modulated, effectively switched ON or OFF, at electrochemical potentials of 0 V and 0.4 V (vs. Ag/AgCl). These results indicate that this biostructure functions as a substrate-powered molecular switch. This technology will provide a scalable pathway for developing single-molecule, glucose-powered bioelectronics.

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Liquid-liquid phase separation of S100A4 protein

Arijus Jaloveckas[1], Darius Šulskis[1]

[1]Sector of Amyloid Research, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania

arijus.jaloveckas@gmc.stud.vu.lt

The main indications of Alzheimer's disease are the accumulation of aggregates called amyloids in the brain, which cause inflammation and neuronal damage [1]. One example are calcium binding and neuroinflammation associated S100A proteins, whose aggregates are found in Corpora Amylacea, spherical bodies of unknown function and origin, that form during the ageing process and several neurodegenerative disorders [2]. One of the initial causes that accelerates amyloid fibril formation is the liquid-liquid phase separation (LLPS) process, where proteins form membraneless droplets based on their affinity for each other and a crowded environment [3]. Presently, the relationship between LLPS and amyloid aggregation of S100 protein is unknown, therefore, the goal of this study is to determine whether a specific S100A4 member undergoes LLPS in a crowded environment and if it causes protein aggregation.

A construct S100A4 fused with an N-terminal eEGP construct was formed using restriction-free cloning. After successfully selecting positive colonies, plasmids containing *eGFP-S100A4* insert were purified and sequenced. For protein expression, BL21 Star™ (DE3) *E. coli* cells were transformed with the pET28b plasmid, containing eGFP-S100A4. Cells were grown until 0.6 optical density and protein production was induced with 0.1 mM IPTG. Harvested cells were lysed using sonication, and the soluble medium was separated via centrifugation. Then, the protein was applied to the Ni-NTA column, and the eluted fractions were concentrated. eGFP-S100A4 was further purified by size exclusion and ion exchange chromatography. Amyloid aggregation was monitored using amyloid-specific ThT dye. LLPS assay was conducted using different concentrations of eGFP-S100A4 and S100A4 mixture (1:99 ratio) and different polyethylene glycol (PEG) concentrations, which were used to imitate cellular environment. LLPS assay was observed using Olympus IX83 fluorescence microscope.

Initially, we observed that S100A4 does not aggregate in a non-crowded environment, except at low pH conditions. The addition of PEG induced the formation of S100A4 droplets at all 4 different protein concentrations (25, 50, 75, 100 µM), although the most prominent droplets were found in 10% and 20% PEG solutions. After successfully perceiving LLPS of S100A4, further research will include characterization of S100A4 state (aggregates, oligomers) in the droplets and their effect on the neuronal cells.

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INVESTIGATION OF HETEROCYCLIC BASE METABOLISM USING *ESCHERICHIA COLI* AUXOTROPHIC STRAINS

Ieva Petkevičiūtė, Augustė Kavaliauskaitė, Daiva Tauraitė, Jaunius Urbonavičius

Department of Chemistry and Bioengineering, Vilnius Gedimino Technical University, Lithuania
ieva.petkeviciute@stud.vilniustech.lt

Purines and pyrimidines are heterocyclic bases that are present in all three Domains of Life and are essential for nucleic acid structure, energy transfer and cofactor function [1]. Enzyme deficiency or overactivity disrupts nucleotide metabolism and can lead to major health disorders [2]. Additionally, many heterocyclic base derivates are used as active ingredients in the therapy of autoimmune diseases, cancer or viral infections [3]. Auxotrophs are strains that have lost their ability to synthetize important small molecules such as purines or pyrimidines. In metabolism research, auxotrophs acts as a powerful *in vivo* system that allows to determine the relationship between bacterial cell growth and protein synthesis [4]. In this study, auxotrophic *Escherichia coli* strains were applied to investigate the metabolism of modified heterocyclic bases using two complementary approaches: metagenomic library screening and transposon mutagenesis.

Four independent metagenomic libraries were constructed using DNA isolated from soil samples and transformed to auxotrophic *E. coli ΔguaB::*Km and *E. coli ΔpurH::*Km strains. Growth complementation was assessed on minimal M9 medium supplemented with modified purine bases whose metabolic pathways are not fully described in the scientific literature. At the same time, as *E. coli ΔguaB::*Km mutant is able to grow using 2-amino-N^6-methylpurine as source of guanine, whereas N^4-methylcytosine is used as source of uracil in *E. coli ΔpyrF::*Km strain [5], transposon mutagenesis was used to identify genes responsible for this phenotype. All transformants were individually screened for the loss of growth complementation on selective minimal M9 medium. Positive candidate mutants were selected for genome sequencing and analysis.

This study demonstrates the applicability of auxotroph-based systems for functional identification of enzymes involved in the metabolism of modified heterocyclic bases. Further investigations will reveal the identity of genes responsible for the observed growth complementation phenotypes.

This project has received funding from the Research Council of Lithuania (LMTLT), agreement No [S-MIP-25-95].

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E18

IN VIVO STUDY OF A NOVEL THOERIS EFFECTOR

Ignė Jačunskytė[1], Deividas Vilutis[1], Arūnas Šilanskas[1], Giedrė Tamulaitienė[1]

[1]Department of Protein-Nucleic Acid Interactions, Institute of Biotechnology, Life Sciences Center, Vilnius University,
Saulėtekis av. 7, Vilnius, Lithuania
igne.jacunskyte@chgf.stud.vu.lt

Bacteria are constantly exposed to viral (bacteriophage) infections and have therefore evolved a diverse repertoire of innate immune defense systems [1]. Among these, the Thoeris antiviral defense system relies on Toll/interleukin-1 receptor (TIR) domain-mediated immune signaling [2]. Thoeris systems consist of two components: a TIR-domain-containing protein that senses phage invasion and generates a specific ADP-ribose-based immune signaling molecule, and an associated effector protein activated by this signal to induce cellular growth arrest or cell death, thereby aborting phage replication. Although several Thoeris system types have been characterized, large-scale genomic analyses have uncovered numerous Thoeris loci encoding previously uncharacterized effector proteins with diverse domain architectures [3]. The frequent genomic association of TIR domains with genes encoding proteins of unknown function suggests that many Thoeris effectors act through yet undescribed molecular mechanisms.

In this study, we investigated the in vivo function of a previously uncharacterized Thoeris effector protein to define its role in TIR-mediated antiviral immunity. For functional analysis, the effector gene was cloned into E. coli expression vectors and introduced either alone or together with a TIR-domain–encoding gene via dual-plasmid electroporation, thereby establishing single-expression and co-expression conditions. Bacteriophage plaque assays demonstrated that expression of the effector alone did not confer antiviral protection, whereas co-expression with the TIR-domain protein resulted in phage resistance. These results indicate that the effector functions as a TIR-activated antiviral protein. Next, the effector protein was heterologously expressed in several E. coli strains, and expression was systematically evaluated across a range of induction conditions and growth temperatures to determine optimal protein production. Following optimization, the protein was purified by affinity chromatography.

These findings establish a functional framework for this previously uncharacterized Thoeris effector and lay the groundwork for future in vitro studies aimed at determining its molecular mechanism of action. Defining the function of this effector will expand our understanding of the mechanistic diversity of Thoeris defense systems and provide broader insight into conserved principles of TIR-mediated immune signaling across diverse biological domains.

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HIGH-THROUGHPUT KINETIC PROFILING OF TNPB FOR PRECISION GENOME EDITING

Marius Vinogradovas[1], L. Krikščikaitė[1], G. Druteika[2], A. Šilanskas[2],
T. Karvelis[2], S. K. Jones Jr.[1]
[1]Life Sciences Center, EMBL Partnership Institute, Vilnius University, Lithuania
[2]Life Sciences Center, Institute of Biotechnology, Vilnius University, Lithuania
marius.vinogradovas@gmc.vu.lt

Genome editing tools enabling precise DNA cleavage have rapidly advanced since the discovery and engineering of programmable RNA-guided nucleases, like CRISPR-Cas9. However, it is still challenging to comprehend these nucleases' mechanisms and to benchmark their specificity – that is, their ability to distinguish programmed targets from all other DNAs. The recently discovered TnpB nuclease, derived from a transposon system, represents a compact genome editing tool with high potential. However, its functionality, kinetics, and editing capabilities are not fully understood [1].

This study aims to profile TnpB's target specificity and kinetics with the high-throughput NucleaSeq platform [2]. Using NucleaSeq, we investigated the time-resolved cleavage rates, cleavage patterns, and specificities across a library of thousands of DNA targets that mispair with TnpB's RNA guide but are nonetheless cut by TnpB.

We observed distinct cleavage rates and patterns that were driven by mispairing between DNA targets and TnpB's gRNA. TnpB works less specifically when compared to the more widely studied Cas12a [1]. When a mispair occurs close to TAM, cleavage happens much slower than it does for targets with mispairs further from the TAM. Analysis across different gRNA and DNA target libraries confirm this feature of TnpB.

We also observed that TnpB repeatedly cuts (i.e. trims) matched and mispaired targets. Mispairs altered where and how much TnpB trims these targets. Currently, we are performing cell-based assays to compare these results with editing specificity and off-target activity in a human cell line.

This research expands our knowledge about the specificity of TnpB, presenting TnpB as a compact CRISPR nuclease alternative for genome editing. Its unique trimming features also provide opportunities for biotech applications.

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PRODUCTION AND SUBSTRATE SPECIFICITY ASSESSMENT OF RECOMBINANT *ESCHERICIA COLI* PURINE DEAMINASES

Ugnius Kamandulis, Jaunius Urbonavičius, Daiva Tauraitė

Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Lithuania
ugnius.kamandulis@stud.vilniustech.lt

Purines are fundamental biomolecules that are found across all living systems. They are building blocks of DNA and RNA, central to cellular energy metabolism (ATP), and participate in intracellular and extracellular signalling through cyclic nucleotides and purinergic signalling networks. Disturbances in purine metabolic pathways can result in harmful accumulation of purine-related metabolites. Purine deaminases – enzymes that are involved in purine metabolism and help to maintain purine homeostasis in cells. These enzymes catalyse deamination reactions in which e.g. guanine is transformed into xanthine or adenine is converted into hypoxanthine [1]. Alterations in purine metabolism and purinergic signalling have been associated with diverse pathological conditions, therefore systematic biochemical characterization of enzymes involved in purine conversion remains relevant for both basic research and potential application-oriented work [2].

Aim of this study was to express recombinant deaminase enzymes in *Escherichia coli* strains, to purify them and to confirm their functional activity using the adenine/adenosine and their modified derivatives as substrates.

The expression vectors pET21a(+) carrying either *add* or *adeC* genes that encode adenosine or adenine deaminases were transformed to *E. coli Rosetta* (DE3) strain. Recombinant protein expression was induced using IPTG C-terminus His-tagged recombinant proteins were purified using Ni^{2+} affinity chromatography. Expression and purity of proteins were assessed by SDS-PAGE. Enzymatic activities were tested using adenine/adenosine and their methylated counterparts as substrates, and TLC and HPLC were used as methods to monitor substrate conversion and quantification of reaction products.

During this study recombinant Add and AdeC proteins were expressed and purified to >99 % of purity. HPLC analysis demonstrated enzymatic conversion of adenine into hypoxanthine in the presence of AdeC enzyme, indicating that the purified protein retained catalytic function after purification. However, the enzymatic activity was not detected when N^6 mono- and di-methylated adenines were used as substrates. Further experiments are aimed at the testing of the enzymatic activity of Add using the adenosine and N^6-methylated adenosines as substrates to establish the enzymatic activity and specificity of purine deaminase tested.

This project has received funding from the Research Council of Lithuania (LMTLT), agreement No [S-MIP-25-95].

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ISOTHERMAL SNP GENOTYPING ON A SINGLE SURFACE

Skomantas Serapinas[1], Deimantė Stakelytė[1], Kornelija Tucĭnskytė[2], Miglė Tomkuvienė[2], Marius Dagys[1], and Dalius Ratautas[1]

[1]Institute of Biochemistry, Life Science Center, Vilnius University, Sauletekio al. 7, LT-10224 Vilnius, Lithuania
[2]Institute of Biotechnology, Life Science Center, Vilnius University, Sauletekio al. 7, LT-10224 Vilnius, Lithuania
skomantas.serapinas@gmc.vu.lt

DNA analyzers have a very broad scope of application from pharmacogenetics, oncology, hereditary disease and infectious disease to environmental and food safety or veterinary medicine. We have developed a pharmacogenetic sensor for the cytochrome P450 2C19 enzyme, responsible for metabolizing a wide range of xenobiotics. A DNA-based approach can infer the enzyme behavior from any cell, without sampling the expression sites such as the liver or the small intestine. Our sensor uses a PCR amplified saliva sample. [1]

To perform the sequence discrimination, we captured the target DNA onto electrodes, measured the kinetic differences in the melting rates (k_d) between perfect match and mismatch duplexes at constant temperature and were able to confidently identify targeted sequences. Thus, we believe we have developed the first isothermal dissociation kinetics-based DNA biosensor. Using this approach, we can differentiate between the homozygous and heterozygous samples on a single electrode. The sensor was able to genotype real saliva samples directly from the PCR reaction mix.

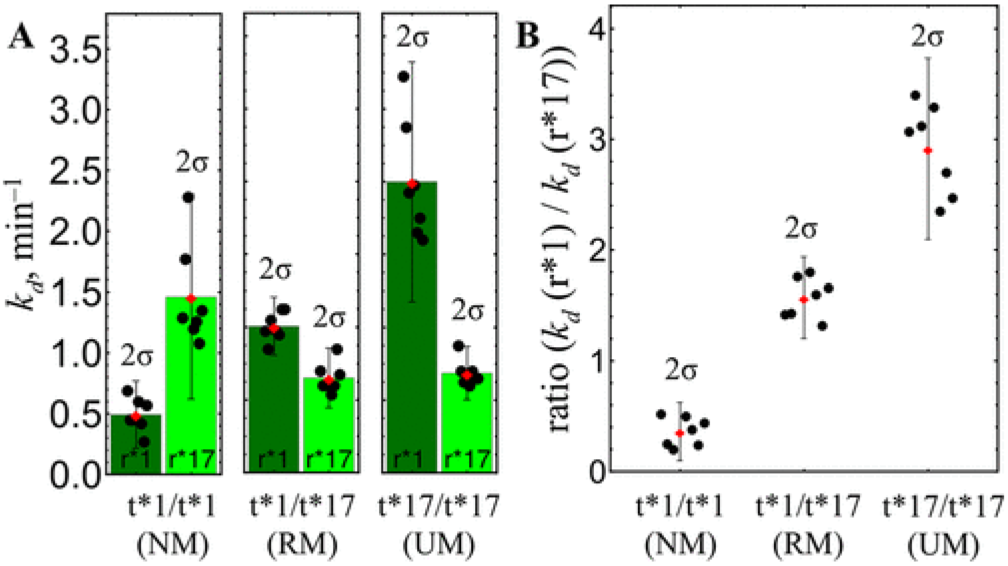

Figure1.Discrimination between synthetic samples. A – dissociation kinetics, B – genotyping. NM – normal metabolizer, RM – rapid metabolizer, UM – ultrarapid metabolizer

Interestingly, the dissociation kinetics also allowed us to estimate the relative binding affinity and consequently, the target sequence dissociation Δ*G*value. While various base substitutions correlated well with theoretical energy values, one sequence had a measured energy offset corresponding to 1.9 ± 0.3 kcal mol^{-1}. In this case, we believe we have measured the loss of a G|A base stacking interaction.

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MATERNAL HIGH-FAT DIET EFFECT ON THE OFFSPRING'S VOLATILOME COMPOUNDS

Patricija Čepauskytė[1], Gintarė Urbonaitė[1], Mantas Norkus[4], Toma Petrulionienė[4], Urtė Neniškytė[1,3]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
[2]Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Lithuania
[3]VU-EMBL Partnership Institute, Life Sciences Center, Vilnius University, Lithuania
[4]National Public Health Surveillance Laboratory, Chemistry Department, Instrumental Research Methods Unit, Lithuania
patricija.cepauskyte@gmc.stud.vu.lt

Aim: One of the main global health concerns is the rising obesity rates. It is known that high-fat can induce physiological, metabolic, behavioral, and neurodevelopmental alterations not only to the mother but their offspring as well [1, 2]. During pregnancy and lactation, maternal diet-induced metabolic and inflammatory changes may be transmitted to offspring [2]. Previous animal studies have demonstrated that mothers that were fed high-fat diet (mHFD) show signs of impaired maternal care. Since mice are olfactory animals, it is thought that high-fat diet disrupts the olfactory system of the mother reducing their ability to recognize and care for their offspring. Therefore, altered olfactory cues may contribute to deficits in maternal behavior [3]. Offspring odor profiles are determined by metabolic activity and are reflected in the volatilome. Changes in the offspring volatilome may modify olfactory signals critical for maternal recognition and care [4]. However, the relationship between maternal diet, olfactory disruption, and offspring volatile profiles remains poorly understood. Investigating the offspring volatilome may provide novel insight into how maternal high-fat diet exposure influences maternal–offspring interactions and early-life programming of metabolic and behavioral outcomes.

Methods: Female C57Bl/6J mice, from weaning and during pairing, pregnancy and lactation, were fed with either control diet (CD, 10% fat) or high-fat diet (HFD, 60% fat). The offspring were weaned to normal rodient diet. P6 pups were placed inside the glass container kept on temperature-controlled heating mat. Volatilome samples were then collected by inserting a sorbent tube and pump operating at a flow rate of 20 mL/min. Collected samples were sent to the National Public Health Surveillance Laboratory.

Results: We evaluated volatilome samples from four litters of control diet (mKD) and high-fat diet (mRD) offspring. A total of 18 volatile compounds were identified and classified into eight functional groups of which aldehydes are naturally occurring compounds commonly found on animal skin and in pheromone mixtures, playing important role in recognition, signaling, and communication between animals. Notably, offspring from mHFD did not emit specific compounds distinguished in mCD offspring. These findings suggest that maternal diet may alter offspring volatile profiles in ways that could affect olfactory-mediated maternal recognition and social interactions and require further research.

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SYNTHESIS OF GALECTIN-7 INHIBITORS

Job Markink[1], Nishant Sewgobind[2], Roland J. Pieters[3]

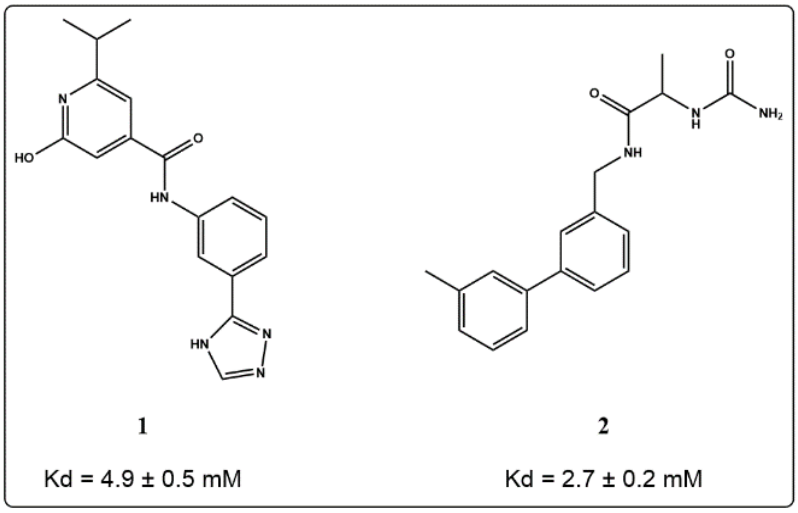
[1]Department of Chemical Biology and Drug Discovery, Utrecht University, The Netherlands
[2] Department of Chemical Biology and Drug Discovery, Utrecht University, The Netherlands
[3] Department of Chemical Biology and Drug Discovery, Utrecht University, The Netherlands
jobmarkink@outlook.com

Galectin-7 is a β-galactoside-binding lectin implicated in epithelial homeostasis, wound healing and cancer pathophysiology, yet its mechanistic roles remain incompletely defined [1]. Chemical probes that selectively inhibit galectin-7 are therefore required to dissect its biological functions and evaluate its potential as a disease biomarker and therapeutic target. Most reported galectin-7 ligands are carbohydrate-based and were originally developed for other galectins. However, high hydrophilicity, limited oral absorption, rapid excretion and insufficient selectivity motivate the development of non-carbohydrate scaffolds for targeted galectin-7 inhibition [2].

In this study, two non-carbohydrate galectin-7 inhibitor chemotypes identified by virtual screening were pursued to establish robust and derivatizable synthetic access. Compounds 1 and 2 (Fig. 1) were previously validated in a fluorescence polarization assay, exhibiting galectin-7 binding with dissociation constants of 4.9 ± 0.5 mM and 2.7 ± 0.2 mM, respectively. Compound 1 comprises an isopropyl-substituted 2-pyridone isonicotinic acid coupled to a 3-(1,2,4-triazolyl)aniline fragment. A concise and cost-efficient route to the pyridone building block was developed using a Guareschi–Thorpe condensation followed by hydrolysis/decarboxylation, enabling straightforward variation of the ketone input. Using this strategy, five additional pyridone derivatives were synthesized to support systematic structure–activity exploration. The triazole aniline fragment was prepared from inexpensive 3-nitrobenzamide via chloral hydrate activation, electrophilic chlorination, cyclization to the 3-aryl-1,2,4-triazole and subsequent nitro reduction. Final coupling afforded compound 1 (50 mg isolated) in an overall yield of 3% over a nine-step synthesis.

For compound 2, the synthetic route commenced with bromination of 3,3-dimethylbiphenyl, followed by nucleophilic substitution with sodium azide and Staudinger reduction to generate the corresponding benzylamine intermediate. Subsequent coupling with Fmoc-protected L- and D-alanine under HATU/HOBt conditions provided enantiomerically defined intermediates in 98% and 70% yield, respectively. Fmoc deprotection using 1-octanethiol as scavenger enabled access to the free amines, establishing a versatile platform for installation of the terminal urea functionality and completion of the biphenyl–alanine–urea scaffold.

Overall, the established synthetic routes provide scalable access to two computationally derived, non-carbohydrate galectin-7 ligand scaffolds and deliver a practical framework for systematic derivatization and future optimization guided by binding assays and structural studies.



1

Kd = 4.9 ± 0.5 mM

2

Kd = 2.7 ± 0.2 mM

Figure 1: Molecular structures of compound **1** and compound **2**.

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E24

TDP-43 PHOSPHORYLATION AND INTERACTION WITH 14-3-3 PROTEIN FAMILY STUDY

Saulė Rapalytė[1], Dominykas Veiveris[1], Darius Šulskis[1]

[1]Sector of Amyloid Research, Institute of Biotechnology, Life Sciences Centre, Vilnius University, Lithuania
saule.rapalyte@gmc.vu.lt

TAR DNA-binding protein 43 (TDP-43) is a multifunctional protein primarily localizing in the cell nucleus, where it binds to RNA and DNA[1]. It consists of 414 amino acids and is composed of four domains: N-terminal domain, two RNA-binding domains (RRM1 and RRM2), and low-complexity prion-like C-terminal domain[2]. RRM domains are flanked by nuclear export and import sequences, thereby enabling protein shuttling between the nucleus and cytoplasm[3]. In both the nucleus and cytoplasm, TDP-43 can form condensates through liquid-liquid phase separation (LLPS) – a biophysical process involved in the formation of membraneless organelles[4]. Due to LLPS, protein cleavage, mutations, and posttranslational modifications, such as hyperphosphorylation, TDP-43 can aggregate and form insoluble neurotoxic species, as well as disrupt downstream cellular processes[5]. TDP-43 aggregates are the hallmarks of several neurodegenerative diseases: amyotrophic lateral sclerosis and frontotemporal lobar degeneration[2]. However, the latest studies have shown that phosphorylation of TDP-43 can greatly suppress the protein's propensity to undergo LLPS and slow TDP-43 aggregation into amyloid fibrils[6]. Alongside this, several members of 14-3-3 protein family, can interact with TDP-43 or kinases and modulate their function[7,8]. Therefore, we aim to identify key kinases implicated in neurodegeneration that can phosphorylate TDP-43 and to investigate the 14-3-3 family proteins impact on kinase activity.

In this study, we initially optimized the purification of full-length TDP-43. This protein is prone to degradation and forms aggregates after cleavage of the purification tag, making it difficult to purify. For the phosphorylation assay, four different kinases were used: CK1α, PKA, PKR, and GSK3β. TDP-43 was mixed with different kinases and incubated at room temperature overnight. Samples were taken right after mixing proteins, one hour after incubation, and the next morning. To investigate 14-3-3 proteins' interaction with TDP-43 and kinases, seven 14-3-3 isoforms were purified: β, γ, ε, ζ, η, θ, and σ. The phosphorylation of TDP-43 was evaluated by mobility shifts in SDS-PAGE electrophoresis.

Our preliminary results imply that CK1α and PKR kinases are the most effective in phosphorylation of TDP-43. After incubating different isoforms of 14-3-3 with kinases (CK1α or PKR) and TDP-43, we observed increased phosphorylation activity in a sample containing 14-3-3 γ, PKR, and TDP-43. To further elucidate molecular mechanisms of phosphorylation and complex formation processes, we will perform a more comprehensive analysis of kinase efficiencies, aggregate species, and structure determination.

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E25

Moonlighting chaperone activity of S100A12 within the S100A family

Orestas Antipas Pelivanov[1,2], Darius Šulskis[1]

[1]Lithuania, Vilnius University, Life Sciences Center, Institute of Biotechnology
[2]Lithuania, Vilnius University, Faculty of Chemistry and Geosciences
orestas.pelivanov@chgf.stud.vu.lt

The S100A family consists of 24 calcium-binding proteins that participate in a wide range of cellular processes, including regulation of cell signaling, immune responses and other vital functions. Dysregulation of S100A proteins has been associated with inflammatory and neurodegenerative diseases [1], [2]. S100A8 and S100A9 are known to form amyloid-like oligomers, fibrils or amorphous aggregates [3], whereas the stability of S100A12 under physiological conditions is unknown, but has been proposed to exhibit moonlighting chaperone behavior as a secondary function [4]. Therefore, we investigated whether S100A12 can alter or inhibit the aggregation behavior of S100A8 and S100A9.

Protein aggregation was monitored in vitro using the amyloid-specific Thioflavin T (ThT) fluorescent probe in a microplate reader in the presence or absence of calcium ions. Protein thermal stability was determined by differential scanning fluorimetry (DSF) by monitoring the fluorescence of the 8-anilinonaphthalene-1-sulfonic acid (ANS) dye.

The results (Fig. 1) showed that in the absence of Ca^{2+}, S100A9 rapidly formed fibrils and S100A8 aggregated more slowly, matching previously reported data [3], whereas the S100A12 sample exhibited no increase in ThT fluorescence, indicating a lack of amyloid formation. On the other hand, co-incubation with S100A12 significantly reduced the aggregation of S100A9 in the presence of Ca^{2+}, while altering the aggregation of S100A8. Furthermore, the melting temperature of S100A12 was higher than that of S100A8 and S100A9, in agreement with the aggregation experiments.

Overall, these results suggest that S100A12 can modulate the aggregation behavior of the S100A protein family and is a stable protein.

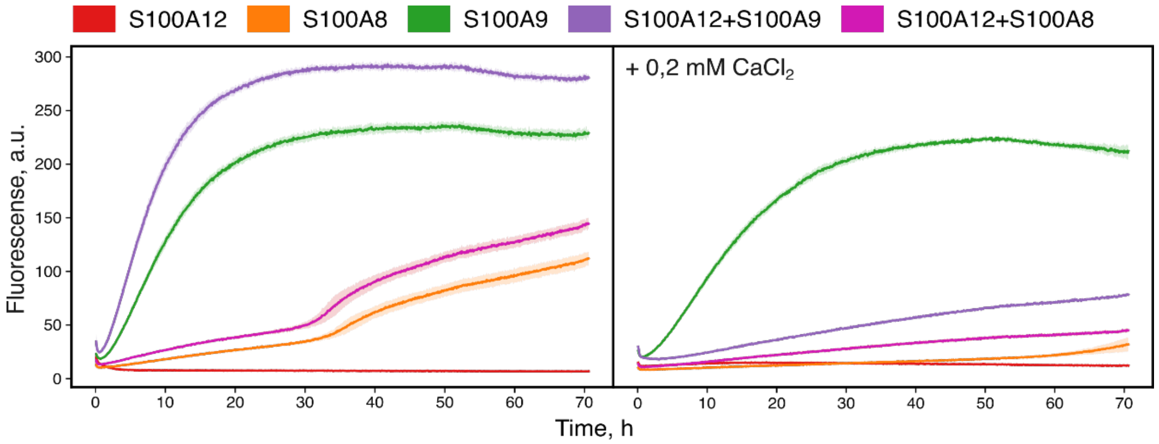

Fig. 1. ThT fluorescence kinetics of S100A8, S100A9 and S100A12 aggregation in the absence and presence of calcium ions

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INVESTIGATION OF THE ACTIVATION MECHANISM OF SPLIT PAGO PROTEINS AND ASSOCIATED HEPN EFFECTORS IN *E. COLI*

Rokas Bružaitis[1], Edvardas Golovinas[1], Mindaugas Zaremba[1]
[1] Department of Protein-DNA Interactions, Institute of Biotechnology, Vilnius University, Lithuania
rokas.bruzaitis@gmc.stud.vu.lt

As genome editing technologies evolve, the scientific community remains dedicated to improving the tools that drive these advancements. Presently CRISPR-Cas (*Clustered regularly interspaced short palindromic repeats - CRISPR associat-ed proteins*) systems dominate the field of genome editing, whereas comparatively little attention has been given to alternatives, such as prokaryotic Argonaute (pAgo) systems. Nonetheless, recent findings in pAgo research indicate that the HEPN domain is vital for the activity of certain pAgo systems [1], although the mechanisms underlying their activation remain unclear.

In this research we examine the possible interactions between a pAgo system and a plasmid containing a CloDF13 origin of replication (*ori*) sequence. The pAgo system analyzed here includes a toxic effector protein harboring a HEPN (*higher eukaryotes and prokaryotes nucleotide-binding domain*) domain. HEPN domain displays toxicity when expressed without its associated pAgo proteins. In some instances, pAgo systems are activated by plasmids that carry a specific CloDF13 *ori* [2]. By investigating an Argonaute-HEPN system in which the CloDF13 *ori* serves as a trigger, we seek to clarify the activation mechanism of this pAgo system. This work establishes a foundation for upcoming studies and holds considerable promises for applications in biotechnology.

This research employs two complementary *in vivo* methods to investigate the mechanisms of activation. Both approaches are designed to evaluate the toxic effects of pAgo system toward host cells. A bacterial plaque assay is used to quantify toxicity by assessing the extent to which activation of the system inhibits host cell growth. In addition to plaque assays performed on solid nutrient medium, toxicity is also evaluated in liquid culture. Therefore, plate reader-based growth curve analysis are conducted to monitor the toxic effects of the activated system.

Exploring bacterial defense activation pathways offers important insight into the complex mechanisms bacteria use to protect themselves from foreign nucleic acids, while simultaneously opening new avenues for innovation in biotechnology and genome editing technologies.

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ESTABLISHING A CELL-BASED SCREENING PLATFORM FOR TNPB GENOME EDITING TOOLS

Bartė Žiliukaitė[1], Marius Baltramonaitis[1], Tautvydas Karvelis[1]

[1]Department of Protein–DNA Interactions, Life Sciences Center, Vilnius University, Lithuania
barte.ziliukaite@gmc.vu.lt

Genome editing is one of the fastest-growing innovations of this century, promising to treat a wide range of genetic diseases. The discovery of CRISPR-Cas systems, responsible for anti-phage defense in bacteria, revolutionized genome editing by offering a selection of programmable nucleases. Currently, the majority of widely used genome editing tools are based on Cas9 and Cas12a effectors because they efficiently induce double-strand DNA breaks and recognize target sequences with high precision. However, directly applying these nucleases in medical research is challenging because the size of Cas9 and Cas12a, together with regulatory sequences, exceeds the limited packaging capacity of adeno-associated viruses that are commonly used in *in vivo* therapy. Since the delivery of genome editing tools into a cell is a crucial step in treatment development, there is a growing interest in researching smaller programmable nucleases that would be more suitable for medical applications.

Recent studies have shown that Cas12 nucleases are derived from TnpB proteins encoded in the bacterial IS200/IS605 family insertion sequences [1]. TnpB proteins also exhibit RNA-guided nuclease activity and are smaller (~400 amino acids) than Cas12 family nucleases (500–1500 amino acids), and therefore are attractive candidates to be used for genome editing. The widely studied TnpB from *Deinococcus radiodurans* (ISDra2) can cleave genomic DNA in eukaryotic cells, but demonstrates lower activity compared to modern genome-editing tools [2]. Therefore, TnpB protein engineering is an appealing strategy to increase its activity, which in turn demands an effective screening system.

Therefore, in this study, we aimed to develop a cell-based platform to identify the active TnpB variants. By introducing DNA-encoded guide RNA and TnpB nuclease variants into cells, followed by direct sequencing, we characterized selected TnpB nucleases. We demonstrate the feasibility of detecting TnpB nuclease activity in a cellular context that can be translated into genome editing and outline the principles of a cell-based platform for screening TnpB variants.

In the future, such cell-based screening could be applied to large-scale studies of protein evolution and to the development of additional nuclease properties such as target specificity or recognition of TAM sequences.

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DETERMINANTS OF PHAGE SENSITIVITY TO THE OLOKUN ANTIPHAGE DEFENSE SYSTEM

Vincentas Raudys[1], Edvinas Jurgelaitis[1], Mindaugas Zaremba[1]
[1]Department of Protein-DNA Interactions, Institute of Biotechnology, Life Sciences Center, Vilnius University
vincentas.raudys@gmc.vu.lt

Bacterial and archaeal populations are continuously exposed to infection by bacteriophages, the most abundant biological entities on Earth [1]. Under this sustained evolutionary pressure, prokaryotes have evolved antiphage defence systems, while phages have concurrently developed strategies to evade them. This coevolutionary “arms race” has driven a substantial diversification of antiphage systems, many of which exhibit unique and largely uncharacterised mechanisms of action. A number of these systems have been harnessed for scientific and medical applications. For instance, CRISPR-Cas systems have transformed genome editing, and restriction-modification systems have been employed in DNA manipulation for over five decades [2].

In this study, we establish a foundation for the characterisation of a novel prokaryotic antiviral defence system – Olokun, identified in the E. coli UMB0934 strain [3]. A phage-escaper workflow was used to select for bacteriophage mutants capable of bypassing Olokun-mediated defence. Putative mutant bacteriophages were isolated and validated. Following purification of phage genomic DNA, sequencing was performed using next-generation sequencing technologies. Subsequent bioinformatic analysis of raw sequencing data enabled the identification of potential determinants associated with phage sensitivity to Olokun.

This work contributes to a deeper understanding of prokaryotic antiphage defence systems and their potential biotechnological applications.

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GFP-based biosensor for screening SARS-CoV-2 M^{pro} protease inhibitors

Augustas Kudarauskas[1], Kristupas Volbikas[1], Nina Urbelienė[1], Constantinos Patinios[2], Rolandas Meškys[1], Darius Balčiūnas [3,4]

[1]Institute of Biochemistry, Life Sciences Center, Vilnius University, Lithuania.
[2]VU LSC-EMBL Partnership Institute for Genome Editing Technologies, Life Sciences Center, Vilnius University, Lithuania.
[3]Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania.
[4]Department of Biology, Temple University, Philadelphia, PA 19122, United States.
augustas.kudarauskas@gmc.vu.lt

M^{pro} protease, also known as 3CLpro or nsp5, is the main protease of SARS-CoV-2 and other betacoronaviruses, making it a prime antiviral target due to its essential role in viral replication. M^{pro} cleaves the viral polyproteins pp1a and pp1ab at 11 sites, processing them into functional non-structural proteins (nsps) essential for viral transcription and replication. Unlike many other proteases that have a catalytic triad, M^{pro} uses a catalytic dyad: Cys145 acts as a nucleophile, attacking the peptide bond of the substrate; His41 acts as a standard base, deprotonating the thiol group of Cys145, thereby activating it. Water molecule replaces the missing third member of the triad, stabilizing His41 via a hydrogen bond [1].

The selection and development of protease inhibitors as antiviral treatments is an important area of modern medicine. More than 1,000 potential covalent inhibitors targeting M^{pro} have been tested, but only nirmatrelvir has been approved by the FDA so far. Hence, clinical trials are ongoing to develop broader-spectrum M^{pro} inhibitors effective against future coronaviruses and SARS-CoV-2 variants resistant to currently prescribed inhibitors. The resistance is mainly due to mutations in the M^{pro} (3CLpro) gene, particularly at the inhibitor binding site.

The goal of this research was to develop a biosensor for detecting M^{pro} protease inhibitors in *E. coli* using positive fluorescence detection. For this purpose, a GFP construct containing M^{pro} protease target sites [2] was created. The results showed that both protease and GFP sensor are well expressed as GST fusions, and the M^{pro} demonstrated proteolytic activity towards GFP constructs in *E. coli* cells. During the process, optimized cultivation and co-expression conditions for M^{pro} protease and GFP sensor were determined. The sensitivity of biosensor was tested with 12 known protease inhibitors. The activity of M^{pro} protease against GFP with target sites was confirmed by Western blotting using antibodies and a Connectase-Cy5.5 detection system.

This proposed system has several unique features:

- Positive selection: suppression of M^{pro} protease enhances GFP fluorescence intensity, unlike most inhibition methods, where a signal decrease is observed;
- High-throughput: the system was optimized for a 96-well plate test and can be adopted for a microfluidic system;
- The effect of inhibitors on Gram-negative bacteria is assessed, which is important for preserving the microbiota;
- The *E. coli* cell wall is a barrier to inhibitors. It can be considered both an advantage and a disadvantage: it complicates the inhibitor from entering cells, but it also provides information about the cell membrane's permeability to the drug.

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VISUALIZING COCONUT: A DUAL-LABELING APPROACH TO DISSECT BACTERIAL ANTIVIRAL IMMUNITY

Domantas Vareika[1], Ugnė Bagdonaitė[1], Aurimas Kopūstas[1], Jonas Juozapaitis[1]

[1]Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
domantas.vareika@gmc.stud.vu.lt

Bacteria and archaea are frequently attacked by viruses and other mobile genetic elements and rely on dedicated antiviral defense systems, such as restriction endonucleases and CRISPR, to survive. The enormous diversity of viruses suggests that more types of defense systems exist than are currently know [1]. The vast McrBC systems fall under the type IV restriction enzymes, which contain at least two components: a dedicated specificity domain that recognizes modified DNA, and an endonuclease domain that cleaves the target [2].

We focus on a previously uncharacterized branch of McrBC system that is denoted CoCoNuTs, named for their defining features: extensive coiled-coil architectures and nuclease tandem domains. CoCoNuTs exhibit remarkable diversity, comprising three distinct types and multiple subtypes. All CoCoNuTs encode domains predicted to interact with components of the translation machinery. Here we aim to characterize type II CoCoNuT system distinct by additional HEPN ribonuclease protein [3].

Previous approaches to studying this system did not yield decisive insights. To overcome this, we developed a fluorescence-based imaging strategy. Two proteins encoded within the CoCoNuT operon were engineered to carry HaloTag fusions, enabling covalent labeling with specific fluorescent dyes. Cells expressing these constructs were infected with a genetically modified bacteriophage λ bearing a fluorescently labeled capsid [4]. Using fluorescence microscopy, we analyze the spatial and temporal dynamics of labeled CoCoNuT proteins and phage particles during infection.

Through this work, we aim to better grasp the mechanism of bacterial defense systems while establishing a broadly applicable imaging-based method. This methodology may prove particularly valuable for studying defense other bacterial defense systems.[1]

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MONITORING OF ARGINASE ACTIVITY VIA UREA BIOSENSOR

Butkevičiūtė M.[1], Gurevičienė V.[1], Šakinytė-Urbikienė I.[1], Sukackienė D.[2], Razumienė J.[1]

[1]Department of Bioanalysis, Biochemistry Institute, Vilnius University, Vilnius, Lithuania
[2]Vilnius University Hospital Santaros Clinics, Vilnius, Lithuania
mante.butkeviciute@gmc.stud.vu.lt

Dysregulation of arginase activity disrupts arginine availability and nitric oxide signalling, causing multiple downstream effects. The importance of arginase in metabolic disorders is evident through diseases such as diabetes mellitus, obesity and metabolic syndrome, non-alcoholic fatty liver disease, cardiovascular disorders, or arginase deficiency (an Inherited Metabolic Disorder) [1]. Thus, monitoring arginase activity is vital for early disease detection, assessing progression and prognosis, and guiding targeted therapies.

This study focused on arginase activity as a biomarker in kidney perfusate due to its close association with key pathophysiological processes, including tissue injury and inflammation. This type of biomarker has not yet been explored, but its biochemical pathway shows potential for its application in the context of kidney perfusion, so monitoring it could provide critical insights into organ viability. For monitoring of arginase activity, amperometric urea biosensor was employed to measure enzyme activity in real time. Since arginase is a key enzyme in the urea cycle, catalyzing the hydrolysis of L-arginine into urea and ornithine, the possibility of applying an amperometric urea biosensor for activity monitoring has emerged. The biosensor has the potential to function even in turbid media that offer significant advantages over conventional methods, such as ELISA, and other sophisticated methods.

In this study, an amperometric urease and thermally reduced graphene oxide based biosensor was developed and applied to evaluate arginase activity in model kidney perfusate samples. The biosensor exhibited high sensitivity and demonstrated excellent selectivity toward urea at 0 V vs. Ag/AgCl, with negligible interference from common electroactive species found in biological media. Recovery tests confirmed good accuracy and repeatability, indicating reliable urea quantification in complex matrices. The linear correlation between biosensor response and arginase activity supports its applicability for monitoring arginase-catalyzed reactions and evaluating enzymatic activity through urea detection in perfusate samples.

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SpCas9off-target activities depend on DNA topology

Uršulė Barkauskaitė[1], Ieva Jaskovikaitė, Stephen Knox Jones Jr.

[1]LSC-EMBL Partnership Institute, Vilnius University, Lithuania
ursule.barkauskaite@gmc.stud.vu.lt

Bacteria rely on CRISPR-Cas (Clustered Regularly Interspaced Short Palindromic Repeats and Associated proteins) systems for adaptive immunity, protecting them against invading bacteriophages and other mobile genetic elements [1]. A prime example, SpCas9, forms a complex with a two-part guide RNA (gRNA) made of the CRISPR RNA and trans-activating RNA. The gRNA directs SpCas9 to its target DNA. However, Cas9's mismatch tolerance can lead to nuclease cleaving DNA sequences that are similar but not identical to the intended target - off-targets. Furthermore, off-target effects depend on DNA topology, as studies show that off-targets are cleaved more frequently when DNA is negatively supercoiled, due to DNA torsional stress, which impacts the accessibility of DNA and Cas9 binding affinity [2], [3].

How to predict Cas9's promiscuous behaviour remains of the utmost importance in terms of avoiding potential off-target effects. Our study focused on a mispaired "insertion" off-target, where an extra, unpaired nucleotide is present in the DNA sequence relative to gRNA, creating a DNA - bulge. An adenine base was inserted between positions 3 and 4 to create an "insertion" off-target, in the study referred to as 3A4. We measured cleavage and nicking rates by performing cleavage assays on supercoiled, linear and relaxed DNA with SpCas9. To assess which catalytic domain is responsible for the initial nicking and final cleavage of the off-target 3A4, HNH-inactive nickase and RuvC-inactive nickase were used. Our results indicate a supercoiling-dependent inactivation of HNH domain, turning a WT SpCas9 into an HNH-inactive nickase.

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FUNCTIONAL ANALYSIS OF LACTIC ACID BACTERIA *LACTOCOCCUS LACTIS* sRNA sLLM2- IN RESPONSE TO CELL WALL-TARGETING ANTIMICROBIALS

Naglis Mykolas Pakštys[1], Milda Mickutė[1], Janina Ličytė[1], Loreta Stankevičiūtė[1], Oskaras Safinas[1], Pascal Courtin[2], Marie-Pierre Chapot-Chartier[2], Giedrius Vilkaitis[1]

[1]Department of Biological DNA Modification, Life Sciences Center, Vilnius University, Lithuania
[2]Universite´ Paris-Saclay, INRAE, AgroParisTech, Micalis Institute, Jouy-en-Josas, France
mykolas.pakstys@gmc.vu.lt

Lactococcus lactis (*L. lactis*) are Gram-positive, immobile, facultatively anaerobic, non-spore-forming lactic acid bacteria that have become an important model organism due to their industrial role as primary components of dairy starter cultures and their impact on public health due to their probiotic properties [1]. To maintain and improve these properties, it is important to understand how these microorganisms adapt to environmental changes, especially when subjected to stress conditions. The bacterial cell wall plays an important role in withstanding environmental threats, such as exposure to cell wall-targeting antimicrobials, that lactic acid bacteria such as *Lactococcus lactis* encounter during application processes. Therefore, it becomes crucial to understand the response mechanisms of these bacteria when exposed to such stressors.

Small regulatory RNAs (sRNAs) are gene-regulating RNAs that regulate the expression of target genes by binding to complementary sequences of messenger RNR (mRNA), thereby altering the stability and/or translation of mRNAs [2]. The contribution of these small RNAs to the regulation of cell wall defense mechanisms is crucial for protecting bacteria from environmental threats, however much of the current understanding of RNA-based regulation in antimicrobial responses stems from studies on Gram-negative bacteria such as *Escheria coli* and *Salmonella*. Thus, expanding our knowledge about Gram-positive species, such as *Lactococcus lactis*, is crucial in order to shed more light on the RNA-based processes that underpin antimicrobial resistance.

In the Department of Biological DNA Modification, it was shown that transcription of several small regulatory RNAs is upregulated/downregulated after *Lactococcus lactis* treatment with cell wall-targeting antimicrobials such as lysozyme and penicillin G. One of the discovered sRNA, annotated as sLLM2-, was discovered to be a potential regulator of *L. lactis* response to mentioned antimicrobials, therefore it is important to understand the mechanism behind this phenomenon. Upon further investigation, a potential sRNA target was discovered – gene *alr*, encoding an alanine racemase interconverting L-alanine to D-alanine.

The aim of this study is to determine the relationship between a small regulatory sRNA sLLM2- and its possible target *alr* in *L. lactis*. Firstly, we analyzed how a strain of *L. lactis* lacking the *alr* gene with and without overexpression of sRNAs responds when grown on agar plates with antimicrobials lysozyme and penicillin G. Then, to confirm the sRNA-mRNA interaction, we utilized MAPS assay technology. Finally, we set out to determine whether regulation occurs at a transcriptional or translational level by rifampicin-based RNA stability assay.

The obtained results will help to understand the mechanisms of sRNAs function in *L. lactis* and their role in gene regulation as well as response to antimicrobials.

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MYCOBIOME COMPOSITION OF THE RESPIRATORY TRACT IN TUBERCULOSIS PATIENTS IN LITHUANIA

Aistė Popandopula[1], Edvardas Danila[2,3], Karolina Kėvelaitienė[3], Rūta Mereškevičienė[3], Vaiva Kumpauskaitė[3], Laima Vasiliauskaitė[3,4], Aleksandras Konovalovas[1], Julija Armalytė[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]Center of Pulmonology and Allergology, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania
[3]Clinic of Chest Diseases, Immunology and Allergology, Institute of Clinical Medicine, Faculty of Medicine, Vilnius University, Vilnius, Lithuania
[4]Laboratory of Infectious Diseases and Tuberculosis, Centre of Laboratory Medicine, Vilnius University Hospital Santaros Klinikos, Vilnius, Lithuania
a.popandopula@gmail.com

Tuberculosis (TB) remains a major global health concern despite advances in treatment and prevention. It is caused by *Mycobacterium tuberculosis* and transmitted through airborne droplets. While exposed individuals might develop latent infection, only a small proportion progress to active pulmonary disease, indicating that host and environmental factors strongly influence disease outcomes. Lithuania has one of the highest TB and drug-resistant TB rates in the European Union, underscoring the need for an improved understanding of disease drivers [1]. Increasing evidence suggests that the respiratory tract microbiome may act as an ecological barrier or facilitator of *Mycobacterium tuberculosis* colonization [2]. Therefore, investigating lung microbiome composition may provide critical insight into TB susceptibility and progression.

Although microbiome research is rapidly expanding, the mycobiome, comprising fungal communities, remains poorly understood, and there is a lack of standardization in detection and identification methods. Different studies use a variety of tools for fungal identification, including amplification of different genome regions. This study aimed to analyze sputum samples using 14 primer pairs, of which the three best-performing pairs were selected and applied to 118 sputum samples, obtained from patients with TB and chronic lung diseases (CLD). A total of 39 sputum samples were later analyzed by fungal ribosomal ITS amplicon sequencing.

Fungal presence detected by PCR was confirmed in 92% of samples, whereas conventional microbiological methods identified fungal species in only 16% of samples. The sputum mycobiome exhibited high species diversity. The genera *Malassezia*, *Saccharomyces*, and *Aspergillus* were more frequently detected in the sputum of TB patients, whereas *Crinipellis*, *Trichosporon*, and *Nakaseomyces* were more common in CLD patients. However, neither alpha nor beta diversity analyses revealed statistically significant differences between the groups. No significant differences in mycobiome composition or diversity were observed between drug-sensitive and drug-resistant TB.

In conclusion, sputum is a suitable and informative sample for respiratory tract mycobiome studies, and the mycobiomes of TB and CLD patients exhibit distinct patterns of dysbiosis. Interpretation of our findings is limited by the small sample size, the lack of standardization in mycobiome research, and the limited literature on respiratory tract fungal communities as well as their interactions with the broader microbiome. Nevertheless, these results provide a foundation for future studies investigating the role of the respiratory tract mycobiome in TB pathogenesis and progression.

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CHARACTERISATION OF 5’-RNA DECAPPING AND SRNA-INTERACTING PROTEINS IN *LACTOCOCCUS LACTIS* AND *LACTICASEIBACILLUS PARACASEI BACTRIA*

Elena Milaknytė[1], Janina Ličytė[1], Giedrius Vilkaitis[1]

[1]Department of Biological DNA Modification, Life Sciences Center, Vilnius University, Lithuania
elena.milaknyte@gmc.vu.lt

Lactococcus lactis and *Lacticaseibacillus paracasei* are gram-positive, anaerobic bacteria. Because of their ability to produce lactic acid they are the members of the lactic acid bacteria (LAB) group and are very important in the food industry for their use as flavour enhancers and starter cultures [1]. During food production LAB face many environmental stressors which can affect their growth and metabolism. To counter these stressors LAB, activate many mechanisms, including the regulation of gene expression, in which small RNAs (sRNA) play an important role [2].

sRNAs are post-transcriptional regulators of genes involved in important cellular processes and often they target proteins or RNA transcripts to control these processes. Various proteins that interact with sRNA influence the stability of bacterial messenger RNA (mRNA) by ensuring the interaction between sRNA and mRNA. Many proteins of this kind are found in gram-negative bacterial species, however in gram-positive bacterial species – they are found less frequently [3].

The identification of the 5’ and 3’ ends of sRNAs is important for characterising sRNA dependent regulatory mechanisms and determining the regulation of sRNA stability, localisation and targets. Recently it has been shown that some sRNA species have nicotinamide adenine dinucleotide (NAD) or diadenosine tetraphosphate (Ap4A) cap structure at their 5’ end, but their function, although associated with sRNA stability, is not yet fully understood [4].

The Department of Biological DNA Modification at the Life Science Center has identified some *L. lactis* enzymes that perform decapping of the NAD or Ap4A structures in the 5' end of sRNA *in vitro* and studies of these proteins showed a higher decapping activity in the presence of Mn^{2+} cations. The aim of our study is to investigate the effect of Mn^{2+} cations on *L. lactis* sRNA decapping proteins, and to develop a method to enrich proteins that interact with *L. lactis* and *L. paracasei* sRNA. Firstly, to determine the effect of Mn^{2+} on decapping activity *in vivo*, L. lactis culture was grown in a medium containing an increased concentration of the Mn^{2+} cation and the level of 5'-NAD modification in RNA was calculated using mass spectrometry. Secondly, to further characterize these enzymes, we analysed decapping activity in different Mn^{2+} concentrations. Lastly, to enrich proteins interacting with sRNA and evaluate the quality of the enrichment method, differrentely biotinylated RNA was incubated with *L. lactis* and *L. paracasei* lysate and the resulting RNA-protein complexes were then purified using Dynabeads™ MyOne™ Streptavidin C1 magnetic particles.

The obtained results will help us to shed the light on the mechanisms of sRNA function and sRNA-dependent stress response regulation in lactic acid bacteria.

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NOVEL DEFENSE SYSTEM - DRT4 – MEDIATED ANTIPHAGE DEFENSE

Augustas Cibulskis[1], Monika Jasnauskaitė[1], Kristina Kazlauskaitė[1], Jonas Juozapaitis[2], Patrick Pausch[1]

[1]LSC-EMBL Partnership Institute for Genome Editing Technologies, Life Sciences Center, Vilnius University, Sauletekio al. 7, 10257 Vilnius, Lithuania
[2]Institute of Biotechnology, Life Sciences Center, Vilnius University, Sauletekio al.7, 10257 Vilnius, Lithuania
augustas.cibulskis@gmc.vu.lt

Bacteriophages, the most abundant biological entities on Earth, drive bacterial evolution through constant selective pressure. As a result, bacteria have evolved diverse defense strategies, including abortive infection (Abi) systems that detect phage invasion and trigger growth arrest or cell death to prevent viral replication. Among these, prokaryotic reverse transcriptase-associated Abi systems, such as AbiK and AbiP2, have garnered attention for their unique mechanisms [1].

Recent discoveries of unknown group (UG) reverse transcriptases (RTs) have led to the characterization of a novel group - defense-associated reverse transcriptases (DRTs) [2]. Notably type 4 DRT (DRT4, UG15) shares structural and functional similarities to AbiK, including a homologous N-terminal RT domain and the assembly of a homohexameric complex [3][4]. Despite these findings, the activation mechanism of DRT4 remains unclear. This study aims to characterize DRT4-mediated defense through bacteriophage infection assays utilizing the BASEL phage collection. Analysis of phage escapers in Bas17 revealed mutations in phage-encoded protein. This protein was subsequently employed to model potential interaction interfaces with DRT4. Mutant DRT4 constructs were then generated based on these predicted binding sites, and their capacity to mediate defense was evaluated against the Bas17 phage.

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Molecular rotor–encoded guide RNAs tune and report CRISPR–Cas9 specificity

J. Mikutavicius, K. Astakhova

Department of Chemistry, Technical University of Denmark, Denmark
jumik@dtu.dk

While most chemically modified guide RNAs rely on sugar and backbone alterations [1], systematic engineering of nucleobase substitutions remains limited due to synthetic challenges and concerns about disrupting Cas9 binding and catalytic activity [2]. Here, we site-specifically introduce fluorescent base analogues (FBAs) into guide RNAs to establish a dual-function strategy in which a single chemical modification both modulates target specificity and reports target interrogation through fluorescence.

We synthesized a library of FBA nucleosides that function as molecular rotors, designed to preserve Watson–Crick base pairing while introducing environment-sensitive fluorescence – and incorporated them at defined spacer positions within guide RNAs. Their activity was evaluated using *in vitro* cleavage assays. Selected substitutions produced position-dependent effects that enhanced mismatch discrimination without substantially reducing on-target activity. Notably, fluorescence measurements distinguished matched from mismatched DNA targets prior to cleavage, enabling real-time monitoring of target engagement.

Collectively, these findings establish internal nucleobase modification of guide RNAs as a dual tuning-and-reporting platform to both control and visualize CRISPR–Cas9 target recognition, with potential implications for improving gene-editing precision.

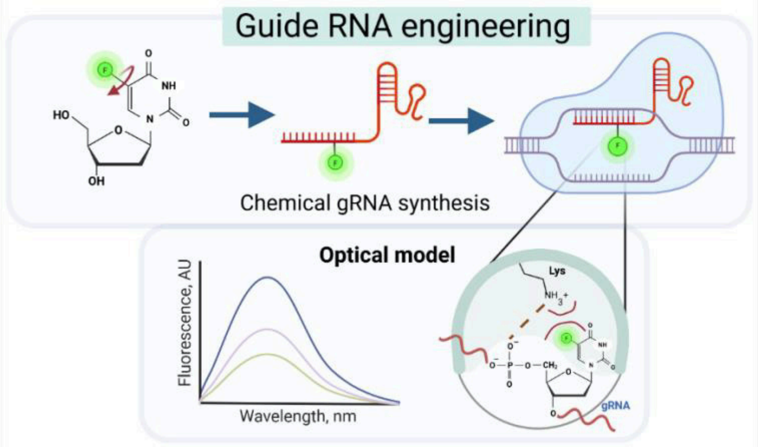

Figure 1. Site-specific guide RNA modifications for fluorescence-based mechanistic insights.

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A FLUORESCENT REPORTER CELL LINE AS A PLATFORM FOR CRISPR-Cas SYSTEM'S VALIDATION

Rugilė Tūskaitė[1,2], Neringa Daugelavičienė[1,2], Eimina Dirvelytė-Valauskė[1,2], Simonas Kutanovas[1,2], Urtė Neniškytė[1,2]

[1]VU LSC-EMBL Partnership for Genome Editing Technologies, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]Institute of Bioscience, Life Sciences Center, Vilnius University, Vilnius, Lithuania
rugile.tuskaite@gmc.stud.vu.lt

CRISPR-Cas systems are adaptive immune mechanisms in bacteria and archaea that protect these prokaryotes from mobile genetic elements. Owing to their programmable nuclease activity, these systems have become widely used tools for genome editing. In bacteria, CRISPR-Cas systems are validated using plasmid interference, PAM depletion, and/or phage assays [1,3]. In mammalian cells, CRISPR-Cas system validation is commonly performed using RT-qPCR, Western blot and DNA sequencing [2,3]. These methods are time-consuming and require specific equipment and expertise, which is why a more straightforward approach for validating novel CRISPR-Cas systems is needed.

The aim of this study was to apply the mouse neuroblastoma Neuro-2a KI: EGFP-mRUBY2-PURO cell line, generated in our laboratory, as a platform for rapid and efficient validation of CRISPR-Cas systems. These cells stably express EGFP (green fluorescent protein) in their nuclei and mRuby2 (red fluorescent protein) in the membrane. To assess whether this cell line is suitable for screening novel gene-editing tools and evaluating their efficiency, Neuro-2a KI: EGFP-mRUBY2-PURO cells were transfected with a plasmid encoding *Staphylococcus aureus* Cas9 (SaCas9) and *EGFP*-targeting gRNA. *EGFP* knock-out efficiency was evaluated by fluorescence microscopy on days 3, 5, 7, and 10 after transfection to determine the optimal time point for *EGFP* knock-out analysis.

The results demonstrated efficient disruption of the *EGFP* gene by SaCas9 at all analysed time points. On day 5 post-transfection, less than 10% of transfected cells remained EGFP-positive, compared to roughly 90% in the control group. These findings indicate that fluorescent reporter Neuro-2a KI: EGFP-mRUBY2-PURO cell line may serve as a simple and effective platform for screening and validation of novel CRISPR-Cas systems in mammalian cells.

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LIGHT-INDUCABLE RUDS DESULFIDASES MITIGATE THE EFFECTS OF UV STRESS IN *PSEUDOMONAS PUTIDA*

Smiltė Sarapaitė, Rapolas Jamontas, Agota Aučynaitė, Rolandas Meškys

Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center, Vilnius University
smilte.sarapaite@chgf.stud.vu.lt

It is known that various tRNA modifications play an important role in the adaptation of bacteria to environmental changes. 4-thiouridine (s^4U) is one of the most abundant post-transcriptional modifications of bacterial tRNA, primarily located at position 8 in the tRNA structure [1]. The tRNA s^4U modification acts as a photosensitive residue in tRNA, crosslinking with structurally nearby cytidine in the 13th position upon exposure to near-UV radiation and consequently delaying bacterial growth by making some tRNAs poor aminoacylation substrates.

tRNA 4-thiouridine desulfidases (RudS) are a novel family of tRNA demodifying enzymes that specifically remove the 4-thiouridine (s^4U) modification from tRNA, thereby directly contributing to the cellular control of sulfur-modified tRNAs under stress conditions [2]. However, the role of 4-thiouridine desulfidases (RudS) in bacterial adaptation to different light conditions and UV irradiation remains poorly understood.

In this study, we investigated RudS-dependent tRNA s^4U demodification and its effect on *Pseudomonas sp.* growth and survivability under visible light (LED) illumination and ultraviolet (UV) irradiation. Using *rudS*-containing and knockout strains, we determined s^4U levels in tRNA after LED illumination using HPLC-MS and analyzed the growth dynamics of bacteria by measuring optical density at 600 nm after UVA/UVC irradiation. Our results show that LED illumination significantly reduced tRNA s^4U levels in *Pseudomonas* strains containing *rudS*, indicating light-induced demodification. Although wild-type and Δ*rudS* mutant strains showed very similar growth dynamics upon UVA exposure, after combined UVA+UVC irradiation the wild-type strain displayed a shorter growth delay than Δ*rudS* mutant. This indicates that functional RudS improves the recovery after combined UVA and UVC-induced damage. Viability assays and gene expression analyses are also being implemented to determine the impact of RudS on bacterial survival and stress responses.

Taken together, our data suggest that RudS-dependent tRNA 4-thiouridine demodification contributes to *Pseudomonas sp.* recovery after UV stress and reveals a novel role for tRNA modifications in bacterial light stress response.

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DEVELOPMENT OF ENHANCED ADOMET COFACTOR ANALOGUES FOR BIOCATALYTIC APPLICATIONS

Aistis Jomantas[1], Bernadeta Masiulionytė[1], Giedrius Vilkaitis[1]

[1]Department of Biological DNA Modification, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
aistis.jomantas@chgf.vu.lt

Enzymatic methylation of DNA cytosines or adenines, RNA adenines or riboses, and histone amino acids is a pivotal epigenetic mechanism involved in essential biological processes, including mammalian development, differentiation, gene expression regulation, maintenance of chromatin structure, disease pathogenesis, and others. Alterations in methylation-dependent processes frequently impair various biological functions, which appear to lead to several diseases, such as cancer, neurodegenerative disorders, and immunodeficiency disorders. The enzymatic methylation reaction is catalysed by a broad class of S-adenosyl-L-methionine (AdoMet)-dependent methyltransferases (MTases), which transfer a chemically inert methyl group to specific biomolecular targets [1, 2]. Recent advances in the development of synthetic AdoMet cofactor analogues and in the engineering of MTases with alkyltransferase activity have enabled novel technologies for deciphering epigenetic and epitranscriptomic changes and their regulation even within cells [3, 4]. Nevertheless, the limited stability of synthetic cofactors may restrict their broader application in biomolecular labelling [5]. To overcome this limitation, S-adenosyl-L-homocysteine (AdoHcy) analogues with modified carboxylate groups were designed to interrupt the dominant degradation pathway of AdoMet – intramolecular cyclisation. To further improve the properties of the derivatives, sulfur was substituted with selenium to enhance their reactivity. Based on these modifications, we hypothesised that the stability and reactivity of the new derivatives would be increased under physiological conditions. Consequently, the AdoHcy analogues were utilised in halide methyltransferase (HMT)-mediated chemoenzymatic synthesis, yielding cofactors with diverse scaffolds suitable for nucleic acid labelling. The functionality of these newly synthesised cofactors was evaluated using nucleic acid-modifying enzymes. In summary, the rational design of more stable and reactive AdoMet analogues, combined with efficient chemoenzymatic synthesis, has the potential to expand the applications of biomolecular labelling and offer advanced tools for the investigation of epigenetic and epitranscriptomic processes.

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BENCHMARKING SGRNAS TARGETING HIF1A AND HIF3A TO ENABLE COMBINATORIAL CRISPR STUDIES IN PDAC

Sofija Aleksandra Paulauskaitė[1], Rūta Matulevičiūtė[1]

[1]Life Sciences Center, Vilnius University, Lithuania
sofija.paulauskaite@gmc.stud.vu.lt

Pancreatic cancer (PC) is one of the deadliest forms of cancer, with an alarmingly low survival rate. The poor prognosis for patients diagnosed with PC is in part due to the complicated nature of the disease and its relationship with the tumour microenvironment. The microenvironment of pancreatic tumours is characterised by hypoxia in relation to normoxic conditions, resulting in changes to the phenotypes of the cancer cells themselves. However, the changes that occur in cancer cells in response to, and as a means of adapting to, these hypoxic conditions are regulated by a transcription factor called hypoxia-inducible factor (HIF). HIF regulates an extensive network of genes and pathways that contribute to pancreatic tumour progression. To study the role that HIF1A and HIF3A play in PDAC, a CRISPR-Cas9 knockout approach can be utilised.

In PDAC, HIF1A is a characterised driver of hypoxia-dependent transcription, while HIF3A may play a similar role but with additional and non-redundant functions. While future studies will investigate both genes in PDAC, the immediate priority is to select single guide RNAs (sgRNAs) with consistent knockout efficiency, particularly for CRISPR combinations where variable guide activity can compromise dual-target editing.

This project aims to establish a workflow to prioritise sgRNAs targeting HIF1A and HIF3A for knockout studies, creating the foundation for future experiments to investigate the role of hypoxia signalling in PDAC. Candidate sgRNAs targeting HIF1A and HIF3A will be selected based on published reports and CRISPR design tools. These guides will be cloned into plasmid templates containing a T7 promoter sequence. The sgRNAs will be transcribed in vitro from these templates, and Cas9-sgRNA ribonucleoprotein (RNP) complexes will be formed through their assembly with recombinant Cas9 protein. The cutting efficiency of Cas9-sgRNA complexes corresponding to candidate sgRNAs will be evaluated using an in vitro Cas9 cleavage assay. This assay will use PCR amplicons spanning each genomic target sequence as substrates. The cleavage assay products will be analysed by agarose gel electrophoresis. The Cas9 cutting efficiency of each sgRNA will be quantified as a ratio of cleaved to uncleaved DNA products.

This is a pre-experimental optimisation and design process, the focus of which is on the experiment preparation rather than the experiment outcome. Outputs will consist of ranked sgRNA libraries for HIF1A and HIF3A, according to the in vitro levels of cleavage activity, the top single guide for each gene for single knockouts, and suggested guide combinations for the pairings needed to enable double knockouts. These outputs represent a functional guide for making decisions related to sgRNA choice that are tied to the robustness of follow-up PDAC hypoxia-pathway experiments.

DEVELOPMENT OF SINGLE-CELL WHOLE GENOME SEQUENCING METHOD USING SEMIPERMEABLE CAPSULES

Karina Račaitė[1], Vincenta Mikulėnaitė[1], Ignas Silickas[1], Juozapas Ivanauskas[1], Simonas Juzėnas[1], Linas Mažutis[1]

[1]Department of Single Cell Analytics, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
karina.racaite@gmc.vu.lt

Genome analysis enables comprehensive investigation of genetic variation and its relationship to biological processes such as development, differentiation, and disease. Whole genome sequencing (WGS) allows detection of single nucleotide variants, insertions, deletions, structural rearrangements, and copy number alterations in a single experiment, and has become central to genomics research and clinical diagnostics. Extending WGS to the single-cell level (scWGS) provides critical insight into cellular heterogeneity and clonal evolution; however, current approaches remain limited by amplification bias, incomplete genome coverage and restricted multi-step processing.

Here, we present a novel scWGS workflow based on semi-permeable capsules (SPCs), a microfluidics-derived platform designed to overcome limitations of droplet-based systems [1]. While droplet-based methods enable high-throughput processing, reagent exchange and complex sequential biochemical reactions are difficult to implement. In contrast, SPCs comprise a hydrogel core enclosed by a thin semi-permeable shell that retains cells and high-molecular-weight genomic DNA while allowing passive diffusion of small molecules. This structure enables harsh lysis, removal of inhibitory components, and sequential enzymatic reactions within intact single-cell compartments.

We optimized multiple library preparation conditions and selected the best-performing workflow based on genome coverage metrics, achieving improved and reproducible coverage across single cells. Species-mixing experiments using human K562 and mouse NIH/3T3 cells demonstrated accurate genome assignment and high-specificity mapping, confirming robust compartmentalization. Together, our results establish SPC-based scWGS as a scalable and reliable platform, with ongoing applications targeting genomic analysis of clinical samples.

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PRECISION ENGINEERING HUMAN CELL LINES FOR SELECTIVE DNA METHYLTRANSFERASE ACTIVITY TRACKING

Joris Dambrauskas[1], Liepa Gasiulė[1], Vaidotas Stankevičius[1]

[1]Department of Biological DNA Modification, Institute of Biotechnology, Life Sciences Centre,
Vilnius University, Lithuania
joris.dambrauskas@gmc.vu.lt

5-Methylcytosine, generated through methylation of CpG dinucleotides in the mammalian genome, is a key epigenetic mark. It regulates gene expression, X-chromosome inactivation, genomic imprinting, and the silencing of retrotransposable elements – processes essential for mammalian development and maintaining normal cellular function [1]. DNA methylation is catalyzed by the DNA methyltransferase (DNMT) family, which transfers a methyl group from the cofactor S-adenosyl-L-methionine (AdoMet) to the 5-carbon position of cytosine within CpG sites. In mammals, DNMTs are commonly divided into two functional groups: the de novo methyltransferases DNMT3A and DNMT3B, which establish genomic methylation patterns, and the maintenance methyltransferase DNMT1, which preserves methylation patterns during cell division [2]. Global DNA methylation profiles derived from epigenomic sequencing reflect the aggregate outcome of locus-specific methylation and demethylation, governed by the coordinated activities of DNMT and TET enzymes [3]. However, methylome profiling alone cannot disentangle the specific contributions of individual DNA methyltransferases to the cellular methylation landscape. To overcome this constraint, recently Dnmt1-Top-Seq experimental platform was developed, which employed structure-guided engineering of the Dnmt1 methyltransferase to enable catalytic transfer of chemical tags onto DNA using synthetic AdoMet cofactor analogue, Ado-6-azide. This, in turn, permitted tracking of DNMT1 methyltransferase specific activity in live genome-edited mouse embryonic stem cells, thereby enabling to dissect activity of individual DNA methyltransferases in vivo [4]. Furthermore, a similar genome engineering of endogenous methionine adenosyltransferase Mat2a in mESCs enabled intracellular biosynthesis of an AdoMet analogue from its precursor azido-methionine and parallel metabolic genome labeling of a DNMT1-specific methylome under physiologically relevant cellular conditions [5].

To apply a similar epigenome labeling strategy in human cells, this study aimed to select state-of-the-art genome editing tools for the successful engineering of endogenous DNMT1, DNMT3A, DNMT3B, and MAT2A genes for subsequent metabolic genome labeling in HEK293 cells. Using a variety of prime editing strategies, we were able to derive HEK293 and HEK293T knock-in cell lines carrying targeted substitutions in the coding sequences of corresponding genes. As a result, a further development of this approach using engineered HEK293/HEK293T knock-in cell lines will provide an experimental foundation for deploying synthetic AdoMet and methionine analogues, allowing downstream epigenomic assays to dissect an individual epigenic landscapes of each DNA methyltransferase in human cells under normal and pathological conditions.

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A SECRET ALLIANCE BETWEEN SPLIT PROKARYOTIC ARGONAUTE PROTEINS AND TYPE I RESTRICTION– MODIFICATION SYSTEMS

Laura Juozepavičiūtė[1], Edvardas Golovinas[1], Mindaugas Zaremba[1]

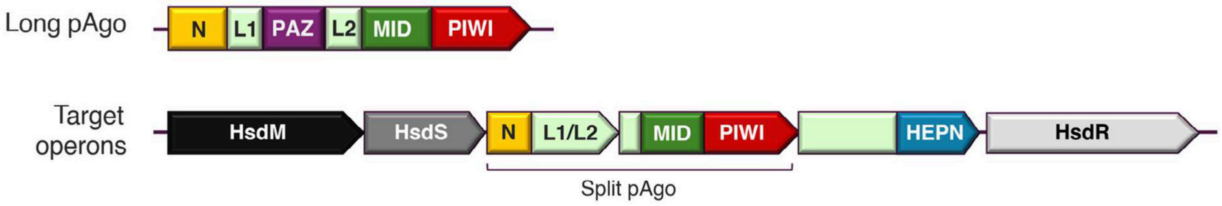
[1]Department of Protein-DNA Interactions, Institute of Biotechnology, Vilnius University, Lithuania
laura.juozepaviciute@gmc.vu.lt

The ability to discriminate host DNA from foreign genetic material at the molecular level is fundamental to bacterial survival and evolution. The bacterial defense forces against genetic elements of exogenous origin are frequently clustered within genomic regions known as defense islands. This pattern suggests that genes of unknown function, which are consistently found near established defense systems, likely serve protective roles or may work synergistically with adjacent systems[1], [2]. Three split prokaryotic Argonaute (pAgo) operons have been identified in the genomes of thermophilic bacteria. Unpublished bioinformatic analyses from our laboratory indicate that these split pAgos, together with associated HEPN domain-containing nucleases, are embedded within operons encoding type I restriction-modification (RM) systems (Fig.1). This genomic co-localization suggests potential cooperation between these defense pathways.

Investigating complex, multi-component bacterial defense systems presents significant challenges, particularly with conventional multi-copy expression vectors that may introduce copy number variability and non-physiological overexpression effects. To mitigate these limitations and better mimic the native conditions, genome integration was implemented. Genetically engineered Escherichia coli strains carrying split pAgo and type I RM defense modules have been constructed using Argonaute-mediated homologous recombination [3]. The constructed E. coli strains have been successfully validated, demonstrating both viability and genetic stability. This creates a solid foundation for subsequent cytotoxicity analyses and functional assays designed to evaluate protection against plasmid acquisition and bacteriophage infection.

Understanding such alliances may provide insight into how bacteria deploy layered immune strategies to limit the constant influx of invading genetic elements.

Figure 1. Schematic representation of the genomic region encoding the target defense systems. For comparison, the domain architecture of a long pAgo is shown.



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CHARACTERIZATION OF THE EC2APEA ANTIVIRAL PROTEIN THROUGH PHAGE INFECTIONS AND TOXICITY ASSAYS

Ema Ežerskytė[1], Jonas Juozapaitis[1], Giedrius Sasnauskas[1]

[1]Department of Protein-DNA Interactions, Institute of Biotechnology, Life Sciences Center, Vilnius University
ema.ezerskyte@gmc.stud.vu.lt

The intense coevolutionary arms race between bacteria and phages has resulted in an unexpectedly wide and diverse array of antiphage defense systems [1]. While well-characterized antiviral systems such as CRISPR-Cas and restriction-modification directly target bacteriophage genetic material, abortive infection (Abi) systems function by activating toxic effectors upon infection, causing inhibition of metabolism or programmed death of the host cell to halt bacteriophage proliferation and protect the bacterial colony [2]. Many Abi systems rely on proteins containing HEPN (Higher Eukaryotes and Prokaryotes Nucleotide-binding) domain, which possesses endoribonuclease activity and is important in a variety of bacterial immune responses [3].

Our research focuses on one such Abi system ApeA which encodes a single protein ApeA with a HEPN domain variant as its key functional feature. While ApeA was previously shown to defend against a limited set of classic T phages [4], the molecular mechanisms were not yet clear. Our earlier work expanded the known antiphage spectrum of ApeA homologs and identified a highly active homolog Ec2ApeA. Structural analysis of Ec2ApeA revealed a loop, prominently changing its conformation during activation. The protein was also co-purified with a dinucleotide, suggesting a potential activation mechanism.

Here we aimed to explore the activation mechanism of Ec2ApeA further by assessing the functional importance of the structurally dynamic loop, as well as the potential dinucleotide signal in the cell. Targeted mutagenesis was used to evaluate the role of the conformationally dynamic loop and co-expression of a dinucleotide cleaving enzyme was employed to test the requirement of dinucleotides as signaling molecules in Ec2ApeA activation.

These experiments demonstrated that both the conformationally dynamic loop and the dinucleotide signal are critical for Ec2ApeA protein activation.

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CHARACTERIZATION OF A NOVEL BACTERIAL ANTI-PHAGE DEFENSE SYSTEM

Miglė Paulikaitė[1], Radvilas Bendorys[1], Patrick Pausch[1]

[1]LSC-EMBL Partnership Institute for Genome Editing Technologies, Vilnius University, Lithuania
migle.paulikaite@gmc.stud.vu.lt

Bacteriophages (phages) pose a constant threat to bacteria, driving the evolution of diverse anti-phage defense mechanisms. Among these strategies, recently identified anti-phage systems involve protein components related to eukaryotic ubiquitination pathways. Characterization of these proteins is essential for elucidating their roles in bacterial immunity and phage evasion.

In this study, we focus on the functional characterization of a bacterial anti-phage defense system using bacteriophage spot titer assays to evaluate protection against phage infection. By introducing and analyzing mutations in system proteins, we assess how specific protein components and their variants contribute to effective defense and directly link protein-level changes to antiviral function. Greater knowledge of this bacterial anti-phage system could help enable the use of its mechanisms as modulable tools in biotechnology.

RAPID CHARACTERIZATION OF PROTEASE-ASSOCIATED CRISPR-CAS SYSTEMS USING *IN VITRO* TXTL AND CONNECTASE ASSAYS

Bingshati Sarkar[1], Dainora Jankūnaitė[1], Adrian Fuchs[2], Carl R. P. Salazar[3], Caglar Yildiz[3], Raymond H. J. Staals[3], Constantinos Patinios[1]

[1]VU LSC-EMBL Partnership Institute, Vilnius, Lithuania
[2]Max Planck Institute for Developmental Biology, Tübingen, Germany
[3]Department of Microbiology, Wageningen University and Research, Wageningen, the Netherlands
bingshati.sarkar@bchi.stud.vu.lt

CRISPR-Cas is a prokaryotic adaptive immune system that protects against mobile genetic elements (e.g., phages) and is classified into 2 classes, 7 types, and 46 subtypes [1]. Among the most complex CRISPR-Cas systems are those belonging to Class 1, Type III. These systems provide RNA-dependent, transcription-coupled immunity, in which target RNA recognition triggers DNA cleavage. In addition, RNA recognition activates the synthesis of cyclic oligoadenylates (cOAs), which function as second messengers. cOAs activate accessory nucleases (e.g., Csm6 or Csx1) to degrade RNA, or engage effector proteins such as SAVED-CHAT or CalpL to induce proteolysis and host cell dormancy [2, 3].

A notable example is the Type III-B system from *Haliangium ochraceum*, which utilizes cOA signalling to strengthen the antiphage response through a cascade of targeted proteolytic events. This system comprises a canonical Type III-B (Cmr1-Cmr6) effector complex together with associated genes encoding SAVED-CHAT, PCaspase (a prokaryotic caspase), the sigma factor PC-σ, the protease inhibitor PCi, and a serine-threonine kinase (PCk). Upon target RNA recognition, Cas10 (Cmr2) produces cyclic tri-adenosine monophosphate (cA_3), which induces SAVED-CHAT oligomerization and activates its CHAT protease domain. Activated SAVED-CHAT specifically cleaves and activates PCaspase, which subsequently functions as a promiscuous protease that degrades multiple protein substrates. *In vivo*, this proteolytic cascade results in a strong abortive infection-like phenotype characterized by pronounced interference and reduced cell viability in *Escherichia coli* [2].

While the Type III-B system from *H. ochraceum* was partially characterised both *in vitro* and *in vivo*, its study relied on laborious and time-consuming approaches, including heterologous protein expression and purification, as well as cloning of large, multi-component operons. These technical challenges complicated experimental workflows and limited scalability. In addition, the putative kinase PCk could not be cloned or functionally characterized due to its apparent toxicity, resulting in incomplete mechanistic understanding of the system. To overcome these limitations and accelerate the characterization of protease-associated CRISPR-Cas systems, we implemented a cell-free transcription-translation (TXTL) platform combined with an in-gel fluorescent Connectase assay [4, 5].

Using this methodology, we demonstrate that cA_3 induction triggers oligomerization and activation of SAVED-CHAT, which cleaves and thereby activates PCaspase. The observed proteolytic activity was inhibited by the PCi, consistent with both our assays and the findings by Steens et al. (2024). Importantly, the full protocol can be completed in less than 20 hours, does not require cloning or recombinant protein production and purification in *E. coli*, and requires minimal hands-on time. Furthermore, our setup enabled efficient expression of otherwise toxic proteins such as PCk. Our initial data highlight the power of this methodology and open the possibility to characterize other protease-associated CRISPR-Cas systems, as well as other bacterial defence systems involving proteolytic cascades.

Our future work will focus on systematic characterization of genomic regions flanking Type III-associated loci and on resolving outstanding mechanistic questions.

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BIOLOGY AND ECOLOGY

Allelopathic Activity and Phytochemical Composition of *Heracleum sosnowskyi*

Aistė Kundrotaitė[1], Asta Judžentienė[1,2], Tatjana Charkova[2], Irena Nedveckytė[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University
[2]Department of Organic Chemistry, Center for Physical Sciences and Technology
aiste.kundrotaite@gmc.stud.vu.lt

Heracleum sosnowskyi is a fast-spreading plant considered invasive in Lithuania and the European Union. This plant should be controlled for several reasons: contact with *H. sosnowskyi* sap and sunlight can cause severe burns to human skin; it can harm herbivorous animals by causing burns to the muzzle and mucous membranes; and it has a negative effect on plant communities and often forms particularly large thickets [1].

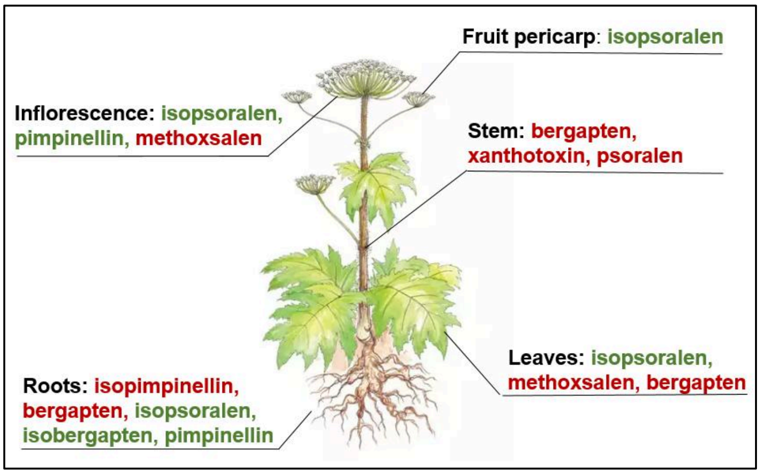
The aim of this study was to determine the phytochemical composition of different morphological parts (flowers, leaves, and roots) of *H. sosnowskyi* and to investigate the allelopathic effects of this invasive plant on a model species, lettuce (*Lactuca sativa*), as well as on three native plant species: perennial ryegrass (*Lolium perenne*), white clover (*Trifolium repens*), and timothy grass (*Phleum pratense*), using aqueous extracts of leaves, roots, and flowers at different concentrations. In addition, the study sought to identify the most allelopathically active morphological part of H. sosnowskyi. The phytochemical composition of the aqueous extracts was analyzed using GC/MS and HPLC/DAD/TOF, while allelopathic activity was assessed through seed germination and seedling growth bioassays conducted using the Petri dish method.

All morphological parts of *H. sosnowskyi* produce phenolic compounds that can potentially inhibit the germination of other plant species [2]. The inflorescences contained predominantly isopsoralen, pimpinellin and methoxsalen (Fig. 1.). The leaf extracts were characterized by the highest concentrations of isopsoralen, methoxsalen and bergapten. The roots of *H. sosnowskyi* exhibited a greater diversity of furanocoumarins (isopimpinellin, bergapten, isobergapten, pimpinellin, isopsoralen), although their concentrations were lower compared to those in the inflorescences and leaves.

The allelopathic results obtained demonstrated the significant inhibitory effects of aqueous extracts of Sosnowsky hogweed. The application of flower and leaf extracts generally resulted in the most significant suppression of germination and growth in the all tested plant species. Leaf extracts had stronger negative effect on seed germination and growth of letuce and clover; flower extracts – on reygras and timothy. The root extracts demonstrated the weakest inhibitory effects on all tested plant seeds.

The results of this study contribute to a deeper understanding of allelopathic interactions and may support the management of invasive plant spread. The findings are relevant for monitoring and controlling the expansion of aggressive *H. sosnowskyi* populations, while also providing a scientific basis for the development of natural herbicides derived from the phytotoxic compounds present in these plants.

Figure 1. Dominant furanocoumarins in different parts of *H. sosnowskyi*. Angular furanocoumarins are marked in green, and linear furanocoumarins in red (prepared by the author).



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MORPHOLOGICAL AND MOLECULAR ANALYSIS OF *MESOCESTOIDES* SPP. IN INTESTINAL SAMPLES FROM RED FOXES (*VULPES VULPES*) IN LITHUANIA

Paula Budrytė[1], Evelina Juozaitytė-Ngugu[2], Petras Prakas[2], Dalius Butkauskas[2]

[1]Life Sciences Center, Vilnius University, Lithuania
[2]State Scientific Research Institute Nature Research Centre, Lithuania
paula.budryte@gmc.stud.vu.lt

Members of the genus *Mesocestoides* (Cyclophyllidea, Mesocestoididae) are found worldwide, and some species are considered zoonotic [1]. These helminths infect canids (family: Canidae), felids (Felidae), and other carnivorous mammals [2, 3]. However, some studies indicate that *Mesocestoides* spp. helminths have also been detected parasitizing humans [4]. Overall, 13 *Mesocestoides* spp. species have been recorded, of which *M. litteratus* and *M. lineatus* are most encountered in Europe [5]. The life cycle of these parasites typically involves three hosts: various coprophagous arthropods (order: Oribatida) or beetles (Coleoptera) serve as the primary intermediate hosts, which become infected by ingesting *Mesocestoides* spp. proglottids or eggs, from which the oncosphere develops. Various vertebrates are identified as secondary intermediate hosts, in which the oncosphere develops into the procercoid and tetrathyridium [3, 6]. The definitive hosts are representatives of various taxa, such as red foxes (*Vulpes vulpes*), which become infected by consuming an animal or its tissues containing tetrathyridia [7, 8].

In this study, we examined 30 intestinal and/or fecal samples from red foxes collected between 2019 and 2025 in Lithuania. *Mesocestoides* spp. were morphologically identified based on key diagnostic features by Khalil et al. [9] using a stereomicroscope and a light microscope. PCR amplification and sequencing of the cytochrome c oxidase subunit I (*Cox1*) gene were used for *Mesocestoides* species identification.

Based on morphological parameters, the prevalence of *Mesocestoides* spp. infection in intestinal and/or fecal samples of red fox was determined to be 56.7% (17/30). The adult *Mesocestoides* helminths were up to 30 cm in length and had an anterior scolex, behind which was the ribbon-shaped, segmented body. The scolex dimensions were 40.9 µm × 79.4 µm, with no distinct rostellum or hooks observed. Behind the tip of the scolex were four elongate muscular suckers. Each mature segment of *Mesocestoides* contained a single set of reproductive organs, called a parauterine organ, 96.9 µm × 46.5 µm in size. Using the *Cox1* gene, *M. litteratus* was identified for the first time in Lithuania. A total of 18 sequences of *M. litteratus* were characterized, revealing 6 haplotypes with a sequence identity ranging from 97.3% to 99.7%. This is the first study to report the prevalence of *M. litteratus* in the intestinal and/or fecal samples from red foxes in Lithuania. Furthermore, we observed high genetic variability within this species, which may indicate high diversity within the parasite population studied. Further research is required to monitor the nationwide distribution of these parasites and to evaluate the public health risks they pose as zoonotic agents.

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The versatility of fungi and yeasts isolated from road construction sites: characteristics, activity and application in the management of environmental by-products

Sabina Myrzakhmetova[1], Saloua Biyada[1]

[1]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Lithuania
sabina.myrzakhmetova@stud.vilniustech.lt

Microorganisms exhibit valuable potential for converting organic by-products into eco-friendly energy sources and monomers that can serve as feedstock for new production processes. They have been recognised as a sustainable approach to address the global environmental emergency, specifically in by-product management (e.g. the food industry accounts for 10% of global warming due to the by-products it generates [1]), which remains a significant ecological and economic challenge, rendering them a real-world model of circular economy implementation.

In this regard, this study aims to conduct an in-depth analysis of the desirable properties of microorganisms isolated from harsh conditions, particularly fungi and yeast, and their implementation in ecological problem issues.

Essentially, chemical analysis of soil from road construction by-products was carried out. A serial dilution method was used to isolate fungi and yeasts from soil using Potato Dextrose Agar (PDA) at 28°C for 5 to 7 days (Fig. 1). The isolated strains were then purified to achieve single-strain colonies for the following profile identification by sequencing. Forward enzymatic activity assessments, notably cellulolytic, lipolytic, and pectinolytic activity, were performed using substrate exposure and a consequent absorbance assay.

Figure 1. Depiction of different microorganisms isolated from samples collected from road construction by-products.

A total of 8 species of fungi and yeasts were identified, exhibiting different lipase, cellulase, and pectinase activities. The isolate “F7” showed the most promising results in this research, expanding its application to the degradation of oil spills, textile waste, and food waste.

Finally, on the basis of the acquired material, a conclusion on the comparable metabolic properties of the microorganisms was drawn and analysed to control by-product disposal for a cost-effective solution in a way of maintaining a sustainable environment.

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F4

THE IMPACT OF THE EDGE EFFECT BY DEFORESTATION ON THE DIVERSITY AND ABUNDANCE OF SOIL FLIES (INSECT: DIPTERA)

Viktorija Amankavičiūtė[1], Dr. Ina Gorban[1]

[1]Institute of Biosciences, Life sciences center, Vilnius University, Lithuania
viktorija.amankaviciute@gmc.stud.vu.lt

The edge effect caused by forest fragmentation leads to significant changes in environmental conditions, affecting both the forest floor and the soil ecosystems [1]. Organisms developing in the soil, especially soil flies, are sensitive to the changes and can be used as bioindicators [2]. The aim of this study is to assess the impact of the edge effect caused by deforestation on the diversity and abundance of soil flies (Insecta: Diptera).

The study was conducted in the Punios šilas forest, in three different habitats: inside the forest, the ecotone (edge zone) and the deforestation site. Flies developing in the soil were collected using emergence traps [Fig. 1]. Based on morphological characteristics and identification keys, more than 30 different families were identified.

Figure 1. Soil emergence trap. (Author: S. Slančiauskas)

The results of the study show that the structure of soil dipteran communities differs significantly depending on the habitat. The highest abundance of specimens was found in the forest, while the highest family richness was found at the deforestation site. The ecotone was distinguished by the lowest values of both abundance and diversity. The results show that the edge effect and the changes that come with the effect in environmental conditions have a significant impact on the diversity and distribution of soil dipterans across different habitats.

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BEETLE DIVERSITY ACROSS FLOWERING STRIPS AND WINTER WHEAT FIELDS: IMPLICATIONS FOR BIOLOGICAL CONTROL AND CONSERVATION

Liepa Uzielaitė[1], Vytautas Tamutis[2], Grita Skujienė[1]

[1]Institute of Biosciences, Life Sciences center, Vilnius University, Lithuania
[2]Tadas Ivanauskas Museum of Zoology, Kaunas, Lithuania

liepa.uzielaite@gmc.stud.vu.lt

Intensive agricultural management is widely recognized as a key driver of biodiversity decline, primarily through habitat loss and altered ecosystem processes. One effective method for naturally increasing insect biodiversity in crop plantations is the use of flowering strips [1]. Nevertheless, farmers remain concerned that flowering strips could promote pest populations. This study aims to evaluate the extent to which these concerns are justified. Specifically, this study assessed the effect of distance from flowering plant strips on beetle (Coleoptera) abundance and species diversity in winter wheat fields in the Kupiškis district, Lithuania (Juodpėnai and Šileikiai villages).

Beetles were sampled over a two-week period from May 18 to June 2, 2023, using Barber pitfall traps (n = 36) arranged in transects placed either directly within the flowering strip or, as a control, along the field margin (Fig. 1, I), as well as at different distances within the adjacent winter wheat field (Fig. 1, II–IV).

A total of 10,835 beetle individuals representing 60 species from 9 families were identified. Carabidae dominated in both species richness and abundance. The most abundant species were *Poeciluscupreus*(Linnaeus,1758) and *Nebria brevicollis(*Fabricius, 1792). Both fields with flowering strips showed higher beetle species richness compared to the control field, although the highest beetle abundance (n = 2,068) was observed in the last transect (IV) of the control field. The highest Shannon diversity index was recorded in the Šileikiai field with flowering strips (H′ = 2.40) (Fig. 1), and it gradually decreased towards the centre of the field (transect IV).

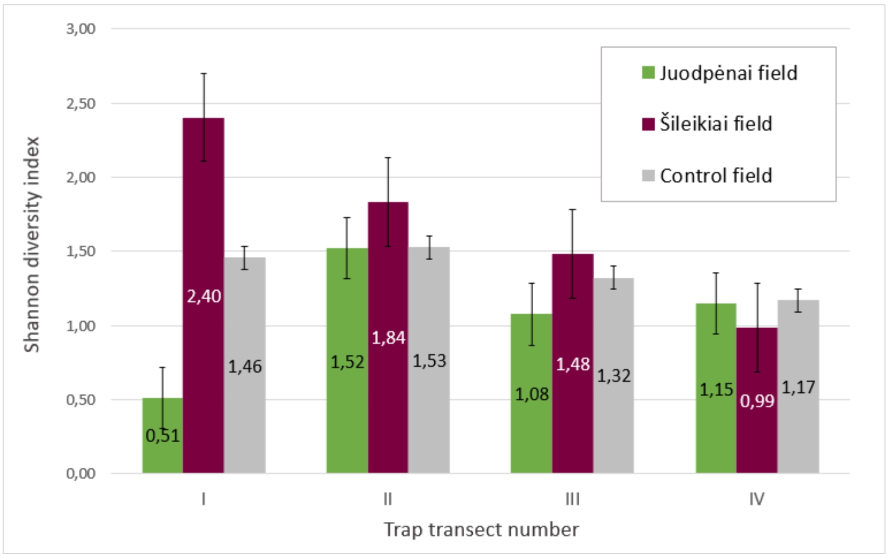

Figure1. Shannon diversity indices (Mean ± SE) of beetles in the Kupiškis district, Lithuania (Juodpėnai and Šileikiai villages), in traps across transects I–IV: I – within the flowering plant strip or control field margin; II–IV – at 5, 25, and 55 m into the winter wheat field.

In summary, beetle diversity showed some variation among transects and field types, especially close to field margins, but only in the Šileikiai field was a clear distance-related gradient evident, indicating a context-dependent spatial influence of flowering strips. The results suggest that flowering plant strips can enhance beetle diversity locally, particularly near field margins, indicating their potential as a site-specific measure for biodiversity conservation. The findings do not indicate an increase in pest insects associated with flowering strips and suggest a potential contribution of predatory beetles to biological control..

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F6

THE EFFECT OF WATER SOURCE ON THE GROWTH OF THE YELLOW MEALWORM (*TENEBRIO MOLITOR* L.)

Felicija Kvedaraitė, Gabrielė Bumbulytė-Žukevičienė
State Scientific Research Institute Nature Research Centre, Laboratory of Chemical and Behavioural Ecology
Vilnius, Lithuania
felicija.kvedaraite@gmail.com

The increasing global demand for sustainable protein sources has intensified interest in insect farming as an environmentally friendly alternative to conventional livestock production. The yellow mealworm (*Tenebrio molitor* L.) is a promising species for food and feed applications due to its high protein content and efficient feed conversion. However, optimizing rearing practices remains essential for sustainable large-scale production, including the choice of larval water source.

In industrial systems, carrots are commonly used to provide moisture, yet their use may be economically inefficient and directly competes with human food resources. Therefore, this study aimed to evaluate alternative water sources that avoid direct competition with the human food chain. Under controlled laboratory conditions, *T. molitor* larvae were reared using eight alternative water-source treatments representing (a) beverage-industry by-products and (b) alternative water thickeners. Larvae were maintained until pupation, and larval body weight was recorded weekly throughout development to characterize growth dynamics.

Distinct differences in growth trajectories were observed among treatments. Certain alternative water sources supported more favorable larval growth patterns, particularly during later developmental stages, whereas others were associated with slower weight gain. Overall, the findings underscore that water-source selection can meaningfully influence mealworm growth performance and should be considered a key parameter in rearing optimization. Evaluating non-food moisture sources, such as industrial by-products and functional thickeners, may contribute to more sustainable and resource-efficient mealworm production systems.

F7

EPN *Steinernema carpocapsae*: predator-prey interaction via chemical cues

Gija Leonavičiūtė[1], Rasa Čepulytė[2], Vincas Būda[3]

Laboratory of Chemical and Behavioral Ecology, State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania
gija.leonaviciute@gmc.stud.vu.lt

Entomopathogenic nematodes (EPNs) are soil-dwelling, non-segmented roundworms that parasitize insects and are widely used as environmentally sustainable alternatives to chemical insecticides. Although chemical cues are known to play a central role in host location, EPN-host interactions may be more complex than currently understood. It remains unclear how EPNs respond to hosts that are already infected with EPNs and dead. Therefore, volatiles released from EPN-infected insect cadavers represent one potential source of such cues, but their functional significance remains largely unexplored. Improved understanding of these processes could enhance the efficacy of EPNs as biocontrol agents.

The behavioral responses of infective juveniles of *Steinernema carpocapsae* (the free-living, non-feeding stage that persists in soil and searches for suitable insect hosts) to 1-undecene, a volatile compound released by EPN-infected insect cadavers, were investigated. A two-choice assay was used to test ten concentrations of 1-undecene. High concentrations (200-20 mM) were attractive, whereas lower concentrations (2-0.02 mM) elicited neither attraction nor repellence. Because attraction was observed at high concentrations only, these results suggest that 1-undecene alone is unlikely to serve as a primary host-location cue under natural conditions. Other chemical signals may therefore play a more important role in EPN host recognition.

F8

SARCOCYSTIS SPECIES IN WILD CANIDS FROM LITHUANIA

Tamara Kalashnikova[1], Donatas Šneideris[1], Evelina Juozaitytė-Ngugu[1], Naglis Gudiškis[1], Petras Prakas[1], Dalius Butkauskas[1]

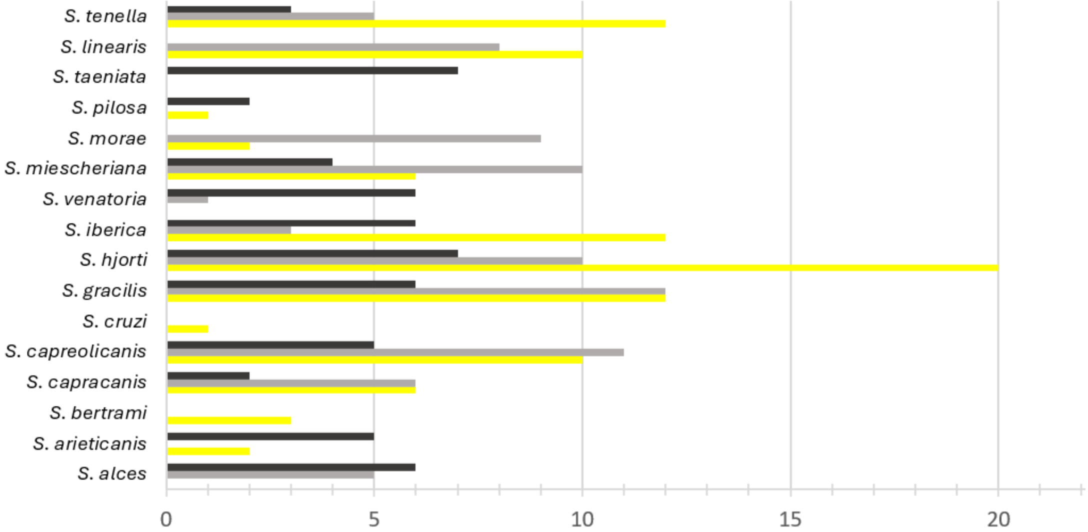
[1]Molecular Ecology Laboratory, The State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania
tamara.kalashnikova@gamtc.lt

Sarcocystis species are protist parasites who can infect a wide range of animals, including reptiles, birds, and mammals. Nowadays more than 220 species are known. The lifecycle of *Sarcocystis* is dixenous and includes prey-predator relationships between hosts. Intermediate host (IH) gets infected through contaminated water or food. Following this, *Sarcocystis* species undergo asexual reproduction in IH's muscles. Definitive host (DH) contracts infection eating meat contaminated with *Sarcocystis* species. Subsequently, sexual reproduction occurs in the intestine and ends with sporulation of oocysts. The sporocysts formed are shed with faeces into the environment where IH can consume it. In this study we aimed to establish the richness of *Sarcocystis* species in intestines of red fox (*Vulpes vulpes*), raccoon dog (*Nyctereutes procyonoides*) and grey wolf (*Canis lupus*)

A total of 67 animals were collected from 2019 to 2023 in Lithuania, namely 28 red foxes, 26 raccoon dogs and 13 grey wolves. Scrapings of the intestinal mucosa were analysed under light microscope. A two-step nested PCR (nPCR) was performed targeting cytochrome c oxidase subunit I (*cox1*). Studies had proven that *cox1* is a universal marker for the identification of *Sarcocystis* spp. that use ungulates as IH [1]. First step of nPCR was conducted with genus-specific primers, second — with species-specific primers. Samples were tested for 16 *Sarcocystis* species. The list of species and the IH (present in Lithuania) are: Caprinae — *S. arieticanis* (sheep), *S. tenella* (sheep), *S. taeniata* (sheep), *S. capracanis* (goat, Barbary sheep, European mouflon); Suidae — *S. miescheriana* (pig and wild boar); Equidae — *S. bertrami* (horse); Bovidae — *S. cruzi* (cattle, European bison); Cervidae — *S. alces* (moose), *S. pilosa* (sika deer, red deer), *S. capreolicanis* and *S. gracilis* (roe deer), *S. hjorti* (moose, red deer, sika deer), *S. iberica* (red deer, sika deer), *S. linearis* (moose, red deer, roe deer, sika deer), *S. venatoria* (red deer), *S. morae* (fallow deer, red deer, sika deer).

In total 27 of 28 (96.42%) red fox, 23 of 26 (88.46%) raccoon dog and 11 of 13 (84.62%) grey wolf samples were positive for Sarcocystis spp. All 16 *Sarcocystis* species have been identified in intestinal samples of canids. Red fox samples were positive for the greatest number of *Sarcocystis* species, 13 of 16, being the only one tested positive for *S. cruzi* and *S. bertrami* (Fig. 1). The highest detection rate was recorded for *S. hjorti* whose IH are cervids. Grey wolf samples were positive for 12 of 16 *Sarcocystis* species and were the only host positive for *S. taeniata*. Lastly, 11 of 16 raccoon dog samples were for *Sarcocystis* spp. Notably, *S. miescheriana* that uses pigs or wild boar as IH, was most often found in raccoon dogs. As these predators usually do not hunt in packs, carcasses are a likely source of infection. Due to high variety of *Sarcocystis* species identified, we can conclude that canids play an important role in the distribution of *Sarcocystis* spp.

Figure 1. Molecular analysis results of the *Sarcocystis* species presence in intestinal samples of grey wolf (black), raccoon dog (grey) and red fox (yellow).



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THERMAL EFFECTS ON EGG SURVIVAL IN *TENEBRIO* BEETLES (*TENEBRIO* SPP.)

Fausta Molotokaitė[1,2], Gabrielė Bumbulytė-Žukevičienė[2]

[1]Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]Chemical and Behavioural Ecology Laboratory, State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania
fausta.molotokaite@gmc.stud.vu.lt

Tenebrio molitor is an extensively studied species widely used in research and industrial mass rearing, including production for human consumption. Its biology, developmental requirements, and responses to environmental conditions under laboratory and farming settings are well documented. In contrast, substantially less information is available for *Tenebrio obscurus*, despite its close relatedness to *T. molitor* and its current use as animal feed. Given its potential for broader application in human food production, a detailed understanding of its rearing requirements is needed; however, key developmental parameters, particularly temperature-dependent development and survival during early life stages, remain insufficiently documented.

Temperature is a major abiotic driver of insect development, especially during embryonic and early larval stages. Therefore, this study assessed the effects of four constant temperatures (21, 23, 25, and 27 °C) on egg hatching success and early larval survival in *T. molitor* and *T. obscurus* under controlled laboratory conditions.

The results showed clear differences between the two species in both egg hatching and larval survival. *Tenebrio molitor* exhibited relatively stable performance across a broad range of temperatures, whereas *T. obscurus* showed stronger sensitivity to temperature variation during early developmental stages. This indicates that temperature plays a key role in shaping early developmental success in *Tenebrio* species and highlights the need for species – specific optimization of rearing conditions. Much remains unknown about the basic biology and rearing requirements of *T. obscurus*, and additional studies are needed to support its potential use in large-scale production and human food systems.

INFLUENCE OF MAGNETIC CHITOSAN NANOCOMPOSITE- AND PROBIOTIC-SUPPLEMENTED DIETS ON GROWTH AND OXIDATIVE STRESS IN COMMON CARP

Aida Bradauskaitė[1], Renata Butrimienė[1], Sergej Šemčuk[1,2], Danguolė Montvydienė[1], Natalia Matvienko[1], Vesta Skrodenytė-Arbačiauskienė[1], Živilė Jurgelėnė[1]

[1]State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania
[2]Department of Environmental Research, Center for Physical Sciences and Technology, Vilnius, Lithuania
aida.bradauskaite@gamtc.lt

Magnetic chitosan nanocomposites (MCNs) are advanced materials that combine the biocompatibility of chitosan with the superparamagnetic properties of magnetite (Fe_3O_4), making them attractive for various industrial and biomedical applications [1,2]. Nonetheless, due to their capacity to bind and deliver biological substances, MCNs could also serve as carriers for functional feed additives in aquaculture. However, despite these promising properties, their suitability as probiotic carriers or enhancers has not yet been evaluated. Moreover, the long-term effects of dietary MCNs supplementation on aquatic organisms are not sufficiently investigated. Consequently, a better understanding of MCNs interactions with fish physiology and stress responses is essential to assess their efficacy and to prevent unintended harm.

This study investigated the effects of dietary probiotics and MCNs on morphophysiological parameters and oxidative stress biomarkers in *Cyprinus carpio* L. over a 21-day feeding period. Fish were divided into four experimental groups: control, probiotic-only, MCN-only, and a combined treatment (MCN+P) and fed twice daily at a ration corresponding to 4% of their average body weight. At the end of the experiment, fish morphophysiological parameters were assessed, and organ samples were collected for biochemical analyses. Antioxidant enzyme activities and oxidative damage markers, including superoxide dismutase (SOD), catalase (CAT), malondialdehyde (MDA), and hydrogen peroxide (H_2O_2), were measured in liver, gill, and muscle tissues.

The results showed that after the 21-day feeding trial, neither MCNs nor probiotics induced significant changes in the morphophysiological parameters of the fish. The body weight, Fulton's condition factor, gill somatic index, and hepatosomatic index did not differ significantly among dietary groups. Differences in MDA levels were also not observed, indicating the absence of enhanced lipid peroxidation. In contrast, H_2O_2 concentrations were significantly reduced in gill tissues of the MCN+P group, suggesting that MCNs together with probiotics could reduce oxidative stress in fish. Activities of SOD and CAT remained stable across treatment groups, further indicating that neither probiotics nor MCNs induced oxidative stress in *C. carpio* under the tested conditions.

Overall, these findings highlight the potential compatibility of MCNs as a carrier system in aquaculture fish diets, as they do not adversely affect fish growth or elicit harmful oxidative responses in *C. carpio*.

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EXPLORING CARBON AND NITROGEN ALLOCATION IN AN EARLY LAND PLANT, *LYCOPODIUM ANNOTINUM* L.

Gerda Rumšaitė[1,2], Anita Elizabeth Clark[1], Andrius Garbaras[2], Radvilė Rimgailė-Voicik[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
[2]Isotopic Research Laboratory, Center for Physical Sciences and Technology, Lithuania
gerda.rumsaite@gmc.stud.vu.lt

Lycophytes are vascular plants that date back to late Silurian 420 million years ago and were crucial for the formation of terrestrial ecosystems [1]. Due to their evolutionary significance, studies on the functioning of extant lycophytes can provide valuable insights into their importance in contemporary ecosystems.

This work focuses on *Lycopodium annotinum* L. (interrupted clubmoss, syn. *Spinulum annotinum* (L.) A. Haines), an extant lycophyte found in temperate and boreal forests of North America, Europe, and Asia [2]. This species is included in the Red List of the International Union for Conservation of Nature (IUCN) and is classified as Least Concern (LC) [3]. The life cycle of these plants consists of two independent stages: gametophytes and sporophytes. Gametophytes are subterranean and usually less than 1 cm in size, while sporophytes can extend tens of meters and make up a significant part of the forest understory layer. There is some data about carbon allocation in different organs of *L. annotinum* [4]; however, due to their peculiar life cycle and clonal growth form in the sporophytic stage, investigations into their functioning (especially nutrient allocation) across different habitats remain exceptionally sparse and pose methodological challenges.

Methods which can be used to assess nutrient allocation in plants include stable isotope analysis (SIA). SIA involves measuring carbon ($^{13}C/^{12}C$, expressed as $\delta^{13}C$) and nitrogen ($^{15}N/^{14}N$, expressed as $\delta^{15}N$) stable isotope ratios. These ratios can be used as ecophysiological integrators which reflect the effects of environmental variations on plants.

Therefore, the main aim of this study was to assess the carbon and nitrogen allocation in *L. annotinum* sporophytes found in different habitats, encompassing segments from different stems and varying age. The main objectives were 1) to evaluate C and N allocation differences between habitats, 2) to assess age-related C and N distribution variation, and 3) to determine C and N allocation in different stem types. Specimens were collected in September 2025 in two different habitats – a forest dominated by European spruce (*Picea abies* (L.) H. Karst.) and a forest dominated by Scots pine (*Pinus sylvestris* L.). The specimens included orthotropic (vertical) and plagiotropic (horizontal) stems of varying age. Specimens were cleaned, dried, separated into parts, and ground. $\delta^{13}C$ and $\delta^{15}N$ of *L. annotinum* biomass was determined via isotope ratio mass spectrometry (IRMS).

Results of this study revealed that habitat type had a clear influence on the isotopic composition of *L. annotinum*. Plants growing in spruce-dominated and pine-dominated forests had different $\delta^{13}C$ and $\delta^{15}N$ values, indicating that environmental conditions strongly shape carbon and nitrogen allocation in this species. In addition, $\delta^{13}C$ values showed a small but significant difference between orthotropic and plagiotropic stems, as well as segments of different age. In contrast, $\delta^{15}N$ values did not differ between organs or shoot age, indicating that internal plant structure has only a minor influence on nitrogen-related processes in *L. annotinum*. Overall, these results illustrate that environmental settings (extrinsic factors), rather than intrinsic factors, may be the main determinants influencing nutrient allocation in *L. annotinum*.

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OCCURRENCE OF ANTIBIOTIC RESISTANCE GENES AND POTENTIAL RESISTANCE PLASMIDS IN FARMLAND-ASSOCIATED BACTERIA

Alper Melih Ucar[1, 2], Juliana Lukša-Žebelovič[1, 2], Elena Servienė[1, 2]

[1]Nature Research Centre, Vilnius, Lithuania
[2]Department of Chemistry and Bioengineering, Vilnius Tech, Lithuania
a.ucar@stud.vilniustech.lt

Agricultural ecosystems integrate soil, water, feed, animals, and microbiota into a connected environmental network in which antimicrobial resistance (AMR) can persist and circulate. Bacteria are a dominant component of environmental microbial communities and play an essential role in ecosystem functioning [1]. Antimicrobial resistance (AMR) is disseminated among diverse bacterial species, leading to the emergence of potential pathogenic antibiotic-resistant bacteria (ARB) that threaten both animal and human health [2]. Agricultural fields act as the hotspots for AMR, where resistance genes transfer across ARB in the interconnected environmental compartments including soil, water, and animal feed [3]. Horizontal transfer of antibiotic resistance genes (ARG) is typically mediated by plasmids [4]. Understanding how ARB are distributed within farmland ecosystems is necessary to establish baseline resistance levels and to assess potential risks to animal and public health. This study investigates the antibiotic resistance genes, and potential resistance plasmids of multidrug-resistant environmental bacteria from ecological dairy farms in Lithuanian.

This study examined selected antibiotic resistance genes (ARGs) and the presence of plasmids in antibiotic-resistant bacteria isolated from ecological dairy farm environments in Lithuania. A previously characterized collection of 44 antibiotic-resistant bacterial isolates originating from farmland-associated matrices was screened by PCR for genes conferring resistance to ampicillin, streptomycin, tetracycline, and chloramphenicol. Plasmid extraction was performed for each isolate using a commercial plasmid miniprep kit.

The *blaTEM* gene was the most abundant resistance gene detected, present in 50% of the isolates and in 22 of 33 ampicillin-resistant strains. Streptomycin resistance genes *strA* and *strB* were predominantly detected together, with *strA* present in 11.36% and *strB* in 9.1% of the isolates. Out of five tetracycline-resistant farmland isolates, three strains (6.82% of tota isolates) carried the *tetA* resistance gene. Furthermore, chloramphenicol resistance genes *floR* and *cmlA* were detected in 11.36% and 2.27% of the isolates, respectively. Plasmids were detected in six isolates, with estimated sizes ranging from approximately 1.2 to 7 kb.

The obtained results show the abundancy in certain resistance genes in multidrug-resistant bacteria isolated from ecological farmland environments. Further work targeting a broader range of resistance genes and characterization of plasmids would help clarify the mechanisms underlying the observed resistance patterns.

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F13

ALIEN SPECIES *OTIORHYNCHUS ARMADILLO* AND *O. PSEUDONOTHUS* (COLEOPTERA: CURCULIONIDAE) IN LITHUANIA: DISTRIBUTION, TROPHIC ASSOCIATIONS, AND MOLECULAR VARIATION

Vytautas Račkauskas, Svetlana Orlovskytė, Sandra Radžiutė

State Scientific Research Institute Nature Research Centre, Lithuania
vytautas.rackauskas@gamtc.lt

Climate change and global trade in plants are promoting the spread of invasive insect pests, posing an increasing threat to agriculture and natural ecosystems. In Lithuania, two *Otiorhynchus* (Coleoptera: Curculionidae) species have relatively recently been recorded: *O. armadillo* was found in 2010 [1] and *O. pseudonothus* was detected in 2023 [2]. Both weevil species are highly polyphagous pests damaging cultivated and ornamental plants. However, their current distribution, host plant range, and genetic diversity in Lithuania remain insufficiently studied. The aim of this study was to determine the distribution of these alien species in Lithuania, their trophic associations, and molecular variation. In total, 344 *Otiorhynchus* specimens were collected from 23 localities (six counties) in Lithuania. Based on morphological characters, 168 specimens were identified as *O. armadillo* and 176 as *O. pseudonothus*. *O. armadillo* had a wider geographical distribution (14 localities in six counties) than *O. pseudonothus* (nine localities in three counties).

O. armadillo was collected on plants belonging to nine families, whereas *O. pseudonothus* was recorded on plants of eight families, indicating a broad host plant range under Lithuanian conditions. Six plant genera were identified as host plants for both weevil species; the most frequent was *Thuja* sp. (*Cupressaceae*).

DNA barcoding based on a 657 bp fragment of the mtDNA CO1 region was performed for 25 Lithuanian specimens (14 *O. armadillo* and 11 *O. pseudonothus*), which was compared with sequences available in GenBank [3] and BOLD [4]. The two analysed species formed distinct clades; however, only *O. pseudonothus* exhibited well-supported topology (99%). *O. armadillo* divided into two branches, suggesting possible cryptic diversity. Eight of 14 European *O. armadillo* mitotypes and four of 11 *O. pseudonothus* mitotypes were detected in Lithuania, several recorded for the first time.

The wide distribution, host plant range, and relatively high intraspecific variability of *O. armadillo* and *O. pseudonothus* in Lithuania indicate that both species are already established in the country. A considerable proportion of mitotypes was recorded exclusively in Lithuania (35% of the detected *O. armadillo* mitotypes and 27% of *O. pseudonothus* mitotypes), suggesting insufficient research of these species in other European countries.

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F14

STUDY OF *APIS MELLIFERA MELLIFERA* POPULATION IN DZŪKIJA NATIONAL PARK

Vincent Tachpaj Judakov[1,2], Laima Blažytė-Čereškienė[2]

[1]Vilnius University, Life Sciences Center, Vilnius, Lithuania
[2]State Scientific Research Institute Nature Research Centre, Laboratory of Chemical and Behavioral Ecology, Vilnius, Lithuania
vincentasjj@gmail.com

Over the centuries, 20 subspecies of honey bees have evolved in Europe, each with its own historical range. Native to northwestern Europe, including Lithuania, is the *Apis mellifera mellifera* subspecies, which is endangered due to habitat loss and genetic introgression from imported non-native honey bee queens and colonies.

The aim of this study was to investigate the honey bee population in Dzūkija National Park and to assess how managed bee colonies and swarms comply with the morphometric and genetic criteria of *A. mellifera mellifera.* It also seeks to identify bee colonies that may be used in further studies and projects related to the conservation and population restoration of *A. mellifera mellifera* in Lithuania.

38 bee colonies originating from known *A. mellifera mellifera* queen bees and 21 captured bee swarms of unknown origin were analyzed. The morphometric characteristics (discoidal shift and cubital index) as well as abdominal tergite pigmentation of the workers in each colony were evaluated. Honey bee swarms were analyzed to determine their maternal ancestry, using the COI–COII intergenic spacer sequences of mtDNA.

Morphometric analysis of bee colonies of known origin indicated that the cubital index of 73,5% of bee colonies and the position of the discoidal shift of 23,7% of bee colonies correspond to the characteristics of the Lithuanian population of *A. mellifera mellifera* described before 1984. Genetic analysis of captured swarms revealed that 52,3% of the swarms were of the M maternal lineage, i.e., belonged to the native *A. mellifera mellifera* subspecies, while the rest belonged to lineages of non-native subspecies. Analysis of the morphometric parameters of the M lineage swarms revealed that 63,6% of swarms had a cubital index and another 63,6% had a discoidal shift that corresponded to the limits of these criteria characteristic to the Lithuanian *A. mellifera mellifera*.

8 honey bee colonies and 5 swarms of M lineage were selected which could be used in further bee breeding and population restoration programs.

F15

HOW LONG CAN WITCHES' CAULDRON FUNGUS FRUITING BODIES LIVE? A STUDY OF *SARCOSOMA GLOBOSUM* LIFESPAN

Eglė Vabuolė[1], Ernestas Kutorga [1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania

egle.semaskaite@gmc.stud.vu.lt

The early spring fruiting Witches' Cauldron (Sarcosoma globosum), an endangered and protected in Lithuania ascomycete species, is associated mostly with old-growth, not or minimally disturbed Norway spruce (Picea abies) forests. Therefore, this fungus is considered a valuable indicator of forest ecosystem naturalness. Due to its fragmented distribution and irregular occurrence of fruiting bodies, the species has long been regarded as poorly studied, with available biological data often limited to isolated records. During the last decade, an increasing number of observations across Europe and North America has drawn attention to the distribution and annual variation in fruiting of S. globosum [1–3]. However, the development and maturation of individual fruiting bodies remain insufficiently documented. Knowledge on S. globosum lifespan is important since this fungus grow and survive quite adverse environmental conditions, such as winter cold and snow, dry periods in early spring, and pest infestations. In particular, little is known about the persistence of S. globosum fruiting bodies and the proportion that actually reach maturity and produce ascospores. These aspects are crucial for assessing the reproductive potential of the species and the long-term stability of its populations.

This study aimed to investigate the timing of appearance, longevity, and maturation dynamics of S. globosum fruiting bodies. The research was based on individual-level observations conducted over three fruiting seasons between 2021 and 2024. Field monitoring of fruiting bodies was carried out in three fungus localities in Lithuania, namely, Grabijolai forest, Antazavė pinewood and Labanoras forest. The localities were surveyed once per month during the fruiting season. Individual fruiting bodies were repeatedly counted, marked and measured (the diameter measured with a digital caliper) during each visit. . The maturity of asci was examined microscopicaly by taking samples from the hymenium of fruiting bodies in spring. Meteorological conditions were characterised using data from the Lithuanian Hydrometeorological Service [4](LHMT) and the Global Historical Climatology Network Daily [5].

The results of the study showed that annual S. globosum fruiting bodies can start their development considerably earlier than traditionally assumed for this species, in some cases already during pre-winter months and in December. Individual fruiting bodies have tough and felty outer surface, gelatinous inside, and could overwinter under snow. The lifespan of fruiting bodies varies, ranging from 2 to 7 months. Substantial proportion of observed fruiting bodies did not reach full maturity, and failed to produce ascospores. This suggests that, although fruiting bodies may be long-lasting, successful maturation may be constrained by various environmental factors, which shapes the reproductive success and population viability of the species.

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F16

CHARACTERIZATION OF BACTERIA AND YEAST, ISOLATED FROM *IPS TYPOGRAPHUS* GUT

Rusnė Žilinskaitė[2], Simona Jaseliūnaitė[1], Goda Mizerienė[1], Dovilė Čepukoit[1], Daiva Burokienė[1]

[1]Laboratory of Plant Pathology, State Scientific Research Institute Nature Research Centre, Lithuania
[2]Life Sciences Center, Vilnius University, Lithuania
rusne.zilinskaite@gmc.stud.vu.lt

The European spruce bark beetle (*Ips typographus*) is one of the most widespread and damaging spruce pest in Europe. Climate change is altering its behavior and population, which is leading to increased forest losses across the continent [1].

Research of the beetle's microbiota provides insights into the diversity of microorganisms living in its gut, their interactions, and the mechanisms that help the insect protect itself from the external environment and overcome the tree's defense systems [2].

The gut microbiota of *I. typographus* includes a variety of bacteria and fungi, such as symbionts, pathogens, and non-pathogenic microorganisms. Numerous bacterial and yeast species have previously been documented in the gut of *Ips typographus*, which motivated the primary objective of this study: the phenotypic characterization of bacterial and yeast isolates obtained from the gut of *I. typographus* using cultivation-based approaches, morphological classification, cell wall property assessment, and enzymatic assays. This study is a continuation of ongoing research conducted at the Laboratory of Plant Pathology. The study focuses on the microbiota associated with *Ips typographus*, a collection of gut-associated microorganisms isolated from the beetle.

Microbial isolates were cultured and grouped according to the colony morphology and cell wall properties. To further characterize the isolates, enzymatic activity tests were performed, and the isolates were evaluated for antagonistic activity against entomopathogenic fungi. One representative from each morphological group was sequenced to determine its taxonomic identity.

Understanding the gut microbiota of *I. typographus* may provide valuable insights into microbe-host interactions that influence beetle fitness and could lead to the development of novel biocontrol strategies for this economically important forest pest.

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Distribution and Species Composition of Entomopathogenic Nematodes in Meadow and Forest Soils of Vilnius County (Lithuania)

Agnetė Kuprytė, Deimantė Tiškevičiūtė, Svetlana Orlovskytė, Rasa Čepulytė, Vincas Būda

Laboratory of Chemical and Behavioral Ecology, Institute of Ecology, Nature Research Centre,
Akademijos 2, 08412, Vilnius, Lithuania
agnete.kupryte@gmc.stud.vu.lt

Entomopathogenic nematodes (EPNs) are among the most efficient biological control agents of insect pests. However, knowledge regarding their distribution and species composition in Lithuania remains extremely limited. Studying the diversity and distribution of locally occurring EPNs is essential for understanding their potential in sustainable pest management. Since native species are often better adapted to local environmental conditions and may therefore control pests more effectively than commercially available strains, assessing their diversity becomes particularly important. The aim of this research was to determine the occurrence, species diversity, and habitat distribution of entomopathogenic nematodes in Vilnius County, Lithuania.

In this study, 20 soil samples were collected from meadow and forest habitats in Vilnius County (Lithuania). EPNs were isolated from soil samples using the *Galleria mellonella* baiting technique [1], infective juveniles were recovered from infected larval cadavers using the White-traps method [2], and species identification was performed by DNA barcoding [3]. A total of five distinct EPN species were identified across all soil samples. These results significantly enhance current knowledge of the occurrence, species composition, and habitat-specific distribution of EPNs in Vilnius County, particularly highlighting differences between meadow and forest habitats.

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F18

FLOWERING STRIPS AS A TOOL TO INCREASE BIRD POPULATIONS

Edgaras Žiūkas[1], Saulis Skuja[1]

[1]Institute of Biosciences, Life Sciences Centre, Vilnius University, Vilnius, Lithuania
edgaras.ziukas@gmc.stud.vu.lt

Farmland birds in Europe are experiencing severe declines, with agricultural species nearly 18 times more likely to decrease than those in other habitats [1]. Similar long-term trends are evident in Lithuania [2]. Flowering strips have been suggested as a potential mitigation measure [3], and this study evaluated their effectiveness in Lithuanian agricultural landscapes.

Bird surveys were conducted on a BASF FarmNetwork farm (owned by Zigmantas Aleksandravičius) in Kupiškis District, Lithuania. Two areas were studied: Juodpėnai-Šileikiai (130 ha) with flower strips, and Puponys II (140 ha) as a control. Surveys were carried out six times per year from April to June, 2021–2025, using a modified cartographic-point methodology. Birds, including breeding species, were recorded in two monthly periods (1st–15th and 16th–end of month), with at least five days between visits at the same site. Differences between fields with flower strip and control fields were analysed using paired t-tests (separately for May and June).

In 2025, 2142 individuals and 84 bird species were recorded. Diversity (Shannon index, H') was highest in Juodpėnai-Šileikiai in May II (H' = 3,24) and lowest in April II (H' = 1,95). Evenness (Pielou's E) peaked in May II and June I (E = 0,81) and was lowest in April II (E = 0,57). Bird density in fields with flower strips was 2,4 times higher than in control fields in May and 1,3 times higher in June in 2025 (Fig. 1).

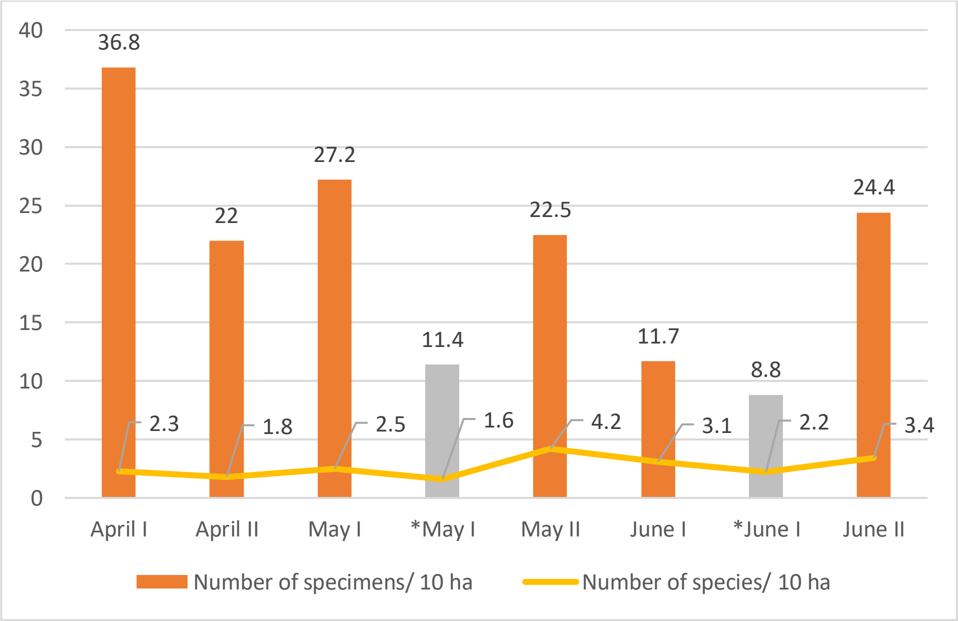

Figure 1. Temporal variation in bird density (individuals per 10 ha) and species richness in farmland during the April–June period, 2025: orange boxes indicate fields with flowering strips; grey indicates control field.

The five-year results indicate that flowering strips contribute to higher bird species richness. Bird density tended to be higher in fields with flower strips compared to control fields; however, the differences were not statistically significant (May I: t = 2,19, p = 0,094; June I: t = 1,78, p = 0,149). In contrast, species richness was significantly higher in flower strip fields in both May (t = 3, p = 0,04) and June (t = 8,28, p = 0,001). These findings suggest that flower strips enhance bird species diversity, although their effect on overall density remains inconclusive over the five-year period.

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F19

MORPHOLOGICAL AND MOLECULAR ANALYSIS OF *PORROCAECUM* SPP. IN INTESTINAL SAMPLES FROM COMMON STARLINGS (*STURNUS VULGARIS*) IN LITHUANIA

Rugilė Pociūtė[1], Evelina Juozaitytė-Ngugu[2], Petras Prakas[2]

[1]Life Sciences Center, Vilnius University, Lithuania
[2]State Scientific Research Institute Nature Research Centre, Lithuania
rugile.pociute@gmc.stud.vu.lt

Members of the genus *Porrocaecum* (Rhabditida, Toxocaridae) are zooparasitic nematodes, which mainly parasitize various birds, occasionally occurring in mammals. Around 40 nominal species of *Porrocaecum* have been reported worldwide [1]. The lifecycle and genetic relationships of *Porrocaecum* species are poorly understood. The parasite exhibits a heteroxenous, two-host prey–predator life cycle, involving an earthworm as the intermediate host and a bird as the definitive intestinal host. The morphological identification is based on the analysis of the specific features as relatively large lips and male reproductive structures.

In this study, ten intestinal samples from common starlings (*Sturnus vulgaris*) collected in Lithuania in 2025 were examined. The genus of *Porrocaecum* was morphologically identified based on key diagnostic features of nematodes by Levin[2] using a stereomicroscope and a light microscope. PCR amplification and sequencing of the of the small ribosomal (*18S* rRNR) subunit were used for *Porrocaecum* spp. identification.

Based on morphological analysis, the prevalence of *Porrocaecum* spp. infection in intestinal samples of common starlings was 60% (6/10). The adult *Porrocaecum* helminths were up to 6 cm in length and had relatively large lips, the dorsal lip has a pair of large, double subdorsal papillae. Between these lips there are well-defined interlabia, appearing as a pointed triangular protrusion of the interlabial cuticle. In males, several pairs of pre-anal and post-anal papillae are present on the ventral surface of the posterior extremity. Females exhibit only one pair of post-anal papillae near the tip of the tail. The spicules in males were almost equal in length [2]. Using the 18S rRNR gene, the genus Porrocaecum was identified in intestinal samples of common starlings for the first time in Lithuania. A total of four identical Porrocaecum spp. sequences were characterized, showing 99.9 % identity with Porrocaecum reticulatum. Previously, Porrocaecum ensicaudatum was reported in intestinal samples of common starlings; however, sequencing of the internal transcribed spacer 1 region is required for accurate species identification. This is the first study to report the prevalence of Porrocaecum spp. in the intestinal samples of common starlings in Lithuania.

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F20

OCCUPANCY STUDIES OF ARTIFICIAL NEST SITES OF THE BLACK STORK (*CICONIA NIGRA*) IN WESTERN LITHUANIAN FORESTS

Greta Nemirova[1], Saulis Skuja[1]
[1]Department of Zoology, Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
greta.nemirova@gmc.stud.vu.lt

The Black Stork (*Ciconia nigra* Linnaeus, 1758) belongs to the order *Ciconiiformes* and the family *Ciconiidae*. The population of Black Storks in Lithuania has been declining since 1976 and according to data from 2017, the population does not exceed 480–720 breeding pairs. One of the conservation measures used to counteract this decline is the installation of artificial nests. This study investigated artificial nests for Black Storks in Western Lithuania with the aim of evaluating their occupancy and effectiveness for breeding. [1][2]

The fieldwork was conducted in Western Lithuania from July 11 to August 10, 2025. During this period, 33 artificial nests were inspected (Fig. 1).

The research was based on previously collected nest data and coordinates obtained from the SRIS (Protected Species Information System) database, which were used for comparison with earlier monitoring records of the same nests. The nest sites were studied using the "Maps.lt" website and the mobile application "Geo Tracker." All nests were inspected in accordance with ethical research principles: loud talking, touching nests, or intentionally disturbing birds was strictly prohibited.[3]

The following equipment was used for nest inspection: 4×10 binoculars, a Sony Alpha 6000 digital camera, an AIR 3S unmanned aerial vehicle (drone), a mobile phone with GPS (Geo Tracker application), Microsoft Excel for data organization, and RStudio for statistical analysis. Nests were evaluated according to 6 specific criteria, including occupancy, nest condition, tree condition, habitat condition, recorded nearby anthropogenic activity and, if possible, number of offspring.

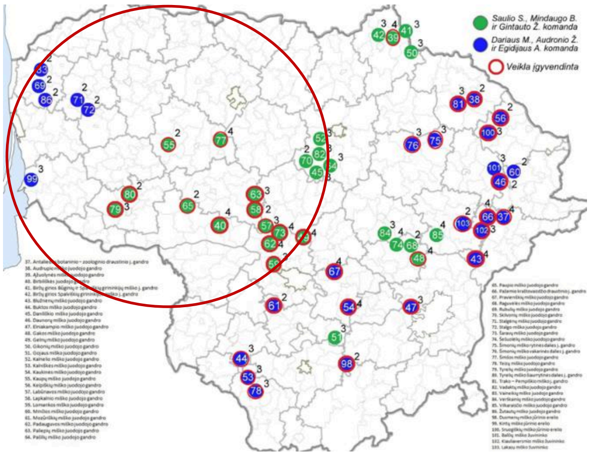

Figure 1. Location of verified artificial nesting sites for Black Storks in Western Lithuania. [4]

The number of occupied nests fluctuated between 2018 and 2025: 6 in 2018 (out of 32), 1 in 2019, and 4 in 2025 (out of 33), and all breeding attempts were successful. Two nests are no longer suitable due to tree falls, and five (21%) require reconstruction. Of the four occupied nests recorded in 2025, the number of nestlings could be determined in only one nest, which contained one chick. Three of the four occupied nests were in oak trees that were in good condition. The surrounding habitat was generally suitable for breeding, although forestry activity was recorded near 18% of nests.

In conclusion, artificial nesting sites have been found to be attractive to these birds, but their use depends on various circumstances. It was estimated that the condition of the artificial nesting sites studied had deteriorated critically over the past seven years: 6% of the nests needed to be restored, 21% needed to be reinforced.

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CHANGES IN THE SPRING MIGRATION PHENOLOGY OF LONG-DISTANCE MIGRANT WILLOW WARBLER BASED ON VENTĖ CAPE RINGING DATA FROM 1983–2024

Augustinas Baronas[1], Gintaras Malmiga[2]

[1]Life Sciences Centre, Vilnius University, Lithuania
[2]Laboratory of Avian Ecology, Nature Research Centre Lithuania
augustinas.baronas@gmc.stud.vu.lt

The timing of spring migration in birds is strongly dependent on climate and its variability. Long-term studies have shown that spring arrival times of long-distance migrant passerines have shifted earlier in recent decades [1]. Those changes are associated with climate change and rising global temperatures. In this study we examine if arrival date timing of spring migration is related to April-May mean temperatures and winter (January-March) NAO (North Atlantic Oscillation) index for a long-distance migrant willow warbler (*Phylloscopus trochilus*) over a 37-year period (1983–2020).

The ringing data was collected in Ventė cape using stationary traps (lower trap (rybachy type), small trap and 4 different zigzag traps) and mist-nets. The data was provided by Kaunas Tadas Ivanauskas Zoological Museum ringing centre. Because the data collection standardization changed throughout the ringing years in the station, we filtered the dataset to avoid data errors. Furthermore, we analysed which climate parameters would be suitable for our study and processed the climate data. We then used GAM (Generalized Additive Model) model (day of the year ~ temperature + NAO) to evaluate the phenological shifts of spring migration.

Mean number of ringed willow warblers per study period over 37-years was 244 ± 134, range 82-712 individuals (fig. 1). Willow warblers advanced their spring migration by 0.147 days a year (t = -2.94, p =0.006, n = 35) or 5.4 days over 37 years. Overall, the model explained 61.7% of the total deviance, with April-May mean temperature having strong influence on arrival (edf = 1, F = 14.834, p = 0.0006), while NAO had smaller, non-linear effect on spring migration timing (edf = 2.789, F = 3.58, p = 0.02).

These results show that a long-distance migrant – willow warbler has advanced their spring migration in relation to warming temperatures. Furthermore, though smaller, NAO had significant non-linear effect on the timing on willow warbler spring migration.

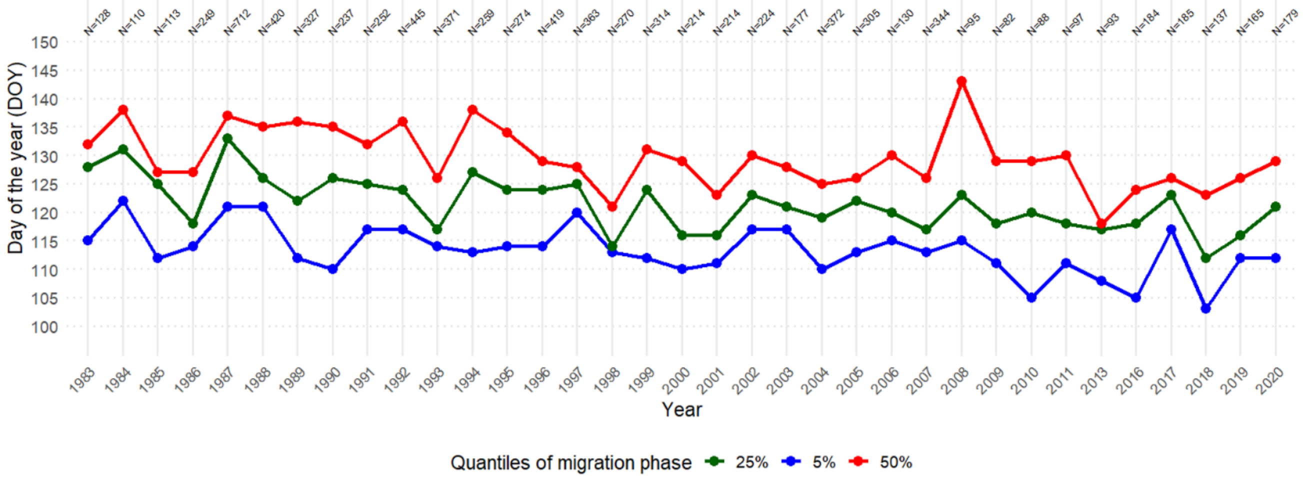

Figure 1. *Phylloscopus trochilus* arrival date graph with different migration phases (5% of all bird arrivals from that year season, 25% and 50%). The upper part shows sample size for the year.

Acknowledgments: We would like to thank Kaunas Tadas Ivanauskas Museum for providing us the ringing data.

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THE IMPACT OF HERBICIDES WITH DIFFERENT MODES OF ACTION ON THE GROWTH AND PHYSIOLOGICAL STRESS RESPONSES OF WINTER WHEAT

Šaltytė, G.[1], Jankauskienė, J.[1], Mockevičiūtė, R.[1], Todorova, D.[2]

[1]Laboratory of Plant Physiology, State Scientific Research Institute Nature Research Centre, Akademijos Str. 2, 08412 Vilnius, Lithuania
[2]Institute of Plant Physiology and Genetics, Bulgarian Academy of Sciences, Acad. G. Bonchev Str., Bl. 21, 1113, Sofia, Bulgaria
greta.saltyte@gamtc.lt

Climate change poses a serious threat to agriculture, increases plant stress, and changes the effectiveness of plant protection products, intensifying abiotic stressors such as temperature fluctuations and water regime instability. This trend increases plant stress and reduces their productivity [1]. 2024 marked the first year in which the global temperature exceeded +1.5 °C above pre-industrial levels, with 11 consecutive months surpassing this threshold [2]. These climatic changes heighten the risk of extreme weather events, including hurricanes, unprecedented high or low rainfall, droughts, and extreme cold periods. Such variability directly impacts plant health, productivity, and resistance to stress, posing considerable challenges to global food security. In particular, staple crops such as winter wheat (Triticum aestivum L.) are vulnerable to these climatic shifts, facing persistent threats from weeds, pathogens, pests, and intensified abiotic stresses [3].

The aim of this study was to compare the effects of two herbicides with different modes of action on the growth and physiological parameters of winter wheat under real field conditions. The study was conducted on farmers' fields in northern Lithuania under natural agronomic conditions. Two widely used herbicides representing different classes of herbicides were selected: one of them acts by inhibiting essential plant growth processes through disruption of hormone regulation, the other by disrupting amino acid synthesis, which leads to faster growth inhibition and metabolic imbalance in weeds. Growth and biomass parameters, including plant height, green biomass, and dry biomass, were evaluated. Additionally, key physiological and biochemical indicators of stress were quantified. The obtained results enabled a comparative assessment of the intensity of herbicide-induced oxidative stress and its impact on plant growth and physiological status.

The differences identified between herbicides allow us to identify the mechanisms of action that influence the physiology of crops under field conditions. These data form the basis for further research aimed at assessing the impact of climate change on herbicide efficacy and plant physiological responses.

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ASSESSMENT OF MOLLUSC DIVERSITY IN SELECTED WETLANDS OF KURTUVĖNAI REGIONAL PARK

Guoda Petraitytė[1], Benediktas Jukonis[1], Grita Skujienė[1]

[1]Department of Zoology, Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
Petraityte.guoda@gmc.stud.vu.lt

Wetlands globally have declined three times faster than forests, making their conservation critically important for biodiversity, water regulation, carbon storage, and other ecosystem services [1]. Molluscs, due to their sensitivity to environmental changes and specific habitat requirements, are widely used as bioindicators in wetland ecosystems, as distinct mollusc communities—including rare and sensitive taxa such as members of the family Vertiginidae—form in response to habitat quality and overall species diversity [2]. This study aimed to determine the structure and species composition of mollusc communities in ten wetlands of Kurtuvėnai Regional Park, Lithuania.

Sampling was conducted between 2019 and 2024 during the peak vegetation period (July–August). A total of 23 samples were collected from ten wetlands (Lygės, Gudmoniškių, Juodlės, Niaukos, Stikliuko, Maksvytės, Pageluvio, Pakėvės, Smirdėlės and Bevardės) representing three habitat types: 7230 (Calcareous fens), 7140 (Transition mires and quaking bogs), and mixed habitats (7230/7140). Samples were air-dried and sieved, and molluscs were manually separated from moss and other organic debris. Species identification was carried out by Dr. G. Skujienė based on shell morphology of adult individuals, following standard malacological keys (Kerney et al., 1983; Pokryszko, 1990).

A total of 29 mollusc species were recorded, including five freshwater species. The highest abundances were observed for *Euconulus alderi* (Davies, 1979), *Vallonia pulchella* (O. F. Müller, 1774), and *Vertigo antivertigo* (Draparnaud, 1801). Species richness (S) ranged from 3 to 16 species per sample, whereas total abundance (N) varied between 121 and 2537 individuals. Shannon diversity index (H′) values ranged from 0.62 to 2.30, indicating moderate to high diversity and relatively even species distribution across most sampling sites. Shannon diversity index (H′) did not differ significantly among habitat types (Kruskal–Wallis test, $p > 0.05$), although transition mires (7140) exhibited lower mean diversity compared to calcareous fens (7230) and mixed habitats (Fig. 1).

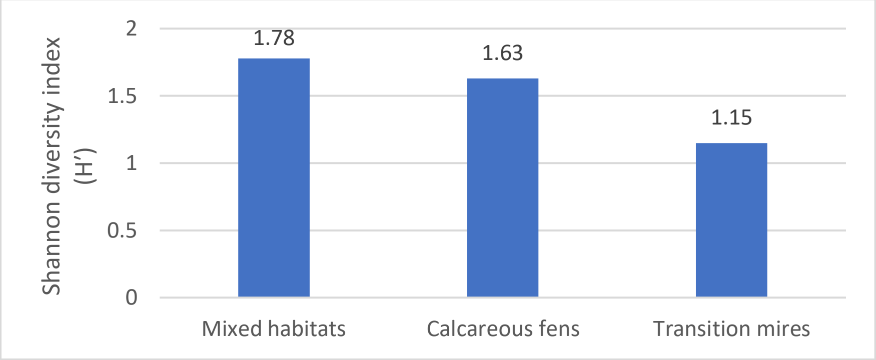

Figure 1. Mean Shannon diversity index (H′) of mollusc communities across different habitat types in Kurtuvėnai Regional Park wetlands.

Two European Community (EC) species were recorded: *Vertigo angustior* (Jeffreys, 1830), detected in a single sample with one adult individual, and *Vertigo geyeri* (Lindholm, 1925), found in 17 samples. Detection of *V. geyeri* varied among habitats, with the highest frequency in calcareous fens (7230; 88.9%) and the lowest in transition mires (7140; 50%), while only mixed habitats hosted both EC species.

The results indicate that calcareous and mixed wetlands support higher mollusc diversity and provide favourable conditions for EC species. Notably, *V. geyeri* showed a clear preference for calcareous fens, where it was most abundant, underscoring the conservation importance of this habitat type. This study provides updated data on mollusc community structure and EC species distribution in Lithuanian Natura 2000 wetlands and highlights the ecological significance of habitat heterogeneity for biodiversity conservation within protected areas.

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HABITATS AND FRUCTIFICATION OF ENDANGERED FUNGUS *GYROMITRA SPHAEROSPORA* IN LITHUANIA

Sam Saddler[1], Ernestas Kutorga[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
sam.saddler@gmc.stud.vu.lt

Xylosaprotrophic fungi play a fundamental role in forest ecosystems by ensuring the decomposition of organic matter and nutrient cycling. Some species, such as *Gyromitra sphaerospora*, are threatened with extinction due to the loss of suitable habitats and removal of substrates, especially – coarse woody debris. The world population of *G. sphaerospora* is relatively small and globally declining. The fungus was assessed in the International Union for Conservation of Nature (IUCN) Global Fungal Red List as Near Threatened (NT) [1] and is nationally red-listed in 9 European countries [2; 3; 4]. In Lithuania, this fungus was first discovered in 2003, it is considered to be very rare, and is listed in the Lithuanian Red Data Book under the Endangered (EN) category [4]. Currently, only three localities of this fungus are known in Lithuania.

The aim of this research was to investigate the habitats, substrates and fructification of *G. sphaerospora* in all three fungal localities in Lithuania, namely, Punia Pinewood Strict Nature Reserve in Nemunas Loops Regional Park, Prienai Pinewood in Drubengis Botanical Reserve in Nemunas Loops Regional Park, and Dusetos Wood in Vosyna Strict Nature Reserve in Sartai Regional Park. The field studies were conducted in 2024-2025. We counted the fruiting bodies of *G. sphaerospora*, identified the substrate type, volume, wood decay stage of coarse *Picea abies* woody debris, and habitat type. For the detailed analysis of habitat disturbance intensity we established 6 permanent sampling plots (circular, 100 m^2 in size) – three plots in Dusetos Wood, and three plots in Punia Pinewood.

The results showed that *G. sphaerospora* grows in three habitat types listed in EU Habitats Directive Annex I, namely, 9010* Western Taiga (Dusetos Wood), 9020* Fennoscandian hemiboreal natural old broad-leaved deciduous forests (*Quercus*, *Tilia*, *Acer*, *Fraxinus* or *Ulmus*) rich in epiphytes (Punia Pinewood and Dusetos Wood), and 9050 Fennoscandian herb-rich forests with *Picea abies* (Prienai Pinewood). The main substrate of this fungal species is *P. abies* logs and stumps in the 3rd stage of wood decay, with an average volume of 1.17 m^3. A new substrate type for this fungus in Lithuania was recorded in Dusetos Wood – *Tilia cordata* log of the 3rd stage of decay.

The fructification of *G. sphaerospora* varied between localities and study years: in Dusetos Wood we observed 166 fruiting bodies in 2024, and 46 – in 2025, while in Punia Pinewood only 4 fruiting bodies were observed in 2024, and 9 – in 2025. In Prienai Pinewood only single fruiting body was observed in both 2024 and 2025.

Only natural disturbances were observed in *G. sphaerospora* habitats, mainly windthrow, *P. abies* canopy dieback and damage by *Ips typographus*. All fungal localities are situated in the protected territories, two of them are in strictly controlled protected areas. However, likely the indirect anthropogenic threats could degrade habitat conditions or induce habitat loss in the future, such as climate change intensified tree windthrow and *P. abies* deadly damage with pest infestations. Although the habitats of *G. sphaerospora* are legally protected, the long-term prognosis for the conservation of this species in Lithuania remains unfavourable due to population fragmentation and natural disturbances of habitats.

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URBAN MEADOWS IN VILNIUS: VEGETATION COMPOSITION AND ECOLOGICAL CONDITION

Martynas Balsys[1], Kęstutis Katilius[1]

[1]Laboratory of Flora and Geobotany, Nature Research Centre, Žaliųjų Ežerų Str. 47, LT-12200 Vilnius, Lithuania
martynas.balsys@gmc.stud.vu.lt

Urban meadows represent important components of green infrastructure and contribute to plant diversity in highly urbanized environments. Despite ongoing urban development, some meadow habitats in Vilnius had persisted as open areas since 1995, providing an opportunity to assess their vegetation structure and ecological condition.

The aim of this study was to evaluate the vegetation composition and ecological condition of long-term urban meadows in Vilnius. Potential sites were identified using orthophotographic maps from 1995–2025, resulting in 35 candidate locations. Field surveys were conducted in 24 sites that met the selection criteria. Vegetation was recorded in 16 m² (4 × 4 m) plots, and species cover was estimated using the Londo decimal scale [1].

A total of 141 vascular plant species belonging to 25 families were recorded. Species diversity was assessed using the Shannon diversity index, and the proportion of alien and invasive species was evaluated as an indicator of anthropogenic pressure. To explore variation in species composition among sites, non-metric multidimensional scaling (NMDS) ordination was applied. Ecological conditions were further interpreted using Ellenberg Indicator Values [2], allowing indirect assessment of environmental gradients based on species composition.

Preliminary analyses suggested variability in species diversity and community structure among sites. Although native species dominated most plots, the presence of alien and invasive taxa indicated ongoing urban ecological influence.

These findings highlighted the ecological relevance of persistent urban meadows in Vilnius and provided a foundation for future monitoring and management of urban green spaces.

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ANALYSIS OF AMPHIBIAN ACTIVITY AND PHENOLOGICAL PATTERNS IN LITHUANIA

Kristijona Kleinaitytė[1], Vita Žvynakytė[1], Andrius Pašukonis[1]

[1]Life Science Center, Vilnius University, Lithuania
kristijona.kleinaityte@gmc.stud.vu.lt

All 13 amphibian species found in Lithuania play an extremely important role as environmental indicators in terrestrial and aquatic ecosystems, reflecting habitat quality and prevailing changes . Due to their permeable skin, their activity and phenological phenomena (such as migration to spawning grounds, reproduction, hibernation, and others) are highly dependent on environmental temperature and weather conditions . Therefore, to accurately assess the impact of these factors and understand phenological changes, long-term systematic observations and recordings of amphibians are necessary. The aim of this study is to collect and systematize data that allow assessment of how temperatures influence the distribution, activity, and phenological characteristics of amphibians in Lithuanian territories, based on the analysis of long-term historical and monitoring data.

Long-term and historical data on spring observations of Lithuanian amphibians were collected from various sources and integrated into a common database. In addition, amphibians were monitored from the beginning to the end of the breeding season in 2025 at three locations in Vilnius, each with different habitats. This data was used to compare activity and phenological characteristics across sites.

The database results showed that most observations were recorded for protected species, with limited long-term data available for more common Lithuanian species. Spring monitoring data from Vilnius revealed significant differences in the occurrence and phenology patterns of amphibians depending on different sites with species diversity and emergence dates primarily influenced by temperature and habitat type. These findings highlight the need for expanded long-term monitoring of common species and integrated climate modeling to predict future phenological shifts under ongoing climate change.

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F27

HALOTOLERANCE OF PHYTOPLANKTON AND INVASION SUCCESS OF NOSTOCALEAN CYANOBACTERIA UNDER FRESHWATER SALINIZATION

Gabrielė Šiurkutė[1,2], Izabelė Šuikaitė[1]

[1]Laboratory of Algology and Microbial Ecology, State Scientific Research Institute Nature Research Centre, Lithuania
[2]Life Sciences Center, Vilnius University, Lithuania

gabriele.siurkute@gmc.stud.vu.lt

Increasing freshwater salinization driven by human activities can disrupt local phytoplankton communities, altering their structure and potentially favoring newly arrived halotolerant species. Such responses depend on the initial community composition and species-specific tolerance to osmotic stress, which may suppress salt-intolerant taxa and shift dominance towards halotolerant groups. These structural changes can facilitate the establishment of alien halotolerant cyanobacteria. *Chrysosporum bergii* and *Sphaerospermopsis aphanizomenoides* are halotolerant cyanobacteria of tropical-subtropical origin that have spread to European waters. The aim of this study was to assess the impact of increasing salinity on native phytoplankton communities and the establishment of alien cyanobacteria in freshwater ecosystems.

Two microcosm experiments were conducted using two alien Nostocalean cyanobacteria, *Chrysosporum bergii* and *Sphaerospermopsis aphanizomenoides*, and phytoplankton communities collected from two mesotrophic artificial reservoirs. The experiments included two groups: a native phytoplankton community (control) and a native community inoculated with the alien species at the beginning of the experiment. Four NaCl concentrations (0, 0.2, 1 and 5 g/L NaCl) were adjusted during 3 days of acclimatization, followed by 10 days of incubation. Samples from each microcosm were fixed with Lugol's solution. Phytoplankton species were identified using a light microscope, and abundance and biomass were estimated using a Nageotte counting chamber.

Local freshwater phytoplankton communities consisted of organisms from five taxonomic groups (Myzozoa, Bacillariophytina, Chlorophytina, Ochrophytina and Cyanobacteria). Increasing salinity from 0 to 5 g/L NaCl affected the biomass of phytoplankton communities differently: in the first experiment it varied with statistically significant differences observed ($p < 0.05$), while in the second experiment, no significant effect was detected ($p > 0.05$). Salt-sensitive algae from the Ochrophytina group disappeared, and halotolerant Chlorophytina genera dominated. The relative biomass of alien cyanobacteria increased with rising salinity, indicating successful establishment of these species in the communities. *C. bergii* biomass remained stable across all NaCl concentrations, whereas *S. aphanizomenoides* biomass increased with rising NaCl concentrations, reaching the highest biomass at 5 g/L NaCl. In conclusion, these results suggest that freshwater salinization can both restructure native phytoplankton and promote the establishment of alien halotolerant cyanobacteria, which may affect ecosystem integrity and functioning.

UNVEILING *SARCOCYSTIS* INFECTIONS IN LITHUANIAN HORSES: FROM MIROSCOPY TO MOLECULAR DIAGNOSTICS

Naglis Gudiškis[1], Mingailė Stanionytė[1,2], Petras Prakas[1], Eglė Rudaitytė-Lukošienė[1], Dalius Butkauskas[1], Donatas Šneideris[1]

[1]Laboratory of Molecular Ecology, State Scientific Research Institute Nature Research Centre, Akademijos 2, Vilnius, Lithuania
[2]Life Sciences Center, Vilnius University, Saulėtekio av. 7, Vilnius, Lithuania
naglis.gudiskis@gamtc.lt

Sarcocystis are protozoan parasites with a two-host life cycle, forming sarcocysts in the muscles of the intermediate hosts, such as livestock, while definitive hosts (predators or omnivores) become infected by consuming meat with those mature sarcocysts [1]. In humans, farm and wild animals, infection can cause muscular, neurological, and gastrointestinal disorders, depending on the *Sarcocystis* species and host [2]. Despite the economic and health relevance of these parasites, data on prevalence and species richness in equids are scarce, due to limited molecular studies [3]. To date, five *Sarcocystis* species have been described in equids: *Sarcocystis asinus*, *Sarcocystis bertrami*, *Sarcocystis equicanis*, *Sarcocystis fayeri*, and *Sarcocystis neurona* [1]. Nevertheless, taxonomic uncertainties persist, particularly regarding *S. bertrami* and *S. fayeri*, which may represent distinct or conspecific species. Microscopy alone is insufficient for species identification in horses due to long sarcocyst maturation periods, highlighting the need for molecular studies [4]. Therefore, the aim of this study was to investigate the species diversity of *Sarcocystis* in different muscle tissues of domesticated horses (*Equus ferus caballus*) and to assess the intensity and prevalence of these parasites.

During 2023–2024, a total of 107 muscle samples were collected from domesticated horses slaughtered at abattoirs in Lithuania. Three types of muscles were sampled: neck muscles (n = 59), cheek muscles (n = 23), and masticatory muscles (n = 25). Microscopical analysis was performed using methylene-blue-stained and unstained muscle squash technique, followed by sarcocyst isolation and DNA extraction. Molecular analysis was conducted through PCR amplification of *cox1*, *18S* rRNA, and *28S* rRNA genes, followed by sequencing and bioinformatic analysis.

Microscopical analysis detected *Sarcocystis* spp. in 23.4% of the examined horse muscle samples, with parasite infection intensity ranging from 1 to 12 sarcocysts per gram of tissue ($\bar{x}$ = 3). Observed cysts were elongated, spindle-shaped, and septated, with lengths of 400–6000 µm and widths of 30–225 µm, containing banana-shaped bradyzoites measuring 10–16 × 2–4 µm. Molecular analyses confirmed all isolates as *S. bertrami*.

These results provide the first molecular characterization of *S. bertrami* in Lithuanian horses and highlight the importance of combining molecular and microscopic methods to resolve taxonomic ambiguities and accurately assess the diversity and distribution of *Sarcocystis* in equids, with implications for animal health and food safety.

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LINKING EARLY-LIFE PERSISTENT ORGANIC POLLUTANTS EXPOSURE TO ENDOCRINE AND IMMUNE SYSTEMS IN BALTIC GREY SEAL (*HALICHOERUS GRYPUS*) PUPS

Kamilė Bužinskytė[1], Caglar Akay[2], Annika Jahnke[2], Simonas Balčiūnas[3], Bjarne Styrishave[4], Tomasz Maciej Ciesielski[5], Mart Jüssi[6], Vaida Survilienė[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[2]Helmholtz-Centre for Environmental Research – UFZ, Department of Exposure Science, Leipzig, Germany
[3]Institute of Chemistry, Vilnius University, Vilnius, Lithuania
[4]Department of Pharmacy, University of Copenhagen, Copenhagen, Denmark
[5]Norwegian University of Science and Technology, Faculty of Natural Sciences, Department of Biology, Trondheim, Norway
[6]PRO MARE MTÜ, Vintriku, Estonia
kamile.buzinskyte@gmc.stud.vu.lt

The Baltic Sea is heavily polluted with persistent organic pollutants (POPs), which bioaccumulate in marine mammal blubber and transfer to offspring during gestation and lactation, increasing early-life exposure [1]. This study investigated concentrations of POPs in relation to endocrine, hematology parameters and morphometric traits in free-ranging (Innarahu, Estonia, 2024) grey seal pups (n=20; 10 females, 10 males).

Blubber POPs concentrations were quantified using gas chromatography–tandem mass spectrometry. Detected groups included: polychlorinated biphenyls (PCBs), organochlorine pesticides (OCPs), polybrominated diphenyl ethers (PBDEs), polycyclic aromatic hydrocarbons (PAHs), triphenyl phosphate (TPP), benzophenone (BPN), benzyl benzoate (BB), diphenyl sulfone (DPS), homosalate (HOM), and bis(4-chlorophenyl)sulfone (BCS). Liquid chromatography-tandem mass spectrometry was used to successfully quantify steroid hormones: cortisol, cortisone, corticosterone, androstenedione, 11-deoxycortisol, 11-deoxycorticosterone, progesterone, pregnenolone, 17-OH-pregnenolone, 17-OH-progesterone, dihydroprogesterone. In addition, hematological parameters (RBC, Hgb, WBC [MONO, LYM, GRA], PLT) were measured using automatic veterinarian hematology analyser.

Generalized linear mixed model (GLMM) was used to assess associations between POPs concentrations and morphometric parameters, sex, and developmental stage (suckling vs. weaning). POPs accumulation was sex-dependent, with males exhibiting significantly higher ΣPCBs and DPS levels. HOM and TPP had a positive association with pup body mass and axillary girth.

Bivariate Spearman analysis revealed significant associations between selected POPs, steroid hormones, and hematological parameters. ΣPAHs and BPN were positively correlated ($p<0.05$) with progesterone, pregnenolone, and 11-deoxycortisol, suggesting potential disruption of steroidogenesis through altered steroid precursor dynamics. In contrast, HOM was negatively correlated with pregnenolone, indicating a possible inhibitory effect on steroid hormone synthesis. Monocyte concentrations were negatively associated with HOM and BB, pointing to potential immunotoxic effects of POPs exposure. Overall, these findings suggest that early-life POPs exposure may disrupt steroid precursor dynamics, steroidogenesis, and immune function in developing marine apex predators, and future studies will further investigate these effects.

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F30

MICROSCOPIC AND MOLECULAR DETECTION OF SARCOCYSTIS IN CATTLE (Bos taurus) RAISED IN LITHUANIA

Mingailė Stanionytė[1], Petras Prakas[2], Dalius Butkauskas[2], Donatas Šneideris[2], Naglis Gudiškis[2]

[1] Life Sciences Center, Vilnius University
[2] State Scientific Research Institute Nature Research Centre
mingaile.stanionyte@gmc.stud.vu.lt

Protozoan parasites of the *Sarcocystis* genus are commonly detected in bovine tissues. Their biological diversity and epidemiological significance have caused them to remain underexplored for a long time [1]. In recent years, an increasing number of studies have reported novel *Sarcocystis* species in cattle, some of which exhibit zoonotic potential and pose risks to food safety and public health [2]. Accurate species identification is complicated by pronounced morphological similarities among *Sarcocystis* species, limiting the reliability of microscopic methods [3]. The aim of this study was to assess the prevalence and species of *Sarcocystis* parasites in the cardiac muscle and diaphragm of cattle raised in Lithuania.

A total of 56 cattle tissue samples, including 28 heart and 28 diaphragm samples, were examined, and parasite abundance was assessed using a methylene blue–stained muscle squash compression microscopy method. Species identification was performed following artificial digestion with pepsin, gDNA extraction, and nested PCR. Mitochondrial *cox1* gene fragments were targeted for species discrimination, and nPCR products were analysed using electrophoresis, sequencing, and bioinformatic analysis.

Microscopical analysis revealed a lower prevalence of *Sarcocystis* parasites in cardiac muscle compared to the diaphragm, and also lower than detected by molecular methods, as only low to moderate parasite loads (0–20 sarcocysts/g) were observed. Using internal species-specific primer pairs, two species, *Sarcocystis cruzi* and *Sarcocystis bovifelis*, were identified in cattle tissues from Lithuania.

These findings indicate that *Sarcocystis* infections are common in cattle cardiac muscle, but species diversity may be underestimated when relying solely on conventional diagnostics. Integration of molecular techniques is essential for accurate identification of zoonotic *Sarcocystis* species and for improving food safety and public health risk assessments.

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Study of *Sarcocystis glareoli* parasite in brains of small mammals from Lithuania

Dovilė Laisvūnė Bagdonaitė[1], Marius Jasulionis[2], Evelina Juozaitytė-Ngugu[1], Vitalijus Stirkė[2], Linas Balčiauskas[2], Dalius Butkauskas[1], Petras Prakas[1]
[1]Laboratory of Molecular Ecology, State Scientific Research Institute Nature Research Centre, Lithuania
[2]Laboratory of Mammalian Ecology, State Scientific Research Institute Nature Research Centre, Lithuania
dovile.bagdonaite@gamtc.lt

Members of the genus *Sarcocystis* are widespread Apicomplexan parasites that can infect mammals, birds and reptiles. These parasites utilize two hosts to complete their lifecycle: the intermediate host gets infected from contaminated food or water, while definitive hosts become infected after consumption of tissues with fully developed sarcocysts. Depending on the species, *Sarcocystis* parasites can form sarcocysts either in muscle tissues or in the CNS of the intermediate host.

To date, more than 40 *Sarcocystis* sp. are known to use small mammals as intermediate hosts, along with a substantial number of novel *Sarcocystis* spp. that have yet to be discovered. In recent years, new species have been described using both morphological and molecular methods; however, species characterised before the widespread use of molecular markers remain largely unexamined with these tools. As such, the lack of data hinders some research, and the need to fill this knowledge gap is immense.

The aim of the study was to assess *Sarcocystis* prevalence in brains of small mammals (mice, voles, and shrews) from Lithuania and to comprehensively characterize their genetic variability using nuclear, mitochondrial, and apicoplast genetic markers. In total, 2.2 % (15/687) of analysed samples were positive for *Sarcocystis glareoli*. This parasite was observed in three rodent species – bank vole, common vole, and yellow-necked mouse, as well as a single eulipotyphlan species – common shrew. This is the first documented case of *S. glareoli* infecting an eulipotyphlan host. Moreover, the *ITS2*, *cox1*, *cytB,* and *rpoB* sequences of *S. glareoli* have been reported for the first time.

In conclusion, results obtained contribute to epidemiological research, improve discrimination of *S. glareoli* from closely related species, and are significant for the phylogenetic studies of *Sarcocystis* spp. that use small mammals as intermediate hosts and birds as definitive hosts.

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A REVIEW OF PHILIPPINE CAMPSOSTERNUS (COLEOPTERA: ELATERIDAE) WITH DESCRIPTIONS OF NEW TAXA

Artūrs Vanags[1]

[1]Institute of Life Sciences and Technology, Daugavpils University, Latvia
arturs.vanags@du.lv

The Oriental click beetle genus *Campsosternus* Latreille, 1834 (Coleoptera: Elateridae) comprises approximately 80 described species distributed primarily throughout the Oriental Region, with a single representative extending into New Guinea. Despite the high diversity of the genus in Southeast Asia, the Philippine fauna has remained poorly understood and taxonomically unstable. Historically, only three nominal species were recorded from the archipelago, but their status has long been unclear due to vague original descriptions and an excessive emphasis on variable external coloration.

This study presents a revision of the Philippine species of *Campsosternus* based on material from the Daugavpils University Beetle Collection (DUBC) and examination of type specimens in the Oxford University Museum of Natural History (OUMNH). The study is based on comparative morphological analysis of external characters and male genitalia, including direct examination of type specimens.

As a result of this revision, one new species and two new subspecies are described. *Campsosternus mindorensis* sp. nov., from Mindoro Island, is characterized by a more slender body and the presence of fine grey pubescence on both dorsal and ventral thoracic surfaces – a feature absent in other Philippine taxa. Within *Campsosternus rutilans*, two geographically structured subspecies are recognized: *C. rutilans mindanaoensis* ssp. nov. from Mindanao, distinguished by its matte dorsum and coarse, dense pronotal punctation, and *C. rutilans cupreus* ssp. nov. from Panay and Negros, characterized by coppery to reddish-bronze coloration and finer punctation. Although coloration varies considerably, consistent differences in punctation, surface sculpture, and genital morphology support recognition of these taxa as geographically differentiated lineages.

Male genitalia were examined and illustrated for all Philippine taxa. In contrast to previous studies on Taiwanese species [1], where these characters were considered of limited diagnostic value, the aedeagus was found to provide reliable species-level characters in the Philippine fauna. However, overlapping variation among subspecies supports their interpretation as geographically differentiated populations rather than distinct species.

Two new synonymies are proposed following direct examination of type material: *C. proteus* Hope, 1843 is synonymized with *C. rutilans* (Chevrolat, 1841), and *C. eschscholtzii* Hope, 1843 is placed in synonymy with *C. leachii* Hope, 1843. These conclusions resolve long-standing nomenclatural confusion originating from mixed type series and color-based diagnoses in early descriptions.

The results clarify species limits within Philippine *Campsosternus* and document geographically structured morphological variation across the archipelago. A diagnostic key to all Philippine species and subspecies is included in the present study.

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PREVALENCE OF DIGENEAN CERCARIAE (TREMATODA: DIGENEA) IN FRESHWATER MOLLUSCS

Eivinas Zableckas[1], Rasa Aukštikalnienė[1]
[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
eivinas.zableckas@gmc.stud.vu.lt

Studies of digenean trematode (Digenea) cercariae, the motile larval stages developing in molluscs as intermediate hosts, are essential for understanding parasite diversity, life cycles, and distribution. Trematodes are of medical and veterinary importance, causing severe diseases (trematodiases) in humans and animals. These infections are also linked to economic losses in livestock production, including reduced productivity and increased mortality [1]. As investigations of cercarial infections in freshwater molluscs remain limited in our region compared to Central Europe, further research is warranted [2].

The aim of this study was to evaluate the prevalence of digenean trematode cercariae in freshwater molluscs from the Babtai (Kaunas District, Lithuania) and to assess the morphological diversity of the detected cercariae. Freshwater molluscs were collected from three different water bodies and transported to the laboratory. Cercarial emergence was subsequently examined, and the released larvae were observed and documented using light microscopy [Fig. 1].

Figure 1. Cercariae released from freshwater mollusks.

Of the 582 freshwater mollusks collected, 187 individuals (32.1%) were infected with digenean cercariae. The prevalence of infection varied depending on the season, peaking in August, and was higher in stagnant water bodies, indicating favorable conditions for transmission. Infections were mainly detected in the mollusk species Lymnaeidae and Planorbidae. Larger, probably older individuals were more susceptible to infection, indicating a link between host maturity and parasite establishment. The results showed that freshwater mollusks are an important aspect in better understanding the ecology and life cycle of the parasite.

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RECIPROCAL REGULATION OF ENERGY DISSIPATION AND TRANSPIRATION CONTROLS LEAF HEAT EMISSION

Pawel Burdiak[1], Roshanak Zarrin Ghalami[1], Maria Duszyn[1], Muhammad Kamran[1],
Piotr Gawronski[1] , Stanislaw Karpinski[1]
[1]Department of Plant Genetics, Breeding and Biotechnology, Warsaw University of Life Sciences, Poland
Email: pawel_burdiak@sggw.edu.pl

Ongoing climate warming increases the frequency of high irradiance, drought, and heat events that promote energy absorption in excess (EAE) in plant leaves. Under such conditions, absorbed light energy must be dynamically redistributed between photochemistry, fluorescence, and thermal dissipation to prevent photodamage and oxidative stress [1, 2]. Although non-photochemical quenching (NPQ) and transpiration are recognized as central photoprotective processes, their integrated contribution to real-time foliar heat emission and thermal balance under EAE remains poorly quantified.

The objective of this study was to (i) directly quantify leaf heat emission under moderate and high EAE, (ii) determine how coordinated or disrupted interactions among NPQ, photosynthetic electron transport, and transpiration shape foliar temperature dynamics, and (iii) evaluate how partial suppression of these pathways alters thermal and physiological stress responses. To address these aims, we combined high-resolution thermal imaging with direct photo-calorimetric measurements to assess spatial and temporal patterns of leaf temperature and heat flux in several plant species under controlled and semi-natural conditions.

Leaves exposed to moderate EAE emitted approximately 103–111 W m^{-2}, corresponding to ~30 ± 7% of absorbed light energy, substantially higher than previous theoretical and modeling estimates. Thermal responses were rapid, spatially heterogeneous, and developmentally dependent. Inhibition of transpiration increased foliar temperature without compensatory NPQ enhancement, confirming the importance of stomatal cooling in maintaining thermal stability. Unexpectedly, partial inhibition of photosystem II electron transport and NPQ reduced maximum foliar temperature under high irradiance, revealing a linear relationship between temperature amplitude and declines in NPQ and Fv/Fm. Under combined drought and excess light, partial suppression of electron transport by DCMU decreased heat emission and attenuated stress responses without increasing membrane damage.

Together, these results demonstrate that reciprocal coordination among photochemistry, energy dissipation, and stomatal conductance governs foliar thermal balance under EAE. When extrapolated to the forest canopy scale, conditional shifts in leaf heat emission could reach terawatt magnitudes per 1,000,000 km^2, highlighting a potentially overlooked and dynamic component of land–atmosphere energy exchange in climate feedback processes.

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Overview of Lithuanian Jumping Plant Lice (Hemiptera: Psylloidea)

Žygimantas Ivanavičius, Andrius Petrašiūnas

Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
zygimantas.ivanavicius@gmc.stud.vu.lt

Jumping plant lice occupy an important ecological niche as highly specialized plant parasites. During feeding, these insects can change the phenotypes of their host plants and transmit pathogens, thus directly affecting entire ecosystems [1]. By damaging cultivated plants such as citrus, apple and pear trees, psyllids also impact the economy [1] [2]. Therefore, a better understanding of the ecology and distribution of these insects is of particular importance, given the significant research gap in this field. While about 100 species are recorded in neighboring countries such as Poland or Sweden [1] [3], only 16 have been identified in Lithuania so far [4] [5]. This study aims to fill the research gap by providing the first comprehensive overview of this important insect group in the country, which will provide a basis for future research.

Insects were collected from 2017 to 2024 by different collectors using nets and Malaise traps in various areas of Lithuania. Also, from 2025-04-12 to 2025-09-23, psyllids were purposefully collected from their host plants using entomological net (Leg. Ž. Ivanavičius, A. Petrašiūnas, E. Sriubaitė) and stored in 70% ethanol. Given that, in many species, only the fifth instar nymphs have established identification keys, nymphs were not collected. Collected insect specimens were observed with stereomicroscopes and identified using identification keys [1] [2] [6] [7].

A total of 1517 identifiable insects were collected, which were later successfully identified to species level. In total, 12 species belonging to the family Aphalaridae (70 individuals), 4 species belonging to the family Liviidae (86 individuals), 24 species belonging to the family Psyllidae (1037 individuals) and 11 species belonging to the family Triozidae (324 individuals) were identified (Fig 1). 35 of the identified species were not previously recorded in Lithuania, including the entire Aphalaridae family.

Figure 1. Members of the four identified families: (1) *Rhinocola aceris*, ♂ (Aphalaridae); (2) *Livia crefeldensis*, ♀ (Liviidae); (3) *Cacopsylla visci*, ♂ (Psyllidae); (4) *Bactericera albiventris*, ♀(Triozidae). Insect pigmentation altered by ethanol exposure (photos by Ž. Ivanavičius).

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PREVALENCE OF TICK-BORNE PATHOGENS IN WILD MUSTELIDS (MUSTELIDAE) IN LITHUANIA

Daiva Šakienė, Asta Aleksandravičienė, Loreta Griciuvienė, Indrė Lipatova, Rasa Vaitkevičiūtė, Algimantas Paulauskas

Research Institute of Natural and Technological Sciences, University of Vytautas Magnus, Kaunas, Lithuania
email:daiva.sakiene@vdu.lt

Ticks are important vectors of numerous pathogens of medical and veterinary significance, including *Borrelia burgdorferi* s.l., *Anaplasma phagocytophilum*, *Rickettsia* spp., *Babesia* spp., and Candidatus *Neoehrlichia mikurensis* [1]. Wild carnivorous mammals may act as reservoir hosts for these pathogens, thereby contributing to their long-term maintenance and circulation within natural ecosystems [2,3]. Mustelids (Mustelidae) are widely distributed by small carnivores inhabiting diverse environments and, due to their ecology and behavior, are frequently exposed to tick infestation [2]. However, data on the occurrence of tick-borne pathogens in mustelids remain scarce in many European countries, including Lithuania [3]. The aim of this study was to investigate the occurrence of tick-borne pathogens in wild mustelids in Lithuania using molecular methods.

Samples from mustelids were collected in various regions of Lithuania between 2013 and 2025. The study included stone marten (*Martes foina; n=28*), pine marten (*Martes martes; n=54*), American mink (*Neovison vison; n=61*), and European polecat (*Mustela putorius; n=55*). Initial screening for tick-borne pathogens was performed using duplex and triplex real-time PCR assays. Positive samples were further analyzed by nested, semi-nested, and conventional PCR targeting pathogen-specific genes: *Rickettsia* spp. (17 kDa, *gltA*), *Bartonella* spp. (ITS, *groEL*, *rpoB*), and *Anaplasma/Ehrlichia* spp. (16S rRNA, *msp4*, *groEL*). Positive PCR products were sequenced for pathogen identification.

Molecular analyses revealed the presence of several tick-borne pathogens in wild mustelids, including *Babesia* spp., *Anaplasma/Ehrlichia* spp., *Rickettsia* spp., *Bartonella* spp., and *Borrelia* spp. Pathogen occurrence varied among host species and sampled tissues. Sequence analysis revealed the presence of *Anaplasma phagocytophilum*, *Rickettsia raoultii*, and *Ehrlichia muris*. *Anaplasma phagocytophilum* was identified in the European polecat, *Rickettsia raoultii* in the stone marten, and *Ehrlichia muris* in the American mink. These findings indicate the potential involvement of mustelids in the maintenance and transmission cycles of zoonotic and other vector-borne pathogens.

This study represents one of the first comprehensive molecular surveys of tick-borne pathogens in wild mustelids in Lithuania and provides new insights into their ecological role in pathogen circulation.

The study was conducted within the framework of the Vytautas Magnus University KLIGEN Foundation project "Tick-borne pathogens of mustelids (Mustelidae) in Lithuania" (project no. P-N-25-03).

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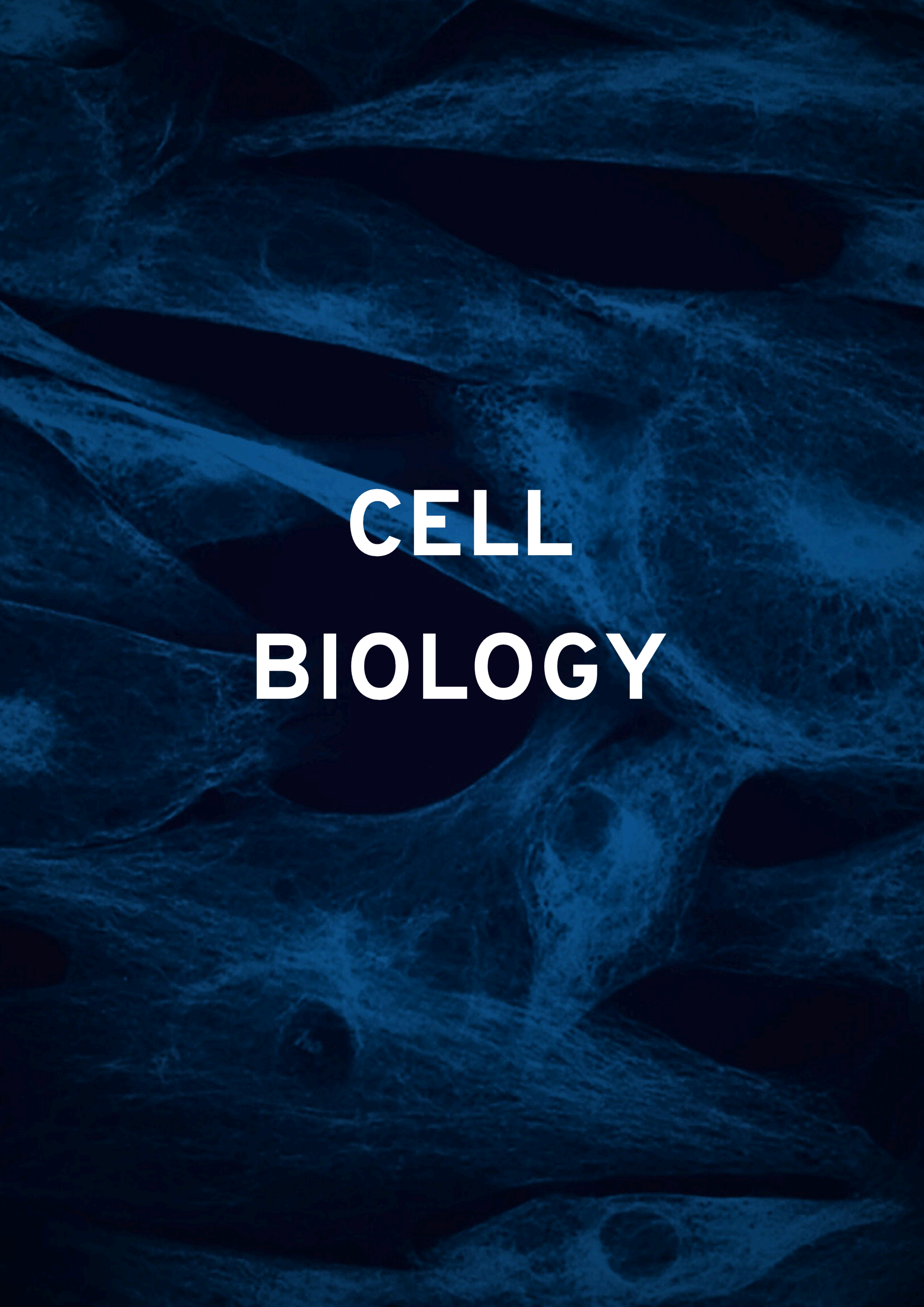
CELL BIOLOGY



IMPACT OF SPHEROID FORMATION METHOD ON THE GROWTH DYNAMICS OF HUMAN MELANOMA SPHEROIDS

Akvilė Žilytė[1], Vilma Petrikaitė[1]

[1]Laboratory of Drug Targets Histopathology, Institute of Cardiology, Lithuanian University of Health Sciences, Lithuania
akvile.zilyte@stud.lsmu.lt

Melanoma is the third most common form of skin cancer and is associated with a high risk of metastasis. Incidence and mortality rates are increasing each year, and it is predicted that the number of melanoma cases will continue to rise rapidly in the coming decades [1]. For these reasons, further research is needed to better understand melanoma biology, particularly the influence of three-dimensional tumour architecture and spheroid size on melanoma growth behaviour in vitro and develop more effective therapeutic approaches [2].

In this study, in vitro 3D culture modelling was used to investigate the formation and growth dynamics of human melanoma spheroids. Two commonly applied spheroid formation methods were employed: the hanging drop method and the non-adhesive surface formation method [3]. These approaches allow the generation of multicellular spheroids that better mimic the three-dimensional architecture of tumors compared to conventional two-dimensional cultures. Two human melanoma cell lines, IGR39 and A375, characterised by different genetic backgrounds, were used to evaluate how the spheroid formation method influences spheroid size and growth dynamics [4]. Spheroids were formed from different initial cell numbers and cultured under identical conditions.

Spheroid growth was monitored over a 10-day period using phase-contrast microscopy. Growth dynamics were assessed by measuring changes in spheroid diameter at defined time points. This approach enabled a quantitative comparison of spheroid development between the two formation methods and between the two cell lines.

On the second day, the A375 spheroids formed using the hanging drop method were 1.2-1.3 times smaller than those formed using the non-adhesive surface method. However, this difference was not observed at later time points, indicating comparable growth dynamics regardless of the formation methods. In contrast, IGR39 spheroids formed using the hanging drop method from 500 and 1000 cells remained consistently larger than spheroids formed on a non-adhesive surface throughout the ten days.

Overall, the results demonstrate that spheroid size and growth dynamics depend on both the used cell line and the formation method. While A375 spheroids exhibit similar growth patterns regardless of the method used, the growth of IGR39 spheroids is influenced by the spheroid formation technique.

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G2

CHARACTERIZATION OF METABOLIC ACTIVITY AND CELL SURVIVAL IN ENDOMETRIAL CANCER 3D MODEL SYSTEMS IN RESPONSE TO TARGETED THERAPY

Laura Marija Račytė[1], Evelina Šidlovska[2], Margarita Montrimaitė[3], Gediminas Januška[3], Rūta Čiurlienė[3], Eglė Žalytė[1]

[1]Department of Biochemistry and Molecular Biology, Institute of Biosciences, Life Sciences Center, Vilnius, Lithuania
[2]National Center of Pathology, Vilnius, Lithuania
[3]National Cancer Center, Vilnius, Lithuania
laura.racyte@gf.stud.vu.lt

Endometrial cancer is the sixth most common cancer in women in the world, and Lithuania and Poland lead the way in Europe in terms of the number of cases of this cancer [1]. The most common risk factors for endometrial cancer are older age, obesity, type 2 diabetes and certain genetic disorders [2], but it is not yet clear why the majority of cases of this cancer are detected in Lithuania and Poland.

The main treatment for endometrial cancer is surgery, but often such therapy helps only in the early stages of endometrial cancer, and the treatment of advanced and/or recurrent tumors requires additional therapy. After surgery, if there is even the slightest risk of tumor recurrence, chemotherapy or radiation therapy is additionally used. Currently, classical chemotherapy drugs such as paclitaxel, cisplatin and carboplatin are mainly used in the clinic, unfortunately, they have a number of disadvantages, such as low efficacy and significant side effects, therefore, it is urgent to search for more effective and specific treatment strategies. As an alternative to classical chemotherapy, promising targeted therapy drugs are constantly being developed and actively investigated, which interfere with various oncogenic molecular pathways essential for the survival and proliferation of cancer cells [3]. Targeted therapy compounds affect specific molecular targets in cancer cells, and therefore, compared to classical chemotherapy, they have a higher selectivity for cancer tissue. This specificity can increase the effectiveness of treatment and reduce toxicity to healthy cells. These compounds are also widely applicable for personalized therapy [4]. The PI3K/AKT/mTOR pathway, receptor tyrosine kinases, various cell cycle, proliferation and survival regulators, PARP polymerase and the nuclear transport system have been identified as potential targets for endometrial cancer therapy.

To accurately evaluate these novel compounds, preclinical models must bridge the gap between laboratory findings and clinical reality. While traditional 2D monolayers have long been the research standard, they fail to replicate the tumor's spatial architecture, cellular crosstalk, and the surrounding microenvironment. In contrast, three-dimensional (3D) models, such as spheroids, better simulate *in vivo* conditions by recreating nutrient diffusion gradients, tumor heterogeneity, and extracellular matrix signaling [5].

In our previous studies the efficacy of targeted therapeutic agents across both 2D and 3D endometrial cancer models was evaluated. In this work initial assessments focused on metabolic activity, which were further refined through fluorescence microscopy to visually differentiate and quantify between metabolic suppression and cell death within the 3D structures. Additionally, quantitative PCR (qPCR) was employed to characterize 3D cultures by changes in the expression of endometrial cancer and stemness markers compared to 2D models. This multi-layered methodological approach allows for a more robust understanding of how endometrial cancer cells respond to precision medicine.

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G3

Dynamics of tight junction protein expression in an *in vitro* blood–brain barrier model

Ariadna Babickaitė[1], Eimina Dirvelytė-Valauskė[1,2], Urtė Neniškytė[1,2]

[1]Life Sciences Center – European Molecular Biology Laboratory (EMBL) Partnership Institute, Vilnius University, Lithuania
[2]Institute of Bioscience, Life Sciences Center, Vilnius University, Lithuania
ariadna.babickaite@chgf.stud.vu.lt

The blood–brain barrier (BBB) is a highly selective, semi-permeable interface separating the systemic circulation from the central nervous system (CNS) (Dotiwala *et al*., 2023). Its functional integrity is maintained by tight junctions between brain endothelial cells, which strictly regulate paracellular and transcellular transport (Alahmari, 2021). While this selectivity protects neural tissue from toxins and immune activation, it also represents a major obstacle for the delivery of therapeutic agents to the CNS. Therefore, evaluating the ability of therapeutic biomolecules or nanoparticle-based systems to cross the BBB is a critical step in CNS drug development.

In vitro BBB models provide a reproducible and ethically advantageous platform for studying transport mechanisms under controlled experimental conditions. These models are commonly based on culturing brain-derived endothelial cells on semi-permeable membrane inserts, where they form a confluent monolayer that mimics key structural and functional properties of the *in vivo* barrier. Such systems allow the quantitative evaluation of particle permeability and facilitate the screening of potential delivery vehicles prior to *in vivo* validation.

In the present study, we aimed to characterize the dynamics of tight junction protein expression in an i*n vitro* blood–brain barrier system established using murine brain endothelial cells. The *in vitro* BBB model was generated by culturing bEnd.3 endothelial cells on transwell membrane inserts (fig.1), allowing the cells to form a continuous monolayer on the porous membrane surface. At defined time points, the development of the endothelial barrier was evaluated by analyzing the formation and maturation of tight junctions. These junctions are essential for maintaining the structural stability and proper function of the blood–brain barrier (BBB). Immunofluorescence analysis was performed to examine the spatial distribution and changes of tight junction proteins—occludin, zonula occludens-1 (ZO-1), and claudin-5, enabling visualization of junctional organization and continuity along cell–cell borders. We then evaluated the integrity and confluency of the cell monolayer on membrane inserts to determine whether bEnd.3 cells form a continuous endothelial layer appropriate for *in vitro* BBB modeling. This approach enables a systematic and reliable evaluation of tight junction formation in bEnd.3 cells by examining both their structural organization and degree of maturation over time. By analyzing how these intercellular junctions develop and become more complex, it is possible to assess whether the cells establish the close cell–cell contacts required for effective barrier function. Through this detailed assessment, the suitability of bEnd.3 cells as an *in vitro* model of the blood–brain barrier can be more accurately determined, particularly in terms of their ability to mimic key structural and functional characteristics of the BBB observed *in vivo*.

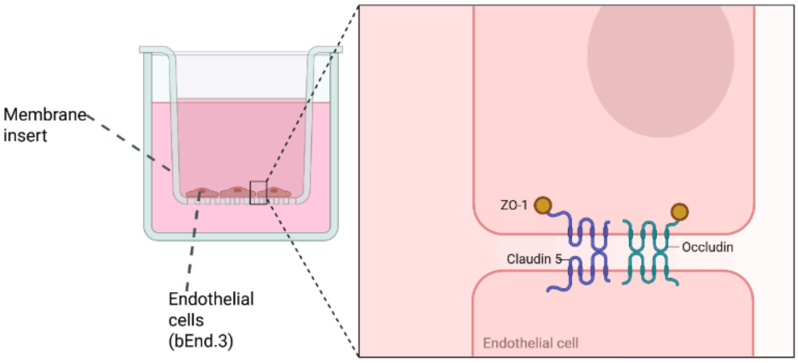

Figure 1. Experimental design of the *in vitro* blood–brain barrier model. Created with BioRender.com.

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ENDOMETRIAL STROMAL CELL DECIDUALIZATION EFFICIENCY AND RESPONSE TO EARLY IMPLANTATION-RELATED SIGNALS IN FEMALE INFERTILITY

Evelina Juškaitė[1], Erika Girniūtė[1], Deimantė Šulijienė[1], Rūta Navakauskienė[1]

[1] Department of Molecular Cell Biology, Life Sciences Center, Vilnius University
evelina.juskaite@gmc.stud.vu.lt

Successful embryo implantation is a highly coordinated process that depends not only on embryo quality but also on the functional competence of the endometrium, as implantation failure and infertility are often associated with abnormalities of the maternal endometrium [1]. In addition, the establishment of early pregnancy relies on effective decidualization of stromal cells and their ability to respond to early pregnancy signals, such as human chorionic gonadotropin (hCG), within the endometrium. However, the molecular mechanisms involved in this process, including the regulation of classical decidualization markers, such as PRL and IGFBP, transcription factors and associated signaling pathways, as well as metabolic reprogramming, remain not fully understood [2,3].

In this study, we aimed to evaluate decidualization efficiency in patient-derived endometrial stromal cells (ESCs) and to examine their molecular response to the early pregnancy signal, hCG. Firstly, primary ESCs were isolated from female patients with fertility disorders, undergoing assisted reproduction procedures, and cultured *in vitro*. Then, decidualization was induced using cyclic AMP and medroxyprogesterone acetate (mPA) for 3 days. Additionally, ESCs were treated with hCG for 24 hours during decidualization to evaluate their responsiveness to early pregnancy signals. Decidualization efficiency and molecular changes were examined by measuring gene and protein expression levels using RT-qPCR and Western blot. Moreover, metabolic mitochondrial function was analyzed using the Seahorse XFp Cell Mito Stress test and ATP rate assay kits.

Our results demonstrate that decidualization significantly increases the expression of classical decidualization markers, including PRL and IGFBP1, as well as transcription factors FOXO1 and WNT4, compared to non-treated ESCs. ESCs’ treatment with decidualization inducers and hCG also modulates the expression levels of receptivity and immunomodulation-related genes such as SPP1, VEGFA, IL8, and others. Furthermore, changes in ESCs' oxygen consumption rate (OCR) and intracellular ATP production were detected after decidualization and hCG induction.

Overall, our findings suggest that these molecular changes play an essential role in decidualization and may contribute to a better understanding of endometrial function, providing a foundation for future infertility research.

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G5

INVESTIGATION OF POLYAMINE PATHWAY REGULATION IN ACTIVATED MICROGLIA

Račkelytė K[1,2], Umbrasas D[1]

[1]Lithuanian University of Health Sciences, Neuroscience Institute, Laboratory of Biochemistry, Kaunas, Lithuania

[2]Lithuanian University of Health Sciences, Faculty of Pharmacy

kamile.rackelyte1@stud.lsmu.lt

Polyamines (putrescine, spermidine, and spermine) are ubiquitous metabolites involved in the regulation of cellular proliferation, redox homeostasis, gene expression, and immune responses. Dysregulation of polyamine metabolism has been linked to neurodegenerative diseases, especially Parkinson’s disease; however, their involvement in neuroinflammation is insufficiently characterized. Chronic neuroinflammation is primarily mediated by activated microglia and it is a key contributor to the progression of neurodegenerative diseases, therefore it is important to understand metabolic pathways that shape microglial inflammatory responses [1].

In this study, we investigated the transcriptional regulation of polyamine synthesis, degradation, and transport in microglia under inflammatory conditions. Murine BV-2 microglial cells were activated using the TLR4 ligand lipopolysaccharide (LPS). Microglial activation was confirmed by measuring extracellular nitric oxide (NO) levels. Transcriptional changes in genes associated with polyamine synthesis (*Odc1*, *Amd1*), transport (*Slc3a2*, *Atp13a2*), and degradation (*Sat1*, *Paox*) were analyzed using quantitative real-time PCR (qRT-PCR).

Our results demonstrate that LPS-induced microglial activation leads to a significant upregulation of *Odc1*, indicating enhanced polyamine biosynthesis. In contrast, expression of *Atp13a2*, a lysosomal polyamine transporter linked to Parkinsonian pathology, was significantly reduced following LPS stimulation. These data suggest that inflammatory activation of microglia is associated with coordinated transcriptional reprogramming of polyamine metabolic pathways, which may influence microglial inflammatory function under neurodegenerative conditions.

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CAIX INHIBITION IN A PRESENCE OF IFNg ALTERS A172 CELL PROLIFERATION AND MOBILITY

Justina Musvicaitė[1], Tomas Vainauskas[2], Mantas Žvirblis[1], Skaistė Šlekytė[1], Agnė Kvietkauskaitė[1], Agne Petrosiute[1]

[1]Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Life Sciences Center, Vilnius, Lithuania
[2]Department of Immunology, Institute of Biotechnology, Life Sciences Center, Vilnius, Lithuania
justina.musvicaite@gmc.stud.vu.lt

INTRODUCTION. Central nervous system (CNS) tumors cause significant morbidity and mortality in patients of all ages [1]. There is an urgent need to identify novel targets in these patients. Carbonic anhydrase IX (CAIX) helps cancer cells survive in a hypoxic environment and can be and attractive target for therapeutics [2]. How immune environment shapes tumor response to CAIX inhibition needs to be investigated to design effective therapies. We have identified a CAIX inhibitor that can cross blood-brain barrier based on preliminary studies. We aimed to study the effects of this compound in vitro in CNS cancer glioblastoma cell line A172 with or without presence of proinflammatory cytokine IFNg [3].

MATERIALS AND METHODS. MZ24-93 and SS25-18 are small-molecule CAIX inhibitors with potential for blood-brain barrier penetration. We performed a flow cytometry analysis for CAIX expression on A172 cells together with IFNg exposure. A172 cell line express minimal CAIX, with some increase with addition of IFNg. Next, we performed wound closure assay and 3D spheroid assay on these cells to assess compound effects on cell proliferation and motility with or without IFNg.

RESULTS. A172 cell line express minimal CAIX, with some increase in expression with addition of IFNg. A 3D spheroid assay performed on A172 cells showed an increased effect of CAIX inhibition under hypoxia with CAIX inhibition. Wound closure assay showed impaired cell motility in A172 cells under hypoxia and in the presence of IFNg.

CONCLUSION. Our data shows that the addition of cytokine IFNg can alter A172 cell response to CAIX inhibition, even though these cells have minimal CAIX expression. This could be off target effect. Further studies are needed to decipher the mechanisms.

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IN VITRO CYTOTOXICITY AND GENOTOXICITY ASSESSMENT OF TITANIUM CARBIDE MXENES

Rusnė Uzielaitė[1], Rokas Mikšiūnas[1,2], Milda Babonaitė[3], Simonas Ramanavičius[4], Arūnas Ramanavičius[2,5]

[1]Department of Regenerative Medicine, State Research Institute Centre for Innovative Medicine, Vilnius, Lithuania
[2]Laboratory of Nanotechnology, Center for Physical Sciences and Technology, Vilnius, Lithuania.
[3]Department of Botany and Genetics, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[4]Department of Electrochemical Material Science, Center for Physical Sciences and Technology, Vilnius, Lithuania
[5]Department of Physical Chemistry, Institute of Chemistry, Faculty of Chemistry and Geosciences, Vilnius University, Vilnius, Lithuania
rusne.uzielaite@gmc.vu.lt

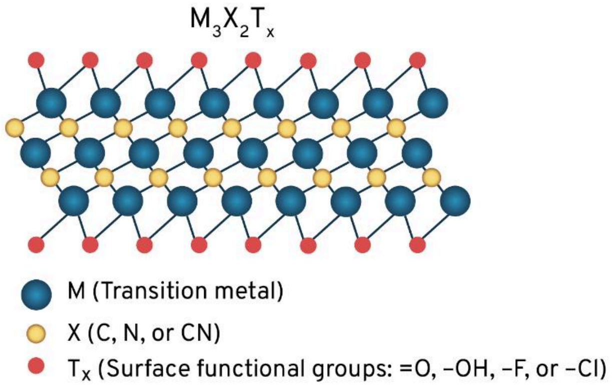
In regenerative medicine, electrically conductive scaffolds are gaining attention because they can mimic the natural extracellular matrix and promote cell proliferation and differentiation, supporting tissue regeneration and wound healing [1]. MXenes, a family of two-dimensional transition metal carbides, nitrides, and carbonitrides, are promising candidates for such scaffolds due to their unique physical and chemical properties [2].

The biological effects of MXenes can vary depending on their synthesis method, chemical composition, surface modifications, and particle size [3]. However, data on their cytotoxicity and genotoxicity are still limited, highlighting the need for careful evaluation before biomedical applications.

In this study, multilayer titanium carbide MXenes were synthesized from a Ti_3AlC_2 precursor by selective chemical etching, and single-layer flakes were obtained by LiCl-assisted delamination (Fig. 1). MXenes were characterized by X-ray diffraction. *In vitro* studies were performed on both multilayer and single-layer MXenes. Cytotoxic effects were examined using human umbilical vein endothelial cells (HUVEC), and genotoxic effects were assessed in human peripheral blood lymphocytes.

These results provide a comparison of cytotoxic and genotoxic effects between multilayer and single-layer MXenes on human cells, offering important insights for their potential use in regenerative medicine.

Figure 1. $M_3X_2T_X$ MXene structure showing metal layers (M), carbon layers (X), and surface groups (T_X)



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G8

METABOLIC ALTERATIONS ASSOCIATED WITH ENDOMETRIOSIS: THE IMPACT OF PROBIOTIC-DERIVED METABOLITES

Elvina Valatkaitė[1], Giedrė Skliutė[1], Rūta Navakauskienė[1]

[1]Department of Molecular Cell Biology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Lithuania
elvina.valatkaite@bchi.stud.vu.lt

Endometriosis is a chronic gynaecological disease characterised by the spread of endometrial tissue outside the uterine cavity [1]. The abnormal growth most commonly happens in the ovaries, fallopian tubes, and pelvic cavity, and in rare cases in other organs, such as the chest or cervix [2]. This condition affects approximately 10–20% of women of reproductive age, and it can cause a wide range of symptoms, including pelvic pain, menstrual cycle irregularities, painful menstruation, dyspareunia, and infertility. The etiology of endometriosis depends on the combined influence of immunological, hormonal, genetic, and environmental factors. Therefore, several theories of the disease's pathogenesis have been proposed, including coelomic metaplasia, lymphatic and vascular dissemination, hematogenous transport, however, retrograde menstruation remains the most widely accepted theory. Endometrial lesions are associated with changes in hormonal status and aberrant immunological responses [2, 3].

Recently, it has been proposed that the microbiome may contribute to the development of various diseases, including endometriosis, by influencing inflammatory processes, hormonal regulation, and immune system activity [4]. The microbiota of patients with endometriosis is associated with reduced abundance of *Lactobacillus* species, an increased abundance of bacteria linked to bacterial vaginosis, and other opportunistic pathogens. Possible explanations for the impact of dysbiosis on endometriosis include activation of the immune system, cytokine-mediated disruption of intestinal function, altered estrogen metabolism, and impaired homeostasis of progenitor and stem cells. This could potentially be used for the treatment of endometriosis since the conventional methods often include treatment with hormonal medications [5]. Moreover, the local inflammatory microenvironment characteristic of endometriotic lesions could be modulated by probiotic microorganisms and their metabolic products [4].

The goal of this study was to characterise the microbial profile of endometriotic tissue and to evaluate the effects of probiotic bacterium *Lacticaseibacillus casei* on stromal cells isolated from endometriotic tissue. Stromal cells were obtained from both endometriotic lesions and endometriotic cysts. The cells were treated with *L. casei* cell-free supernatant (CF-S), and the probiotic-mediated effects were evaluated by studying cellular proliferation, metabolic phenotype, and gene expression profiles. Proliferative activity was assessed using the XTT assay at 24, 48, and 72 hours. Metabolic phenotype was evaluated under basal conditions and after treatment with CF-S after 24 hours by assessing mitochondrial function. Finally, the expression of genes associated with metabolic function was carried out by employing RT-qPCR.

Analysis of the 16S rRNA sequencing data demonstrated distinct microbial profiles between endometriotic lesion and cyst-derived tissues. The predominant genera in lesion samples were *Corynebacterium* (26%) and *Prevotella* (20%), in contrast, in cyst tissue, *Lactobacillus* (48%). XTT assay revealed that higher concentrations of *L. casei* CF-S notably reduced proliferative activity of stromal cells derived from both tissue types. On the contrary, lower concentrations of CF-S induced modest modulation of proliferation, but did not have a cytotoxic effect on stromal cells. Furthermore, analyses of metabolic phenotype and gene expression indicated subtle alterations in their profiles dependent on both tissue type (lesion or cyst) and CF-S concentration.

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3D Scaffolds for Next-Generation BBB Model Systems

L.註Ališauskas[1], V. Petrikaitė[1,2]
[1] Laboratory of Drug Targets Histopathology, Institute of Cardiology, Lithuanian University of Health Sciences
[2] Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania [1]
lukas.alisauskas@lsmu.lt

The development of physiologically relevant *in vitro* systems has become increasingly important for reducing the reliance on animal experimentation and supporting the 3R principles in animal testing (replacement, reduction and refinement) [1]. Three-dimensional (3D) cell culture scaffolds offer advantages over two-dimensional (2D) models by allowing more realistic cell-matrix and cell-cell interactions, thereby improving the physiological accuracy of relevant in vitro models [2]. These advantages are especially critical for modelling complex structures such as the blood-brain barrier (BBB), where 3D matrices can better replicate the extracellular architecture and barrier function compared to 2D substrates [3]. Before such scaffolds can be used in novel BBB model development, it is essential to assess their potential cytotoxicity in accordance with ISO 10993-5, which outlines standardized *in vitro* methods for evaluating the cytotoxicity of materials intended for biomedical applications. Ensuring scaffold biocompatibility at an early stage is crucial for reliable BBB modelling and for advancing relevant research alternatives.

Our study aimed to evaluate the cytotoxicity of newly developed 3D scaffolds and test their biocompatibility *in vitro*.

In vitro cytotoxicity was assessed in accordance with ISO 10993-5 using L929 mouse fibroblasts and the MTT assay on the extracts. Extracts were prepared from 3D scaffolds at a ratio of 0.1 g of material per 1 ml of culture medium. These extracts were added to the L929 cells undiluted and at dilutions of 1:2 and 1:4. Cell viability was evaluated after 24 and 72 h of incubation. For the assessment of biocompatibility under direct contact conditions, L929 cells were seeded onto the 3D scaffolds and cultured for 1, 3, and 7 days, with culture medium renewed every 3 days. All experiments included positive and negative controls and were performed in triplicate.

The MTT assay results demonstrated high cell viability on the 3D scaffolds. After 24 hours of incubation, L929 cells seeded onto the scaffolds exhibited a relative metabolic activity of 203 ± 25%, indicating enhanced proliferation compared to the 2D control. Prolonged incubation for 3 and 7 days further supported this trend, with scaffold-cultured cells displaying higher relative activity than cells maintained in 2D conditions (106 ± 20% and 122 ± 15%, respectively). Cytotoxicity testing of scaffold extracts showed no evidence of cytotoxic effects across the tested dilutions. After 24 hours, relative viability values ranged from 92% to 110%, while 72-hour exposure resulted in viability values between 91% and 96%. As ISO 10993-5 specifies that cell viability above 70% indicates non-cytotoxicity, all extract conditions met the criteria for biocompatibility.

Conclusion: The results demonstrate that the newly developed 3D scaffolds support high L929 cell viability and show no cytotoxic effects, as all extract-treated conditions exceeded the viability threshold of 70%. The confirmed biocompatibility of scaffolds and their extracts indicates that these scaffolds are suitable for use in the development of advanced BBB models.

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EFFECTS OF PRMT INHIBITION ON TUMOR CELL INVASIVENESS AND METASTASIS

Skaistė Kasnauskaitė[1], Veronika Viktorija Borutinskaitė[1]

[1]Department of Molecular Cell Biology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Lithuania
skaiste.kasnauskaite@gmc.stud.vu.lt

Tumor hypoxia is a critical component of the tumor microenvironment, promoting cancer progression, immune evasion, and therapeutic resistance [1]. Hypoxia-driven activation of HIF-dependent transcriptional programs contributes to metabolic adaptation, invasiveness, and the establishment of immunosuppressive (“cold”) tumor phenotypes [1,2]. Epigenetic regulators, particularly protein arginine methyltransferases PRMT1 and PRMT5, have emerged as key modulators of transcriptional plasticity and hypoxia-associated signaling [3]. Increasing evidence suggests that PRMT inhibition may not only suppress tumor growth but also modulate the immune phenotype of cancer cells [4].

The aim of this study was to evaluate how PRMT1 and PRMT5 inhibition affects tumor cell invasiveness and immunophenotype under normoxic and hypoxic conditions. Human solid tumor cell lines with distinct phenotypic characteristics were analyzed using flow cytometry and Transwell migration assays following treatment with selective PRMT inhibitors.

Hypoxia induced cell line-specific phenotypic adaptations, particularly in more invasive models. PRMT inhibition predominantly affected immune- and invasion-related surface markers in aggressive cell lines, while epithelial, less invasive lines remained relatively stable. Notably, modulation of CD73 (*NT5E*), CD44, CD54 (*ICAM1*), and PD-L1 (*CD274*) expression suggested that PRMT targeting may interfere with hypoxia-associated immunosuppressive and pro-invasive pathways.

Overall, these findings support the concept that PRMT1 and PRMT5 represent promising epigenetic targets for modifying hypoxia-driven tumor plasticity and potentially shifting tumors toward a more immunogenic phenotype.

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G11

EVALUATION OF CAIX INHIBITION IN COMBINATION WITH IFNγ IN HUMAN GLIOBLASTOMA 3D CELL MODEL

Skaistė Šlekytė[1], Emma Schlecker[1],
Mantas Žvirblis[1], Agne Petrosiute[1], Agnė Kvietkauskaitė[1]

[1]Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Life Sciences Center, Vilnius, Lithuania

skaiste.slekyte@chgf.stud.vu.lt

INTRODUCTION. Despite significant advances in glioblastoma (GBM) treatment, outcomes remain poor [1]. Hypoxia is an important factor in GBM progression [2]. Carbonic anhydrase IX (CAIX) enzyme helps cancer cells survive in tumor hypoxia by maintaining the physiological pH in cancer cells and acidifying the tumor microenvironment [3]. Our laboratory has developed several CAIX inhibitors (MZ24-93, SS25-18, SS25-27). Among these, MZ24-93 has demonstrated the ability to cross the blood–brain barrier in preliminary studies. We aimed to study the effects of these compounds *in vitro* in the GBM cell line U87 and to determine whether additional factors, such as exposure to interferon-gamma (IFNγ), can alter the response to these inhibitors in the U87 cell 3D spheroid model.

MATERIALS AND METHODS. CAIX expression under hypoxia, with or without IFNγ, was assessed by flow cytometry in U87 cells. A 3D spheroid growth assay was subsequently performed to evaluate the effects on these cells of CAIX inhibitors SS25-18, SS25-27, MZ24-93 as monotherapy and MZ24-93 in combination with IFNγ.

RESULTS. The U87 cell line exhibited high CAIX expression under hypoxia compared to normoxia. The addition of IFNγ to these cells upregulated CAIX expression minimally. In the 3D spheroid model, treatment with the compounds alone did not produce a significant effect on spheroid growth. However, diminished spheroid growth under hypoxia was observed when MZ24-93 was delivered in combination with IFNγ.

CONCLUSION. Our data show that the addition of cytokine IFNγ enhances the efficacy of CAIX inhibition in a 3D GBM model. The underlying mechanisms of this interaction require further investigation.

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G12

SIGNIFICANCE OF B7 FAMILY PROTEINS FOR COLORECTAL CANCER CELL RESISTANCE TO CHEMOTHERAPEUTIC DRUGS

Matas Miliukas, Andrius Jasinevičius, Aušra Sasnauskienė

Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
matas.miliukas@gmc.stud.vu.lt

Colorectal cancer is an oncological disease with one of the highest mortality rates worldwide. A major challenge in its treatment is chemoresistance, which limits the effectiveness of conventional chemotherapy [1]. Cancer cells that resist the initiation of cell death may activate molecular pathways that not only confer chemoresistance but also modulate the expression of molecules, regulating immune response. Immune checkpoint proteins are responsible for communication between somatic and immune cells and may enable cancer cells to evade the immune response [2]. Immune checkpoint proteins, including members of the B7 family, have been reported to exert functions beyond immune suppression. We hypothesize that these molecules may directly contribute to chemoresistance, and elucidating their cancer cell-intrinsic roles could reveal novel mechanisms of therapy resistance.

The aim of this study was to determine the significance of B7 family proteins CD80, ICOSLG, NCR3LG1, PD-L1 and VTCN1 for the chemoresistance of HCT116 colorectal cancer cells to 5-fluorouracil and oxaliplatin. Specific shRNAs were transduced into HCT116 cells to achieve stable knockdown. Cell viability was evaluated following treatment with 5-fluorouracil or oxaliplatin. We have found that reducing the levels of *CD80*, *ICOSLG*, *NCR3LG1*, *PD-L1* or *VTCN1* increases the sensitivity of HCT116 cells to 5-fluorouracil. Furthermore, we have shown that reduced levels of *ICOSLG*, *NCR3LG1*, *PD-L1* or *VTCN1* increase the sensitivity of these cells to oxaliplatin. Our findings indicate that B7 family proteins regulate molecular pathways that contribute to chemoresistance.

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BIOINFORMATICS

H1

ACHROEX: INTERACTIVE WEBAPP FOR *ACHROMOBACTER* SPP. RESEARCH

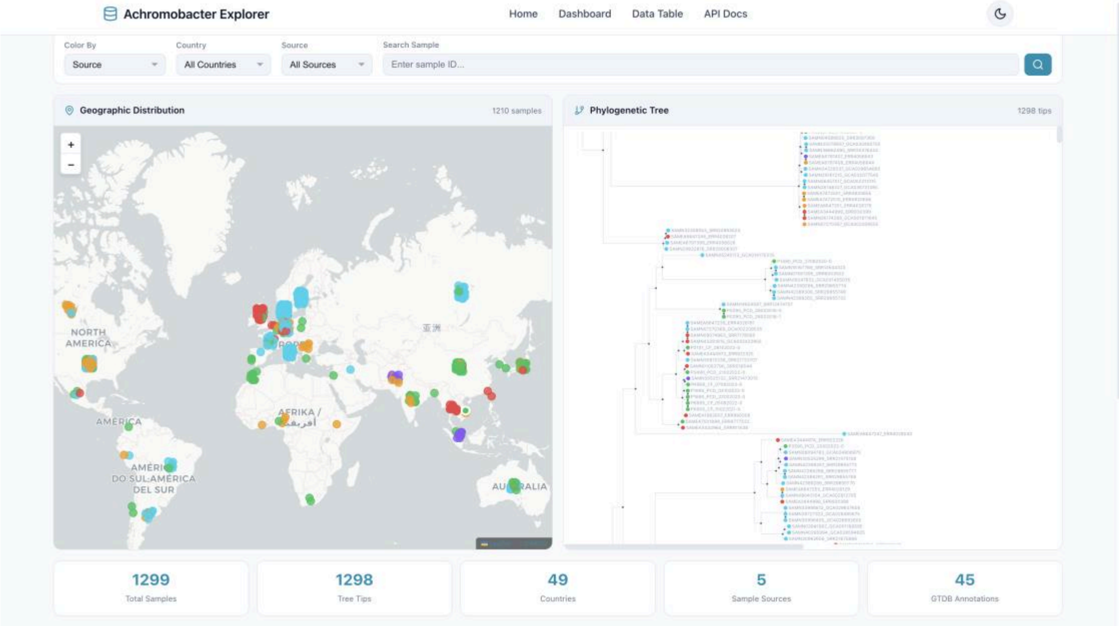
Lukas Pranskūnas[1], Gytis Dudas[1], Migle Gabrielaite[1]
[1]Deparment of Eukaryotic Gene engineering, Institute of Biotechnology, Vilnius University, Lithuania
lukas.pranskunas@gmc.stud.vu.lt

Achromobacter species are Gram-negative opportunistic pathogens that cause chronic pulmonary infections in people with cystic fibrosis (pwCF). These bacteria pose a growing public health concern due to their innate and acquired resistance to antibiotics and their increasing prevalence[1-3].

Here, we are developing an interactive web application for visualising the geographical distribution of *Achromobacter* spp. isolates and their phylogenetic relationship, following the design principles of platforms such as Nextstrain [4] and Microreact [5].

The web application features a decoupled architecture, where the R backend and React frontend are hosted in separate Docker containers. The backend is built using the Plumber API, which handles HTTP requests and routes them to the R-based analytical engine [6]. The frontend was developed using the Vite React Template to ensure seamless integration with the backend [7]. Visualisation of large datasets is an essential component of this application; geographic data are visualised using the Leaflet.js [8] library, while phylogenetic trees are rendered using the D3.js visualisation framework and the phylotree.js library [9, 10].

Following its initial release, AchroEx will support professionals worldwide in epidemiological research and public healthcare. The platform will enable researchers to visualise the geographical spread of strains and their phylogenetic relationships. Furthermore, AchroEx will constitute the largest publicly available *Achromobacter* spp. metadata and phylogenetic dataset, facilitating more robust and conclusive analyses in *Achromobacter* spp. research.



Color By: Source
Country: All Countries
Source: All Sources

1299 Total Samples
1298 Tree Tips
49 Countries
5 Sample Sources
45 GTDB Annotations

Figure 1. A screenshot of ACHROEX – The Interactive *Achromobacter* Explorer.

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EXPLORING THE EVOLUTION OF INFLUENZA-LIKE VIRUSES: SEQUENCE AND STRUCTURE-BASED ANALYSIS OF PB1 AND GLYCOPROTEINS

Aušrinė Žvirblytė[1], Gytis Dudas[1]

[1]Department of Eukaryote Gene engineering, University of Vilnius, Lithuania
ausrine.zvirblyte@gmc.stud.vu.lt

Advances in high-throughput sequencing and reduced costs in metagenomic analysis have significantly expanded the known diversity of the *Orthomyxoviridae*, a family of RNA viruses that includes influenza and infectious salmon anemia, revealing a wide array of influenza-like viruses in aquatic vertebrates and invertebrates [1], [2]. Within this family, influenza-like virus viral entry and exit are mediated by two glycoprotein types: multifunctional HEF (hemagglutinin-esterase-fusion) protein found in influenza C and D viruses, and HA (hemagglutinin) protein, which lacks an esterase domain and requires a separate NA (neuraminidase) protein to release viruses, as seen in influenza A and B viruses [3]. However, it is still unknown whether HA emerged from ancestral HEF or *vice versa*.

To gain a better understanding of the evolution of influenza-like viruses we reconstructed phylogenetic trees of PB1 (coding for the catalytic subunit of the RNA-dependent RNA polymerase) and HA/HEF proteins using amino acid sequence and structural representation (3Di) alignments. Protein structures were predicted using AlphaFold3, although there were significant limitations in predicting the structures of divergent HA/HEF sequences of viruses discovered in tunicates which we could not align reliably. Structural alignments were performed using FoldMason and phylogenetic inference was conducted using IQ-TREE 2 and Phylobayes. To determine the roots of the trees we used outgroup rooting using isaviruses (a group of orthomyxoviruses that includes infectious salmon anemia) as the outgroup for both PB1 and glycoprotein trees.

The topologies of both amino acid and 3Di-based trees consistently placed viruses containing HEFs in basal positions, when rooted with isaviruses. Analysing the amino acid alignment, we were able to identify the FGDSR motif conserved amongst HEFs that contains the serine of the esterase catalytic triad [3], [4]. The region was also found in some sequences that were previously thought to be HAs - DBA45440.1 (Sturgeon influenza-like virus) [1] and WWP49491.1 (Orthomyxoviridae sp., frog).

Our current results indicate that HEF is the ancestral glycoprotein form of influenza-like viruses. Furthermore, the distribution of hosts across the phylogeny reveals broad host plasticity in influenza-like like viruses, that includes both vertebrates and invertebrates. The viral tree topology does not mirror the host evolutionary history, suggesting a history of host jumping among divergent taxa inconsistent with virus-host co-divergence.

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APPLICATION OF DEEP LEARNING MODELS FOR THE ANALYSIS OF INTERGENIC REGIONS IN HOMOLOGUES OF THE CAS7-LIKE PHAGE PROTEIN

Kristupas Užkurnys[1], Praneet
Prabhanjan Bejavada[1], Darius Kazlauskas[1]

[1]Department of Bioinformatics, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
kristupas.uzkurnys@gmc.stud.vu.lt

CRISPR-Cas is an adaptive immune system found in most bacteria and archaea that protects against invading viruses. It recognizes and destroys foreign nucleic acids with the help of effector complexes. Class I CRISPR-Cas systems use multi-protein effector complexes to perform this function and comprise 90% of all CRISPR-Cas loci [1].

One of the proteins, Cas7, has an RNA-Recognition Motif (RRM) domain, forms the basis of Class I CRISPR-Cas effector complex and plays a key role in binding and protecting crRNA guide sequence [2]. Cas7 homologue (gp87) was identified in *E. coli* VpaE1 bacteriophage which is a member of the Felix01likevirus genus. Experimental results show that gp87can bind RNA. In addition, the three-dimensional structures of type III Cas7 homologues and the gp87 protein are similar, even though the sequences themselves are very different.

RNA sequencing of gp87-RNA complex helped us to identify intergenic regions and detect GGTNN repeats. However, it is not known whether there are repeats in all other gp87 homologue groups, as they are different and come from different organisms: viruses, archaea and bacteria.

The aim of this research was to find and model the repeat regions that contain high amounts of GG and CC, because gp87 protein might interact with these regions. To achieve this goal, deep learning models (AIDO, DNABERT-2, Evo2) were used for sequence classification task. Fine-tuned models were deployed to find repeats in 794 genome regions. Sequences containing repeats were subjected to AlphaFold modelling of complexes together with gp87. Detailed results will be presented during the poster session.

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GENOTYPE-PHENOTYPE ASSOCIATION ANALYSIS BASED ON WHOLE GENOME SEQUENCING OF *ACHROMOBACTER* GENUS

Idas Jasaitis[1], Helle Krogh Johansen[2,3], Rasmus L. Marvig[4,5,6], Gytis Dudas[1], Migle Gabrielaite[1]
[1]Department of Eukaryote Gene Engineering, Institute of Biotechnology, Vilnius University, Lithuania
[2]Department of Clinical Microbiology, Rigshospitalet, Denmark
[3]Faculty of Health and Medical Sciences, Institute of Clinical Medicine, University of Copenhagen, Denmark
[4] Department of Genomic Medicine, Rigshospitalet, Denmark
[5]Department of Clinical Biochemistry, Rigshospitalet, Denmark
[6]Department of Health Technology, Technical University of Denmark, Denmark
idas.jasaitis@gmc.stud.vu.lt

Achromobacter genus bacteria are emerging opportunistic pathogens commonly linked to chronic infections in people with cystic fibrosis. Their combination of intrinsic and acquired resistance to numerous antibiotics presents a growing challenge and threat in clinical treatment.

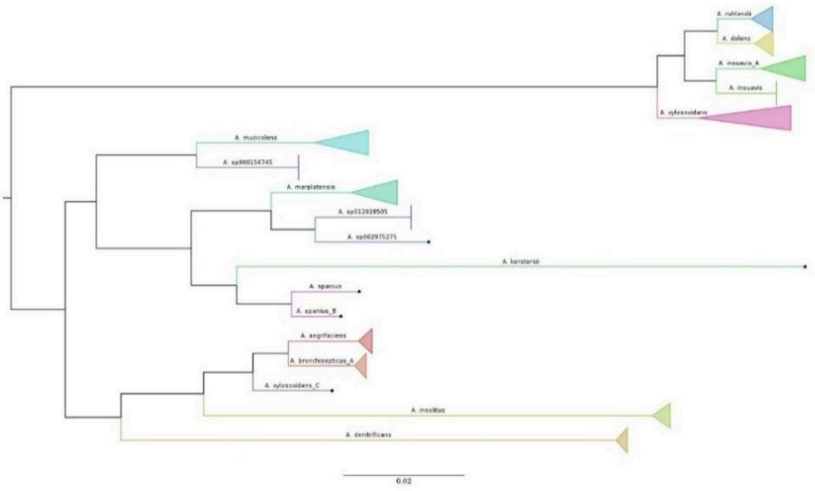
The aim of this study was to inspect, identify, and compare genetic factors contributing to antibiotic resistance in *Achromobacter* spp. whole genome sequenced isolates using multiple analytical approaches: reference resistance databases (AMRFinderPlus), ML-based resistance identification tools (DeepARG), and genome-wide association tools (Pyseer and DBGWAS).

A total of 302 clinical *Achromobacter* spp. samples were analysed together with their antibiotic resistance profiles. A pangenome comprising 33,344 genes was constructed using Panaroo. The resulting phylogenetic tree was subsequently analysed and recombination-corrected using ClonalFrameML (Fig. 1).

To comprehensively assess the genetic basis of antimicrobial resistance, four distinct analytical pipelines were employed: (1) AMRFinderPlus was used to annotate all well-characterized antimicrobial resistance genes across the 302 isolates; (2) DeepARG, leveraging machine learning algorithms, predicted both recognised and potential novel resistance determinants, including non-standard gene variants; (3) Pyseer was used to conduct three complementary analyses based on unitigs, gene presence-absence matrices, and single nucleotide variants to detect associations between genomic features and resistance phenotypes at multiple scales; (4) finally, with DBGWAS we identified top 10 most significant genomic components for each of the 10 tested antibiotics.

AMRFinderPlus showed that our isolates carry many well_known resistance genes, including several beta_lactamases, target modifications and efflux pumps. DeepARG revealed a resistome dominated by vancomycin_related genes and multiple multidrug efflux systems, plus additional genes for beta_lactam resistance, pointing to strong multidrug resistance potential. Pyseer indicated that significant hits are related to COG categories for metabolism and cellular processes/signaling, with information storage/processing also being common, suggesting that resistance involves many genes affecting basic cellular functions rather than a single pathway. DBGWAS identified a smaller set of variants in comparable COG categories, supporting the idea that resistance is driven by both classical AMR genes and broader changes in housekeeping, regulatory and transport functions.

Figure 1. Phylogenetic tree of *Achromobacter* species isolates used in the analysis.



H5

A WORKFLOW FOR IDENTIFICATION AND CONTEXTUAL ANALYSIS OF ANTIMICROBIAL RESISTANCE AND DEFENSE MECHANISM GENE ORIGINS

Viktorija Ramonaitė[1], Helle Krogh Johansen[2,3], Rasmus L. Marvig[4,5], Migle Gabrielaite[6]

[1]Faculty of Mathematics and Informatics, Vilnius University, Lithuania
[2]Department of Clinical Microbiology, Rigshospitalet, Denmark
[3]Faculty of Health and Medical Sciences, Institute of Clinical Medicine, University of Copenhagen, Denmark
[4]Department of Clinical Biochemistry, Rigshospitalet, Denmark
[5]Department of Health Technology, Technical University of Denmark, Denmark
[6]Institute of Biotechnology, Life Science Center, Vilnius University, Lithuania
viktorija.ramonaite@mif.stud.vu.lt

Antimicrobial resistance (AMR) genes encode proteins that allow bacteria to survive antibiotic treatment, making infections increasingly difficult to treat [1]. Defense mechanism genes encode systems that protect bacteria from various environmental threats, including bacterial competitors, bacteriophages, and host immune responses [2]. Both AMR and mechanism genes can be transferred between bacteria through horizontal gene transfer (HGT), thereby increasing the spread of resistance and survival traits [3]. Understanding the genomic context and origins of these genes is essential for identifying transmission pathways, tracking resistance spread across bacterial populations, and recognising high-risk mobile elements [4].

In this study, we developed a workflow to identify AMR and defense mechanism genes and characterise their genomic context. The bioinformatic workflow was built using Snakemake [5] integrating Python scripts and bioinformatics tools including Bakta [6] for genome annotation, ABRicate [7] with NCBI AMRFinderPlus [8], CARD [9], Resfinder [10] databases for AMR gene detection, eggNOG-mapper [11] for functional classification and COG category assignment, and Metagraph [12] for analysis of defense mechanism (COG V) genes. Ultimately, the pipeline enables rapid and reproducible identification of AMR and defense mechanism genes directly from FASTA sequences, facilitating efficient gene content analysis. Future developments will extend the pipeline with additional functional annotation and gene origin inference modules.

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DESIGNING CORE GENOME MLST PROFILE FOR *ACHROMOBACTER XYLOSOXIDANS*

Safina Abdul Razzak[1], Helle Krogh Johansen[2,3], Rasmus L. Marvig[4,5], Migle Gabrielaite[1]

[1]Institute of Biotechnology, Life Science Center, Vilnius University, Lithuania.
[2]Department of Clinical Microbiology, Rigshospitalet, Denmark.
[3] Faculty of Health and Medical Sciences, Institute of Clinical Medicine, University of Copenhagen, Denmark.
[4] Department of Clinical Biochemistry, Rigshospitalet, Denmark.
[5] Department of Health Technology, Technical University of Denmark, Denmark
safina.razzak@gmc.vu.lt

Achromobacter xylosoxidans is an emerging opportunistic Gram-negative pathogen increasingly recovered from chronic respiratory infections, with prevalence rising from ~2–3% in the early 2000s to 20% by 2023 [1-4]. It is detected most frequently in individuals with cystic fibrosis and in healthcare-associated settings, and it commonly exhibits both intrinsic and acquired antimicrobial resistance [1–3]. Current *Achromobacter* spp. identification in clinical settings still relies largely on biochemical assays, MALDI-TOF MS, API N20 typing, or marker-based approaches such as OXA-gene profiling, none of these provide reliable species-level assignment [5]. cgMLST has become a widely adopted approach for high-resolution genomic epidemiology across many pathogens; however, *A. xylosoxidans* lacks a community adopted cgMLST nomenclature resource comparable to those established for other well-studied species [6-7]. Here we aim to develop and validate a species-specific cgMLST scheme for *A. xylosoxidans* using 489 *A. xylosoxidans* whole genomes, enabling standardised allele-based comparisons across the genomes.

Initially, 489 *A. xylosoxidans* assemblies underwent quality control to remove low-quality or incomplete genomes, followed by k-mer entropy filtering to exclude low-complexity sequences. The resulting high-quality genomes were processed with Panaroo (v1.6.0) and chewBBACA (v3.5.1) to construct and compare core genomes. cgMLST scheme development followed the chewBBACA workflow, including Prodigal training, paralog detection, recombination filtering, and evaluation of locus stability using dN/dS. A provisional allele catalog was then iteratively refined to generate a curated, species-specific cgMLST scheme and allele matrix for downstream comparative analyses.

Out of an initial set of 489 *A. xylosoxidans* genomes, after applying assembly quality and k-mer entropy filters, 471 genomes were retained. To improve gene prediction accuracy during schema construction, 50 high-quality genomes were randomly selected to train Prodigal, which subsequently identified 21,603 loci with no paralogous genes. Using core-definition of 95%, 99%, and 100% yielded cgMLST schemes containing 3,875, 2,292, and 780 core loci, respectively. In parallel, Panaroo identified 3,327 core loci using a 95% threshold. chewBBACA retains more loci as core, whereas Panaroo applies a more conservative approach. Despite these differences, we confirmed a strong overlap between the two core sets. The core genome aligns with the expected genomic diversity of *A. xylosoxidans* and is comparable in scale to established schemes for other diverse Gram-negative pathogens such as *Pseudomonas aeruginosa* (3,867 loci) and *Burkholderia pseudomallei* (4,221 loci). Allele profiling across the 489 isolates resolved multiple lineages and offered strong discriminatory power among closely related strains, reflecting the pronounced genomic heterogeneity of *A. xylosoxidans*.

The species-specific cgMLST scheme provides a unified framework for classifying *A. xylosoxidans* isolates, resolving circulating lineages, and distinguishing outbreak-related strains. It enables consistent comparisons across studies and laboratories, improving transmission tracking, population-structure analysis, and detection of emerging clones. This scheme strengthens genomic epidemiology and supports more effective clinical and public-health surveillance.

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H7

SHORT VERSUS LONG-READ SEQUENCING FOR *MYCOBACTERIUM TUBERCULOSIS* GENOME DYNAMICS

Martynas Abraitis[1], Aleksandras Konovalovas[2]
[1]Author, Faculty of Mathematics and Informatics, Vilnius University, Lithuania
[2]Supervisor, Department of Molecular Biology and Biochemistry, Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
martynas.abraitis@mif.stud.vu.lt

Mycobacterium tuberculosis (Mtb) remains a major global health threat, and multidrug-resistant disease continues to challenge clinical control in Lithuania. Short-read whole-genome sequencing is widely used for lineage assignment and drug-resistance prediction; however, it has limited ability to resolve GC-rich repetitive regions and genome-architecture changes. This is particularly relevant for rapidly evolving compartments such as the PE/PPE gene families, which account for roughly 10% of Mtb coding capacity and are often under-resolved in short-read analyses.

The aim of this study was to quantify how short and long-read sequencing differ in the reconstruction of repetitive loci and detection of genome-architecture variation in Mtb, beyond routine SNP-centric genotyping.

Publicly available multi-technology data from the European Nucleotide Archive have been leveraged to analyse 213 Mtb isolates, using sequencing data from multiple approaches. Short and long-read strategies using read-based genotyping for lineage and drug-resistance prediction have been compared, alongside assembly-centred reconstruction to assess genome continuity, access to repetitive loci, and detection of structural variants and rearrangements.

Lineage calls and predicted resistance to first-line drugs have been largely concordant between short and long-read data. The major differences have emerged beyond SNP-centric genotyping: long reads have improved reconstruction across repetitive loci and enabled clearer detection of structural variants and genome rearrangements that are often fragmented or ambiguous with short-read data. These findings show that public multi-technology datasets can be used not only to benchmark routine genotyping but also to quantify how sequencing strategies shape what we can infer about Mtb genome evolution and architectural dynamics.

DIVERSITY OF DOMAINS IN RIBONUCLEASE H FOLD PROTEINS

Jyotika Pachauri[1], Česlovas Venclovas[1], Darius Kazlauskas[1]

[1]Department of Bioinformatics, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania

jyotika.pachauri@gmc.stud.vu.lt

Class 2 CRISPR-Cas effector proteins (C2EPs) such as Cas9 and Cas12 are large, multidomain RNA-guided nucleases that likely evolved from the IS200/IS605 transposon-associated superfamily, IscB and TnpB.[1,2] However, the evolutionary relationships among IscB, TnpB, and C2EPs in terms of domain accretion and architectural diversification remain insufficiently characterized. A key feature conserved among these proteins is the RuvC nuclease domain, a member of the Ribonuclease H (RnaseH)-like fold superfamily and represents the catalytic core responsible for nuclease activity. In this study, we systematically investigated the diversity of domains and protein architectures associated with IscB/TnpB/C2EPs-related proteins by focusing on the conserved RuvC nuclease domain.

We collected a large dataset of Cas9/Cas12/IscB/TnpB representative proteins from published studies and expanded it with new homologs identified through structural similarity searches in the PDB using DALI. These proteins were split into domains, RuvC domains were identified, and homologs were searched in the UniRef50 database using Foldseek.[3] The hits were further divided into domains using boundary predictions provided by the DPAM-AI tool [4] and classified by structural comparison with the ECOD, CATH, and PDB databases. We collected ~68k RuvC homologs, containing ~148k domains, of which ~133k were classified after iterative structural clustering and database searches. From this dataset, we reconstructed the domain architectures of RuvC homolog proteins, calculated the frequency of each unique architecture, and the average domain coverage across all corresponding proteins.

Our results indicate that proteins containing single or double RNase H fold domains are the most abundant, collectively constituting ~55% of all architectures, followed by several cases where this domain architecture integrates other DNA-binding domains, such as winged HTH, MutS domains, and P-loop NTPase domains. Additionally, we identified several protein architectures with <70% domain coverage, suggesting the presence of uncharacterized domains. Overall, our preliminary findings primarily describe the diversity of domain organizations associated with the RNase H fold and establish a structural dataset that can be used for subsequent evolutionary and functional investigations.

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AI-ASSISTED FUNCTIONAL ANNOTATION OF TSEM2 PHAGE

Praneet Prabhanjan[1], Darius Kazlauskas[1]

[1]Department of Bioinformatics, Institute of Biotechnology, Life Sciences Center, Vilnius University,

Lithuania.

praneet.b@bti.stud.vu.lt

Bacteriophages play crucial roles across environmental and clinical domains. Their functional annotation is often limited by sequence divergence. While homology-based methods annotate well-characterized sequences, novel proteins lacking detectable homologs and with unique evolutionary trajectories leave a large fraction of the proteome functionally uncharacterized [1]. In this study, a combination of AI-based structure and functional model predictions [2,3] was employed to investigate these unannotated regions. For initial structural models with low confidence, a structural refinement strategy incorporating custom alignments and modeling parameters was applied. Model quality was assessed using the tessellation-based VoroMQA [4], revealing improved local inter-atomic packing for a subset of previously unresolved proteins. Improved structural models and disorder-function aware AI predictions [5] suggested that some hypothetical proteins exhibit nucleic acid binding potential, which was further supported by high interface scores (iPTM) from complex modeling. Conversely, a subset of hypothetical proteins (<50-100 aa in length) remained unannotated by both sequence- and structure-based approaches. Mathematical representations from Evo2 revealed that some of these sequences cluster with non-coding regions rather than protein-coding. While their biological roles remain unclear, they may represent false-positive predictions from gene callers or sequences unique to this phage. Together, these results indicate that integrating structure-based refinement with AI-based computational models can assist in functional investigation of divergent viral proteins that are otherwise missed by standard annotation methods.

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H10

MAPPING LONGEVITY CLAIMS TO EVIDENCE IN AGING RESEARCH WITH ATOMIC CLAIM UNITS

Arnas Stučinskas[1,2], Audronė Jakaitienė[1,2]
VU Institute of Data Science and Digital Technologies, Vilnius University, Lithuania
Faculty of Medicine, Vilnius University, Lithuania
arnas.stucinskas@mf.stud.vu.lt

Background: Longevity research is expanding rapidly across model organisms, observational cohorts, and clinical trials. Yet claims such as “X slows aging” are hard to evaluate because studies differ in species, populations, endpoints, and the proximity of outcomes to meaningful healthspan. Evidence is therefore scattered and difficult to compare consistently.

Objective: We develop an updatable computational framework that maps longevity-related claims to supporting or contradicting evidence in the biomedical literature. The core representation is an Atomic Claim Unit (ACU): a minimal population–intervention/exposure–endpoint statement with effect direction and study context.

Methods: PubMed records are retrieved and filtered into interventional and observational sets. We extract ACUs from abstracts using schema-constrained large language model prompts, then normalise interventions/exposures and endpoints to canonical labels. Endpoints are assigned to four proximity tiers (E1: survival/mortality; E2: clinical/functional; E3: physiological/imaging; E4: molecular/ageing clocks) and mechanistic endpoints are mapped to the 12 hallmarks of ageing [1]. ACUs are linked back to publications and assigned evidence tiers based on study design (e.g., RCT vs observational) and endpoint proximity. The resulting map enables querying by intervention or outcome across all normalised claims.

Expected results: We anticipate generating a large, continuously updatable set of normalized ACUs that enables: (i) fast search for interventions and outcomes, (ii) systematic identification of agreement and contradictions across studies, and (iii) transparent summaries of evidence strength and indirectness from animal to human endpoints.

Significance: A claim-centric, normalized evidence map can help researchers and non-specialists navigate longevity literature, prioritize interventions for follow-up, and highlight where evidence remains indirect or uncertain.

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MICROBIOLOGY AND BIOTECHNOLOGY

CHARACTERIZATION OF MICROORGANISMS FOUND ON THE SURFACE OF DEAD HONEY BEES

Gintarė Grainytė[1,2], Vilija Lapinskaitė[1], Elena Servienė[1]

[1] State Scientific Research Institute Nature Research Centre, Akademijos str. 2, Vilnius 08412, Lithuania
[2] Life Sciences Center, Saulėtekio av. 7, Vilnius University, Vilnius 10257, Lithuania
gintare.grainyte@gmc.stud.vu.lt

Honey bees *(Apis mellifera)* are primary pollinators that support global agriculture, ensure biodiversity in the natural environment, and contribute to human well-being. Nowadays, the increasing mortality of honey bees has become a serious concern, with one possible cause being the spread of pathogenic and opportunistic microorganisms. Microorganisms found on the surface of dead bees can reflect both environmental microbial contamination and the health status of the colony, as well as potential outbreaks of infectious diseases. The identification of microorganisms found on the surface of dead honey bees is a crucial step for early disease diagnosis, allowing for the optimization of preventive measures and the containment of mass pathogen spread within apiaries.

In this study, cultivable bacterial and fungal microorganisms on dead honey bees were isolated and investigated using microbiological and molecular methods. The honey bees were collected in 2025 early spring from different apiaries across Lithuania (Derviniai, Antazavė, Maniuliškės, Kašėtos, Šakiai, and Vilkaviškis). In the laboratory, honey bees were incubated in MD media, and the collected outwashes were plated on selective media for the isolation of cultivable microorganisms and their identification. Colony size, shape, and color were conducted as well as cells were analyzed morphologically. Subsequently, molecular analyses were performed - gDNA extraction, PCR amplification of marker genes (region between the 18S and 28S rRNA genes for yeasts; 16S rRNA genes for bacteria), restriction fragment length polymorphism (RFLP) analysis using the restriction endonucleases *HinfI* and *HhaI*, electrophoretic visualization of the obtained DNA fragments, and sequencing. The most frequently isolated bacteria on dead honey bees in Derviniai, and Kašėtos apiaries were *Serratia* sp. (98,7%, 75,6%, respectively). The greatest bacterial diversity was found in Vilkaviškis apiary, with dominating *Hafnia* (23%), *Pseudomonas* (14%), and *Enterococcus* (12%) species. Among fungal microorganisms, only *Debaryomyces* sp. was recorded in Šakiai (39%), Vilkaviškis (22%) and Kašėtos (3%) apiaries.

These data are important for understanding the causes of honey bee mortality and for developing effective strategies to protect bee health. This study has received funding from the Research Council of Lithuania, agreement No. S-MIP-24-55.

THE IMPACT OF ESSENTIAL OIL ON THE DIVERSITY OF HONEYCOMB-ASSOCIATED MICROORGANISMS

Urtė Busilaitė[1,2], Živilė Strazdaitė-Žielienė[1], Elena Servienė[1]

[1]State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania
[2]Life Science Center of Vilnius university, Lithuania
urte.busilaite@gmc.stud.vu.lt

Honeybees (*Apis mellifera*) play a vital role in natural ecosystems and agricultural production. In addition to their importance as pollinators, honeybees provide valuable hive-derived products, including honey, propolis and beebread, which are widely used for nutritional and medical purposes. Honeybee colonies harbor diverse microbial communities, due to ubiquitous presence of microorganisms in the environment. In recent years, honeybee populations have declined as a result of multiple stressors, such as pesticide exposure, pathogens, parasites and environmental factors, highlighting the importance of maintaining hive health. Effective hive health management requires the identification and characterization of microorganisms associated with the hive environment. Plant essential oils (EO), hydrophobic mixtures of secondary metabolites derived from plants, are known for their antimicrobial properties. The aim of this study was to analyse the impact of mandarin (*Citrus reticulata Blanco*) essential oil on the diversity of honeycomb-associated microorganisms.

In this study, mandarin essential oil was applied by diffusion inside the hive over one-month period, with two to three drops added weekly to the top of the hive. Following the treatment, honeycomb samples were collected from EO-exposed and control hives and subjected to microorganism isolation. Distinct morphologies exhibiting colonies were purified and identified using molecular methods, including PCR amplification, restriction analysis, and DNA sequencing. In addition, bacterial and yeast cultures grown on solid media were exposed to mandarin EO vapors under laboratory conditions to evaluate their effects on microbial growth. Antibacterial and antifungal activities were assessed by measuring growth inhibition zones.

In control honeycomb samples, three bacterial (*Pantoea*, *Staphylococcus* and *Enterobacter*) and three yeast (*Aureobasidium*, *Debaryomyces* and *Zygosaccharomyces)* genera were detected. Honeycomb samples from EO-exposed hives showed differences in microbial composition, with a higher abundance of Gram-negative bacteria, including *Klebsiella*, *Pseudomonas*, *Serratia* and *Enterobacter*, as well as the Gram-positive genus *Staphylococcus*. In addition, the Gram-positive *Kocuria* was detected while *Pantoea* was no longer observed. Following EO exposure, only two yeast genera (*Aureobasidium* and *Cystobasidium*) were detected, whereas *Debaryomyces* and *Zygosaccharomyces* were absent. Based on sequencing results and literature data, representative bacterial (*Pseudomonas*, *Bacillus*, *Staphylococcus*, *Streptococcus*, *Levilactobacillus* and *Lactiplantibacillus*) and yeasts (*Aureobasidium*, *Debaryomyces*, *Zygosaccharomyces* and *Cystobasidium*) strains associated with *Apis mellifera* were selected for further analysis.

This study indicates that mandarin essential oil diffusion may influence the diversity of microorganisms associated with honeycombs and suggest its potential as a natural antimicrobial in beekeeping. Further research is required to determine optimal application strategies and to evaluate the long-term effects on hive health.

This study has received funding from the Research Council of Lithuania, agreement No. S-MIP-24-55.

I3

YEAST-BASED PRODUCTION OF β-CAROTENE: OPTIMIZATION OF GROWTH CONDITIONS AND CULTURE MEDIA

Erikas Abrutis[1, 2*], Regina Losinska-Sičiūnienė[1], Živilė Strazdaitė-Žielienė[1], Elena Servienė[1]

[1]State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania
[2]Life Sciences Center, Vilnius University, Vilnius, Lithuania
abrutis.erikas135@gmail.com

β-Carotene and other structurally related compounds, including α-carotene, γ-carotene, and lycopene, belong to the carotenoid group. Carotenoids are organic molecules of isoprenoid origin that occur in cells as red, orange, or yellow pigments. Among them, β-carotene is one of the most important carotenoids for human health, as it exhibits anticancer and antimicrobial activities, protects skin cells from oxidative stress, slows skin aging, and may inhibit the development of neurodegenerative diseases. Due to the increasing global demand for carotenoids, considerable interest has emerged in alternative and sustainable production approaches, including carotenoid production using yeast. Yeast-based production of β-carotene is advantageous because it is cost-effective, easily scalable, and more sustainable than traditional sources such as plants or chemical synthesis. However, only a limited number of pigmented yeast species have been extensively studied, and carotenoid yields may be significantly improved through optimization of cultivation conditions. Therefore, the aim of this study was to optimize the growth conditions of little-studied pigmented yeasts and to identify suitable culture media for efficient β-carotene production.

Yeasts were isolated from environmental samples, including water and berries. Based on the morphological characteristics of the cultures, pigmented yeast strains were selected and subsequently identified using molecular biology techniques. In total, eight yeast strains were selected for further analysis (*Cystofilobasidium capitatum, Cystobasidium. minutum, C. psychroaquaticum, Sporobolomyces pararozeus, S. ruberrimus, S. roseus, Erythrobasidium hasegawianum, and Krasilnikovozyma spp.*). The cultivable strains were grown at 4 °C, 16 °C, 22 °C, 26 °C, and 37 °C on various solid media, including PCA, SMA, SDA, CASO, MRS, YEPD, and MHA, to evaluate the effects of temperature and medium composition on yeast growth. Additionally, growth dynamics were assessed in liquid YEPD medium at 22 °C and 26 °C over a 42-hour cultivation period. The results showed that *C. psychroaquaticum*, *Krasilnikovozyma* spp., *S. pararozeus*, *S. ruberrimus*, *C. minutum*, and *S. roseus* exhibited stable growth across a wide temperature range (16–26 °C) on YEPD and MRS media. Moderate growth was observed on SMA and CASO media at these temperatures, whereas growth at 4 °C was minimal or absent for most strains. Only *Krasilnikovozyma* spp. and *S. roseus* demonstrated moderate growth at 37 °C on SMA, CASO, MRS, and MHA media. Growth in liquid YEPD medium was further assessed at 22 °C and 26 °C. During 42 hours of cultivation, *C. psychroaquaticum*, *S. pararozeus*, and *Cystofilobasidium capitatum* grew more efficiently at 22 °C, whereas *S. roseus* and *Erythrobasidium hasegawianum* showed improved growth at 26 °C. In contrast, *S. ruberrimus*, *Krasilnikovozyma* spp., and *C. minutum* exhibited similar growth rates at both temperatures.

Overall, the obtained results provide a foundation for further optimization of cultivation strategies aimed at maximizing pigmented yeast biomass, which can subsequently be used for efficient β-carotene biosynthesis.

DEVELOPMENT OF NOVEL SYNTHETIC APPROACHES FOR THE PREPARATION OF MULTIFERROIC MATERIALS

P. P. Mandal[1], A. Dastankyzy[2], D. Karoblis[2], A. Kareiva[2]

Institute of Chemistry, Vilnius University, Naugarduko 24, Vilnius LT-03225,
FACULTY OF CHEMISTRY AND GEOSCIENCES, INSTITUTE OF CHEMISTRY, CATHEDRAL OF INORGANIC CHEMISTRY
prava.mandal@chgf.stud.vu.lt

Multiferroic materials are compounds that simultaneously exhibit two or more ferroic orders, such as ferroelectricity, ferromagnetism, and ferro elasticity, making them highly attractive for spintronics, data storage, sensors, and energy efficient electronics [1-3]. Among these, hexagonal manganites (RMnO3, where R = Y, In, Sc, Ho-Lu) are of particular interest due to their layered structure and strong coupling between lattice, magnetic, and ferroelectric properties. However, the synthesis of pure hexagonal RMnO3 phases is challenging [4]. Problems such as oxygen stoichiometry control, multiple Mn oxidation states, phase competition, and secondary phase formation often arise [5-8]. To address these issues, soft chemistry approaches offer advantages such as low temperature processing, homogeneous mixing at the molecular level, and precise morphology control.

This study compares two soft chemistry methods, chemical bath synthesis (CBS) and sol-gel polyacrylamide synthesis, analyzing their effectiveness in producing phase pure RMnO3 materials. Main practice objectives:

1) To synthesize hexagonal RMnO3 (R = Y, In, Sc, Ho-Lu) using CBS and sol-gel polyacrylamide methods.
2) To investigate the effects of coprecipitation agents, complexing agents, and annealing temperatures.
3) To compare the phase purity and structural control provided by each method.

The chemical bath method relied on controlled coprecipitation using ammonia in combination with urea or hydrogen peroxide, followed by high temperature annealing. While phase pure YMnO3 could be obtained under optimized conditions, the method showed limited applicability to other rare earth manganites and often resulted in impurity phases. In contrast, the sol–gel polyacrylamide method produced a homogeneous precursor through polymer network formation, enabling improved cation distribution and phase control. This approach allowed the successful synthesis of monophasic hexagonal RMnO3 for most rare earth elements at lower annealing temperatures. Structural and morphological characterization by X-ray diffraction and scanning electron microscopy revealed nanosized particles with porous morphology. Overall, the sol–gel polyacrylamide route proved to be a more versatile and reliable method for the preparation of hexagonal manganites.

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Phenotypic Analysis of Antibiotic-Resistant *Enterococcus faecium* Clinical Isolates

Ernest Oleinik[1], Agnė Kirkliauskienė[2], Marharyta Sytaya[2], Greta Vizujė[3], Jolanta Miciulevičienė[4], Tatjana Kirtiklienė[1]

[1]Microbiology and Biotechnology, Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania.

[2]Faculty of Medicine, Institute of Biomedical Science, Vilnius University, Vilnius, Lithuania

[3]Republic Vilnius University Hospital, Vilnius, Lithuania

[4]Vilnius City Clinical Hospital, Vilnius, Lithuania

ernest.oleinik@gmc.stud.vu.lt

Enterococcus faecium is an opportunistic pathogen that is commonly antibiotic-resistant in healthcare settings and causes severe infections, including urinary tract infections, bacteraemia, and endocarditis [1]. The increasing clinical relevance of *E. faecium* is strongly influenced by the presence of antimicrobial resistance genes, which poses a challenge in infection control and clinical treatment strategies [2].

The aim of this study was to characterize resistance genes and evaluate their phenotypic expressions in clinical *E. faecium* isolates. A total of 59 clinical *E. faecium* isolates were collected from two Lithuanian hospitals between January and November 2024. Antimicrobial susceptibility testing was performed using the disk diffusion method in accordance with EUCAST recommendations, with quinupristin-dalfopristin (15μg), trimethoprim (5μg), teicoplanin (30μg), ampicillin (2μg), imipenem (10μg), linezolid (10μg), ciprofloxacin (5μg), nitrofurantoin (100μg), gentamicin (30μg) and trimethoprim-sulfamethoxazole (1.25–23.75μg) disks. In addition to the disk diffusion method, the E-test was used to characterize and detect vancomycin resistance. Molecular typing using the multiplex PCR method was used to detect genes associated with adhesion, biofilm formation and antibiotic resistance. Biofilm formation by enterococci was determined using microtiter plate method. Statistical analysis of gene and virulence factor distributions and biofilm formation type was performed using the Chi-squared and Fisher's exact tests.

Antimicrobial susceptibility testing showed that all tested clinical *E. faecium* isolates were resistant to imipenem and ampicillin. Linezolid was effective against all tested isolates, whereas ciprofloxacin was active against only one isolate. Resistance to trimethoprim-sulfamethoxazole, trimethoprim and nitrofurantoin was observed in more than half of the tested isolates (73.2%, 66.1% and 66.1%, respectively). Teicoplanin (21.4% resistant isolates), quinupristin-dalfopristin (28.6%) and gentamicin (41.1%) were more effective against the tested *E. faecium* isolates. Evaluation of E-test results showed that most isolates (94.6%) were vancomycin-resistant.

Molecular analysis revealed six distinct vancomycin resistance genes: *vanA*, *vanB*, *vanC1/2*, *vanD*, *vanE*, and *vanG*. *VanA* resistance gene was the most prevalent vancomycin resistance gene, detected in 26 isolates (44.07%). Furthermore, all tested virulence factors (*esp*, *ace*, *acm*, *gelE*, *cylA)* were identified. The most common virulence factor was *acm*, found in 58 isolates (98.3%). The microtiter plate method revealed that 44 isolates (72.13%) formed biofilm, whereas 17 isolates did not. The detection of resistance and virulence genes suggests a potential contribution of genetic traits, elevated biofilm formation and antibiotic tolerance.

This research demonstrates the potential threat of *E. faecium* biofilms to the tolerance of commonly used antibiotics in healthcare institutions and may be linked to underlying genetic determinants and healthcare-associated infections. These findings emphasize the need to design new and effective infection control strategies in clinical environments.

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OPTIMIZATION OF RECOMBINANT LIPOXYGENASE PURIFICATION

Vytautė Blekaitytė, Dovilė Daunoraitė, Inga Matijošytė

Sector of Applied Biocatalysis, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
vytaute.blekaityte@gmc.stud.vu.lt

Lipoxygenases (LOX) are enzymes that catalyse the peroxidation of unsaturated fatty acids into fatty acid hydroperoxides, which serve as key intermediates for the generation of a wide range of value-added compounds. These products are used in diverse applications, including the production of oleochemicals and flavour compounds, and the synthesis of signalling molecules. While lipoxygenases from human and plant sources have been extensively studied, bacterial lipoxygenases have only recently been identified, and their potential remains largely unexplored [1]. To facilitate the investigation and application of bacterial lipoxygenases, access to a purified and catalytically active enzyme is required.

The object of this study is the bacterial *Pseudomonas aeruginosa* lipoxygenase (PaLOX), which is distinguished by its broad substrate spectrum, oxidising linoleic, linolenic, arachidonic, and oleic acids [2]. In previous studies, transgenic *Pichia pastoris* strains carrying the integrative vector with the *palox* gene were constructed. The expression system enabled methanol-induced gene expression and secretion of the recombinant enzyme into the culture medium. Initial purification attempts did not achieve sufficient lipoxygenase purity; therefore, the aim of the present study was to optimise the purification of recombinant PaLOX.

PaLOX was purified from the yeast culture supernatant using immobilised metal affinity chromatography (IMAC) via an engineered histidine tag. Purification conditions were optimised by varying the elution gradient, the composition of purification buffers, as well as the incubation time of the protein with the IMAC sorbent. Protein recovery and purity were evaluated using protein concentration measurements and SDS-PAGE analysis, while catalytic activity was monitored using the lipoxygenase activity assay. A comprehensive presentation of the results will be showcased at the poster session.

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DEVELOPMENT AND STUDY OF A DELIVERY SYSTEM FOR NATURAL COLORANTS

Indrė Bernatavičiūtė[1], Aistė Galinskaitė[1], Gabija Siaurusaitytė[1], Rūta Gruškienė[1]

[1]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania
indre.bernataviciute@stud.vilniustech.lt

In the industrial sector, attention has been given to the use of vegetable residual materials in the production of bio-colorants. Betalain pigments extracted from red beetroot (Beta vulgaris) waste provide a natural alternative to synthetic red dyes. These pigments have been successfully used in commercial food coloring, primarily due to their natural, non-toxic properties; rich color and hydrophilicity. However, as pigments, they are slightly unstable and sensitive to environmental changes, such as pH, oxygen, and light, but mainly temperature [1]. To overcome these limitations, various delivery systems are being developed.

The aim of this work was to increase the stability and functionality of betalains by using κ–carrageenan, one of the most abundant seaweed polysaccharides extracted from marine red algae. κ-Carrageenan has various beneficial biological properties (including antitumor, antihyperlipidemic, anti-aging, anticoagulant and antioxidant activities) and, being biodegradable and nontoxic, is widely used in the cosmetics and food industries. κ-Carrageenan's biocompatibility, mechanical strength, and water-holding capacity support its use as a gelling and stabilizing agent, while low cost and biodegradability enable broad application in encapsulation systems [2,3].

Betalains were extracted from Cylindra beetroot waste by stirring it for 1 hour in water using a rotator stirrer. The resulting extract was mixed with carrageenan solutions of different concentrations. A delivery system mixture containing 50% extract and carrageenan at concentrations of 0.3%, 0.5%, and 1% was processed using either spray-drying or freeze-drying. The acquired samples were stored under various conditions: at 4°C in the dark, at room temperature in the dark and light, as well as at 40 and 80°C in the dark, and their stability was measured at appropriate time intervals. Other properties were also evaluated, including polyphenol content, antioxidant activity and colour parameters.

The resulting encapsulated particles exhibited greater stability in dry form compared to the unencapsulated extract. Under milder conditions, particles stability showed minimal change over four weeks, whereas at 40°C, it decreased to 80% within two weeks. Meanwhile, the unencapsulated extract lost 30–40% of its activity within two weeks even under mild conditions. The stability of the particle solutions increased insignificantly compared to the unencapsulated extract. However, lyophilized and spray-dried capsules containing 0.5% κ-carrageenan showed more promising results compared to the others. A higher content of phenols and antioxidant activity was observed in particles with a lower percentage of carrageenan.

In conclusion, the additional coating of betalains with polysaccharides helps to obtain more functional and stable formulations, which have prospects for wider use.

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Can yeast be used for a biosensor system assessing SARS-CoV-2 major protease activity?

Livija Sakotytė[1], Monika Repšytė[1], Arūnė Verbickaitė[1], Darius Balčiūnas[1,2]

[1]Department of Eukaryote Gene Engineering, Institute of Biotechnology, Life Sciences Center, Vilnius University, Lithuania
[2] Department of Biology, Temple University, Philadelphia, PA 19122, United States

livija.sakotyte@gmc.vu.lt

At the end of 2019, a novel coronavirus disease (COVID-19, Coronavirus Disease 2019) emerged in Wuhan, Hubei Province, China. Its causative agent, severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), is responsible for the global pandemic that emerged in late 2019 and rapidly spread worldwide [1]. During viral replication, the main SARS-CoV-2 protease, Mpro (Major protease), mediates the proteolytic cleavage of viral polyproteins. This protease is indispensable for viral replication and lacks structural homologs in the human genome, making it an attractive target for antiviral drug therapy [2]. With the appearance of resistant variants, concerns about the long-term efficacy of Mpro inhibitors have increased. Mutations in the Mpro active site and surrounding regions have been linked to reduced susceptibility to protease inhibitors, including clinically used compounds, indicating a growing need for the development of novel antiviral inhibitors [3]. To enable rapid and efficient screening of potential Mpro-specific inhibitors, various biosensor-based approaches can be employed. The objective of this study is to develop a fluorescence-based biosensor in yeast and to apply it for the assessment of SARS-CoV-2 Mpro activity.

A sfGFP engineered to contain Mpro protease cleavage sites was co-expressed with either the active or a catalytically inactive Mpro protease. In the inactive variant, the catalytic cysteine at position 145 is substituted with alanine, preventing formation of the acyl–enzyme intermediate and thereby abolishing proteolytic activity [4]. Recombinant protein expression was induced by galactose-regulated promoters. Transformed yeast were cultivated on glucose-containing (non-inducing) and galactose-containing (inducing) media, and fluorescence intensity was evaluated using imaging systems.

Expression of the catalytically inactive mutant (Mpro_C145A) in *Saccharomyces cerevisiae,* Gcn2 strain*,* did not affect fluorescence intensity or cell growth. On the other hand, induction of active Mpro in *S. cerevisiae* resulted in stagnant cell growth, indicating cytotoxicity. Similar growth inhibition upon SARS-CoV-2 Mpro expression in *S. cerevisiae* has been reported recently. Reaserch group from Poland identified that active Mpro caused respiratory suppression and alterations in mitochondrial morphology, suggesting a direct impairment of mitochondrial function [5]. To assess whether the observed cytotoxicity is specific to *S. cerevisiae* or represents a general effect in yeast cells, the same experimental approach was extended to other yeast species, like *Kluyveromyces marxianus* or *Kluyveromyces lactis*. As a phylogenetically related yet physiologically distinct yeast species, *Kluyveromyces* spp. provides a comparative model for evaluating host-dependent effects of Mpro expression. Comparable growth inhibition in both species would indicate perturbation of conserved fungal cellular processes, whereas divergent responses would indicate species-specific effects of Mpro expression.

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BIOFILMS ELECTROGENICITY AS A TOOL FOR *IN-SITU* METABOLIC ACTIVITY MONITORING AND DRUG ASSESSMENT

Rugilė Jonaitytė[1,2], Evelina Lukaitė[1], Marius Butkevičius[2], Eglė Malachovskienė[3], Algimantas Paškevičius[3], Marius Dagys[2], Rokas Žalnėravičius[1,2]

[1]Department of Electrochemical Materials Science, Center of Physical and Technology Sciences, Vilnius, Lithuania
[2]Department of Bioanalysis, Life Sciences Center, Vilnius University, Vilnius, Lithuania
[3]Laboratory of Biodeterioration Research, Nature Research Centre, Vilnius, Lithuania
rugile.jonaityte@gmc.stud.vu.lt

Antibiotic resistance remains a major global public health concern, emphasising the need for reliable and efficient methods to assess bacterial metabolic activity and antibiotic susceptibility [3]. In recent years, electrochemical biosensor-based approaches have emerged as promising tools for rapid monitoring of bacterial biofilm responses to antimicrobial agents [1], [2]. *Staphylococcus aureus* is a clinically important pathogen often associated with persistent infections, while biofilm formation increases antibiotic tolerance and alters metabolic activity [4]. The aim of this study was to evaluate the applicability of chronoamperometry as a simple, inexpensive tool for monitoring metabolic activity and antibiotic susceptibility in *S. aureus*-based biofilms.

Chronoamperometric measurements were performed for 24 h using a three-electrode system consisting of a carbon cloth working electrode, an Ag/AgCl reference electrode, and a titanium foil counter electrode. Experiments were conducted at an applied potential of 0.0 and 0.1 V.

Chronoamperometric profiles revealed antibiotic-dependent changes in current response. Among the tested antibiotics, four, including penicillin G sodium, ampicillin sodium salt, gentamicin sulphate, and erythromycin, exhibited pronounced effects on bacterial extracellular electron transfer (EET). Penicillin G sodium caused the most significant decrease in current (Fig. 1), with mean values declining from 0.259 ± 0.016 μA during active growth (2–6 h) to 0.052 ± 0.007 μA after prolonged exposure (15–22 h), indicating a substantial reduction in electrochemical activity.

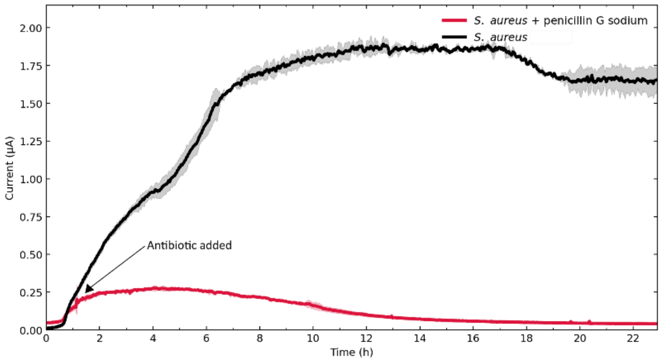

Figure 1. Chronoamperometric investigations of biofilms hosting a carbon cloth electrode in LB media inoculated with $\sim10^6$ *S. aureus* at 35 °C. The Ti foil was used as a counter electrode. The applied potential: 0.1 V.

These findings demonstrate that chronoamperometry enables sensitive, real-time monitoring of bacterial responses to antibiotics and highlights its potential as a rapid method for evaluating *S. aureus* susceptibility.

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PRODUCTION OF ALGINATE MICRODROPLETS FOR PHOTODYNAMIC ANTIMICROBIAL THERAPY

Laura Milaknytė[1,2], Adei Abouhagger[1], Dr. Wanessa De Cassia Martins Antunes De Melo[1]

[1]Department of Functional Materials and Electronics, Center for Physical Sciences and Technology, Lithuania
[2]Life Science Center, Vilnius University, Lithuania
laura.milaknyte@gmc.stud.vu.lt

Chronic wound infections are a major clinical challenge, largely due to the persistence of bacterial biofilms and the increasing prevalence of antimicrobial resistance. Conventional antibiotic therapies often fail to eradicate these infections, highlighting the need for alternative antimicrobial strategies. Antimicrobial photodynamic therapy (aPDT) offers a non-antibiotic approach based on the light-induced generation of reactive oxygen species (ROS), which cause rapid and irreversible damage to bacterial cells without promoting resistance. However, the effectiveness of aPDT depends on precise spatial and time control of photosensitizer activity within the infected tissue [1].

Methylene blue (MB) is a clinically established photosensitizer with broad antimicrobial activity and strong absorption in the red spectral region (660–670 nm), making it well suited for treating infected wounds where deeper light penetration is required [2]. To localize photodynamic action and reduce off-target effects, MB is commonly immobilized in alginate-based hydrogels that are biocompatible and supportive of wound healing. Despite their biological advantages, current alginate systems—typically bulk gels or polydisperse beads produced by batch emulsification—provide limited control over bead size and MB loading. Consequently, photodynamic dosing at the biological level remains poorly defined, and the relationship between ROS generation and antimicrobial effectiveness is difficult to predict. In addition, ROS production is rarely quantified at the level of individual beads, and changes in photodynamic performance over repeated light-activation cycles remain largely unexplored.

In this project, we present a microfluidic strategy to overcome these limitations by enabling controlled delivery and quantitative evaluation of aPDT at the microscale. A water-in-oil droplet microfluidic platform based on a polydimethylsiloxane (PDMS)-on-glass chip with a cross-junction geometry was used to fabricate highly monodisperse alginate microbeads. Droplet formation and calcium-mediated gelation were systematically optimized by adjusting flow conditions, alginate concentration, surfactant content, and crosslinking parameters. The resulting microbeads were characterized by optical microscopy to assess size uniformity and structural stability. Following methylene blue encapsulation, ROS generation was quantified on a per-bead basis under controlled light activation, enabling direct comparison of photodynamic output across monodisperse microbead populations. This approach provides a basis for assessing antimicrobial photodynamic activity against bacterial biofilms, a major barrier in chronic wound therapy.

By combining precise microbead fabrication with quantitative evaluation of photodynamic and biological responses, this work introduces a platform for localized and reusable antimicrobial photodynamic therapy. Such control enables rational tuning of photodynamic performance for clinically relevant conditions. This approach may support future applications in chronic wound care, antimicrobial medical device coatings, and flow-based disinfection systems in infection-prone environments.

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STUDIES ON THE BIOLOGICAL EFFECTS OF IBUPROFEN ON ALIIVIBRIO FISCHERI BACTERIA

Atėnė Petraitytė[1], Virginija Kalcienė[1]

[1]Life Sciences Center, Vilnius University, Lithuania
atene.petraityte@gmc.stud.vu.lt

Ibuprofen is widely used in humans as an antipyretic, analgesic, and anti-inflammatory drug to reduce fever and various types of pain [1]. Since 70–80% of the therapeutic dose is excreted from the body as the parent compound and/or metabolite, ibuprofen is continuously released into wastewater systems. The inefficient removal of ibuprofen by conventional wastewater treatment technologies leads to its frequent detection in aquatic ecosystems [2, 3]. Thus, assessing the ecotoxicological effects of such contaminant on model organisms is essential for evaluating its potential environmental risks.

This study aimed to assess the toxic potential of ibuprofen by exploring a variety of biological endpoints, such as bioluminescence inhibition, viability, antioxidant power, and reactive oxygen species production in the model organism *Aliivibrio fischeri*.

Effects of ibuprofen at a range of different concentrations on bioluminescence was assessed using an acute (30 min) *A. fischeri* microplate assay. Antibacterial properties of ibuprofen were evaluated using a Spot test, while impact on reducing potential was assessed via the ferric reducing antioxidant power (FRAP) assay. ROS production in *A. fischeri* bacteria exposed to ibuprofen was quantified by fluorescence measurement following incubation with fluorogenic probe 2',7'-dichlorodihydrofluorescein diacetate.

The evaluation of the response of *A. fischeri* bacteria to different concentrations of ibuprofen showed a clear negative effect on bioluminescence intensity, with a proportional decrease with increasing ibuprofen concentration. The median effective concentration (EC_{50}) of ibuprofen after 30 minutes was determined to be 2.00 mg/L. The results of a Spot test revealed that ibuprofen inhibits bacterial viability at a minimum inhibitory concentration (MIC) of 6.25 mg/L. Analysis of the reducing power showed a decreasing trend, related both to higher concentrations of ibuprofen and to longer duration of exposure. Ibuprofen at concentrations ranging from 1.56 mg/L to 25.00 mg/L showed a statistically significant reduction in the antioxidant power of *A. fischeri* after 15 minutes, whereas this effect was not statistically significant at other incubation times. Preliminary results of ROS assay indicate that ibuprofen at the range of tested concentrations (0.39 to 25.00 mg/L) induced the formation of reactive oxygen species in *A. fischeri* bacteria; however, this effect was not statistically significant.

The results of this study demonstrate that ibuprofen exerts adverse effects on *A. fischeri*, significantly impairing bioluminescence, bacterial viability, and reducing capacity in a concentration and time dependent manner. The acquired knowledge about the sensitivity of different biological endpoints to ibuprofen is important for selecting appropriate biological methods for early warning systems and developing biosensors sensitive to environmental pollutants.

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I12

ANALYSIS OF (MICRO)PLASTIC BIODEGRADATION-ASSOCIATED GENES IN ENVIRONMENTAL MICROORGANISMS

Alena Koida[1], Gintarė Jakštienė[1], Eglė Lastauskienė[1]

[1]Department of Microbiology and Biotechnology,
Life Sciences Centre, Vilnius University, Lithuania
alena.koida@gmc.stud.vu.lt

Insufficient plastic waste management and plastic's resistance to natural degradation have made plastic pollution one of the greatest environmental issues of the 21st century. Microplastics and nanoplastics formed from larger plastic parts are even more problematic due to an increased surface area and ubiquity. These fragments disrupt aquatic and terrestrial ecosystems and have been shown to adversely affect human health [1,2]. Plastic particles can adsorb and transport hydrophobic organic pollutants, heavy metals, and antibiotics [3,4]. Due to the variability of microbial metabolism, some microorganisms' enzymes can degrade plastic [5]. Large-scale application of these enzymes could be a sustainable solution to mitigate global plastic pollution.

This study aimed to explore natural and artificial sources of microbial genes that encode enzymes associated with the degradation of various types of (micro)plastics. For that, metagenomic DNA from 34 samples of garden and forest soil from Molėtai district, soil and leachate from the operating Kazokiškės landfill, soil from the closed Kariotiškės landfill, as well as plastic particles and sludge from the "Energesman" waste management facility were isolated. The DNA was subjected to PCR analysis for functional genes associated with plastic biodegradation, including alkane hydroxylases, active against pretreated polyethylene (PE); styrene monooxygenases, active against styrene; polyethylene terephthalate (PET)-active cutinases; *Lysinibacillus fusiformis* SCO2 urethanase, active against urethane; and *Pseudomonas chlororaphis* polyurethane (PUR)-active lipase; followed by amplicon sequencing. BLASTx analysis of the amplicon sequences was performed to predict their origin.

It was revealed that the microbiota of garden and forest soil has the potential to degrade pretreated PE, styrene, urethane, as well as to surface-modify PET. This is possible due to the detected presence of alkane hydroxylases, styrene monooxygenases, *Lysinibacillus* urethanase, and *Fusarium solani* cutinase genes. The microbiota from the closed landfill harbors genes encoding *Thermobifida fusca* cutinase 1, alkane hydroxylases, and styrene monooxygenases, active on PET, pretreated PE, and styrene. In the operating landfill, analysis revealed the presence of genes encoding alkane hydroxylases and styrene monooxygenases, indicating potential for biodegradation of pretreated PE and styrene. Microbiota of the "Energesman" waste management facility showed the highest presence of genes associated with plastic degradation, being potentially capable of depolymerizing PET and PUR, degrading urethane and styrene, as well as degrading pretreated PE; harboring genes of *Fusarium solani* and *Thermobifida fusca* cutinases, *Pseudomonas chlororaphis* lipase, *Lysinibacillus* urethanase, styrene monooxygenases, and alkane hydroxylases.

The results of this study prove that prolonged contact of microbiota with contaminants may increase the prevalence and diversity of genes associated with plastic biodegradation; however, broadening the focus of the search to different environments may be reasonable to find new enzymes.

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THE APPLICATION OF KAPPA-CARRAGEENAN FOR NISIN STABILIZATION

Agnė Česnulevičiūtė[1*], Rūta Gruškienė[1], Jolanta Sereikaitė[1]

[1] Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Lithuania
*agne.cesnuleviciute@stud.vilniustech.lt

Nisin (molecular weight of 3.5 kDa), produced by *Lactococcus lactis*, is a well-known food preservative due to its strong antimicrobial activity against Gram-positive bacteria and other spoilage microorganisms. However, its stability can be limited by external factors such as pH changes, temperature variations, and enzymatic degradation. In alkaline conditions, nisin undergoes structural modifications that reduce its antimicrobial activity, while proteolytic enzymes may hydrolyze peptide bonds and cause activity loss. In addition, interactions with food matrix components, such as proteins and lipids, may decrease its bioavailability and effectiveness. To overcome these limitations, biopolymer encapsulation has been proposed as a strategy to improve nisin stability and functionality [1].

κ-Carrageenan is a natural sulfated polysaccharide extracted from red algae, known for its good biocompatibility and biodegradability. Due to the presence of negatively charged sulfate groups, κ-carrageenan is able to interact with positively charged nisin molecules and form stable complexes. These interactions are mainly electrostatic in nature, although hydrogen bonding and hydrophobic interactions may also contribute to system stabilization. Therefore, κ-carrageenan is considered a promising carrier for nisin encapsulation in food systems [2].

The aim of this work was to prepare nisin–κ-carrageenan particles and evaluate the suitability of κ-carrageenan as a carrier for nisin encapsulation. Nisin-loaded κ-carrageenan particles were prepared using the complexation method. It was determined how the encapsulation efficiency depends on the ratio of components used for complexation. The formation of the complexes was confirmed by Fourier-transform infrared (FTIR) spectroscopy. The dominant non-covalent interactions involved in complex formation were identified in order to better understand the stabilization mechanism. The proteolytic resistance of encapsulated nisin was also investigated to assess the protective effect of κ-carrageenan against enzymatic degradation. In addition, release studies were performed to evaluate the behavior of nisin under varying conditions.

The obtained results demonstrate that κ-carrageenan is an effective biopolymer for nisin encapsulation, providing improved stability and protection of the antimicrobial peptide. These findings highlight the potential application of nisin–κ-carrageenan systems in food preservation.

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I14

EXTRACTION OF SOLUBLE DIETARY FIBER FROM BEETROOT WASTE

Gabija Jatkauskaitė[1], Rūta Gruškienė[1], Jolanta Sereikaitė[1]

[1]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania
gabija.jatkauskaite@stud.vilniustech.lt

Every year, about one-third of all food produced worldwide is wasted. A large part of this waste consists of fruit and vegetable processing waste, which account for up to 42% of all discarded food [1]. Nowadays, food waste is not only an environmental challenge but also an untapped opportunity. For example, beetroot processing waste, which is often considered a low-value product, is a valuable source of dietary fiber. Its use allows for the creation of sustainable, natural solutions and gives waste a new, higher technological value [2].

In recent years, beetroot has been receiving increasing attention due to its bioactive components, especially betalain pigments, which have strong antioxidant and anti-inflammatory properties. Beetroot processing generates large amounts of waste, especially pomace, which is rich in dietary fiber whose potential benefits remain largely unexplored. Soluble dietary fiber extracted from beetroot, most of which is pectin, can be used to develop products with higher added value [3]. Pectin is a natural polysaccharide widely used in the food and pharmaceutical industries because of its gelling and stabilizing properties [4].

The aim of this work was to optimize the extraction of soluble dietary fiber from beetroot waste and to characterize the extracted pectin to evaluate its suitability for nisin encapsulation, thereby contributing to the sustainable use of agricultural resources. Beetroot pomace used for pectin extraction was freeze-dried and subsequently powdered. Pectin was extracted using a conventional extraction method and two non-conventional techniques: microwave-assisted extraction (MAE) and pulsed ultrasound-assisted extraction (PUAE). For MAE the beetroot powder was mixed with water acidified with hydrochloric acid (pH 1, 2, and 3) at a solid-to-liquid ratio of 1:10 and exposed to microwaves at power level of 400 W for 30 s. Under optimal conditions (pH 1), the pectin extraction yield was 3.4%. For conventional extraction, the pH 1 condition identified as optimal in MAE was used. The 1:10 mixture was heated at 90 °C for 60 or 120 min. The optimal extraction conditions were pH 1 and 120 min of heating, resulting in a pectin yield of 12.5%. For PUAE, the beetroot powder was mixed with water or acidified water (pH 1 and 2) at a 1:10 ratio and treated with pulsed ultrasound at an amplitude of 80% for 10 min. The optimal extraction conditions were also pH 1, yielding 6.4% pectin. Pectin characterization included measurement of zeta potential to confirm its negative surface charge, Fourier-transform infrared (FT-IR) analysis to verify its chemical structure, determination of total carbohydrate content using the phenol-sulfuric acid method, and assessment of protein impurities using the Bradford method. Thermal extraction results in a sufficient yield of soluble fibers with favorable functional properties, indicating their potential suitability for nisin encapsulation.

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SALT-INDUCED DEAGGREGATION AND ACTIVITY RECOVERYOF GLUCOSE OXIDASE FOLLOWING SOLVENT-MEDIATED AGGREGATION

Nojus Grigorevičius[1], Vygailė Dudkaitė[1], Gintautas Bagdžiūnas[1]

[1] Group of Supramolecular Analysis and Bioelectronics at Institute of Biochemistry, Life Sciences Center, Vilnius University, Lithuania

nojus.grigorevicius@gmc.stud.vu.lt

Protein aggregation is mediated by a complex interplay of noncovalent interactions, driven by conformational changes and environmental stress. This phenomenon is critical across various fields, ranging from the pathology of neurodegenerative diseases, such as Alzheimer's and Parkinson's, to challenges in the food industry and biotechnology.[1] Recently, we observed that glucose oxidase, a specialized enzyme that catalyses the oxidation of D-glucose, aggregates into particles several hundred nm in size following bioconjugation in organic solvents. Notably, while these aggregates exhibit a significant decrease in biocatalytic activity, their tertiary structure remains essentially unchanged.[2]

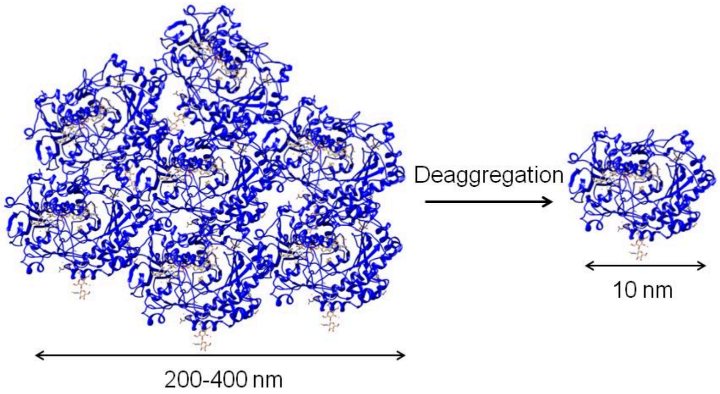

Figure1.The deagregation of glucose oxidase

Inspired by the observations of Singla et al.[3] regarding the salt-induced dissolution of protein clusters, this work investigates the deaggregation of glucose oxidase using various inorganic salts and Triton X-100 as a phase-transfer reagent. We evaluated the biocatalytic activity of the enzyme across the aggregation and deaggregation states. To monitor structural transitions, we employed UV-Vis absorption and fluorescence spectroscopy, along with dynamic light scattering for particle size analysis. These findings will provide a viable strategy for recovering enzyme functionality after the bioconjugation in organic media.

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I16

SELECTION AND FUNCTIONAL ASSESSMENT OF ENDOPHYTIC PLANT GROWTH-PROMOTING BACTERIA FROM WINTER WHEAT (Triticum aestivum L.)

Yuliia Lysak[1], Daiva Burokiene[1], Emilija Eklevaite[2], Dovile Cepukoit[1], Ieva Soke[1], Simona Jaseliunaite[1]

[1]Laboratory of Plant Pathology, The State Scientific Research Institute Nature Research Centre, Lithuania
[2]Life Sciences Centre, Vilnius University, Lithuania
yuliia.lysak@gamtc.lt

The detrimental environmental effects of agrochemicals and pesticides are well documented. Therefore, to promote environmentally friendly agricultural practices and produce safer food, the use of plant growth-promoting bacteria (PGPB) as inoculants represents a promising strategy for sustainable agricultural development. A wide range of PGPB significantly enhance vegetative growth and grain yield in many crops. Their growth-promoting effects are mediated through multiple mechanisms, including nitrogen fixation, production of phytohormones such as auxins, cytokinins, gibberellins, abscisic acid and ethylene; antagonistic activity against phytopathogenic microorganisms via the production of siderophores and hydrogen cyanide; competition for nutrients; induction of systemic host resistance; and enhancement of mineral availability, particularly phosphorus [1] [2].

A key objective in the development of effective inoculation technologies is the evaluation and selection of efficient endophytic bacterial strains. In this study, endophytic bacteria were isolated from winter wheat (*Triticum aestivum* L.).

Plant samples were collected from the April to July 2025 during the wheat growing season. Plant material for bacterial isolation was obtained from four agricultural fields located in eastern Lithuania, with a total area of 4.71 ha. Using culture-dependent methods, a total of 566 culturable endophytic bacterial isolates were obtained from the surface-sterilized root fragments of *Triticum aestivum* L. through plant tissue extraction [3].

The highest number of isolates was obtained from samples collected in April (360 isolates), whereas the lowest number was recorded in June (87 isolates). These findings suggest that periods of intensive aboveground and root system development may be associated with the greater diversity and abundance of plant associated microbial communities.

The purified isolates were stored for subsequent identification to the species level based on 16S rRNA gene sequence analysis. Characterization of selected isolates for indole-3-acetic acid production, phosphate-solubilizing capacity, siderophore production, presence of the *nifH* gen, and their ability to promote growth *in vitro* is ongoing.

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PATATIN PURIFICATION FOR ENCAPSULATION OF BIOACTIVE COMPOUNDS

Vika Valaikaitė[1], Jolanta Sereikaitė[1]

[1]Department of Chemistry and Bioengineering, Vilnius Gediminas Technical University, Vilnius, Lithuania
vika.valaikaite@stud.vilniustech.lt

Proteins are widely used in encapsulation systems due to their ability to form structured matrices, as well as stabilize and protect bioactive compounds from environmental effects. The prospects for the application of protein nanoparticles depend on a number of factors, including versatility, biodegradability, availability, relatively low immunogenicity and cost. Protein nanoparticles can exhibit both protein-specific functions and nanoparticle-specific features, such as controlled release, improved bioavailability and stability. Compared to other nanoparticle platforms, the idea of using proteins to design delivery systems is not a new one. Various proteins are used to form protein nanoparticles and their development is not limited to only natural proteins, since recombinant protein technologies are already being used effectively [1].

In recent years, the food industry has been paying more attention to plant-based proteins in order to create more sustainable food products and reduce dependence on animal-based raw materials. Patatin, the main protein in potatoes, has unique functional properties that make it suitable for use in various food systems. Patatin represents a group of glycoprotein isoforms with a molecular weight ranging between 40 kDa and 45 kDa [2]. This potato protein contains many amino acids that are essential for the human body and is rich in lysine, which is lacking in other edible plants [3]. Due to its excellent foaming, emulsifying and gel-forming properties, patatin is often used in the food and pharmaceutical industries and is also a promising candidate for the protein-based encapsulation of bioactive compounds [3]. The aim of this work was to obtain highly purified patatin for its potential use in protein-based encapsulation systems.

Potato protein powder obtained from Roquette Amilina, UAB was used as a starting material. The sample was dissolved in 25 mM phosphate buffer at pH 6.5 and transferred into dialysis tubing (12-14 kD MWCO). Dialysis was performed against 25 mM phosphate buffer at pH 6.5 to remove low-molecular-weight impurities. Patatin purification was further carried out using sequential ion-exchange chromatography on DEAE-Sepharose and SP-Sepharose resins to obtain patatin fractions. Protein purity was determined by SDS-PAGE. After electrophoretic separation protein bands were visualized using silver staining, which allows sensitive detection of low-abundance proteins. SDS-PAGE analysis showed that the molecular weight of the major band matched the expected size of approximately 40 kDa. The relative purity of the target protein was assessed by analysis of the electrophoretic bands using ImageJ software. Based on the ratio of patatin band intensity to total protein content in the lane, the purity of the isolated patatin was estimated to be around 90 %. Such purity is essential for effective protein-based encapsulation.

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HYPOXIA-INDUCED PLASMA MEMBRANE VISCOSITY CHANGES IN CANCER CELLS AND THEIR RELEVANCE TO CHEMOTHERAPY RESISTANCE

K. E. Kuliešiūtė[1], R. Bagdonaitė[2]

[1] Vilnius University, Faculty of Medicine
[2]Vilnius University, Life Sciences Center, Institute of Biochemistry, Department of Bioelectrochemistry and Biospectroscopy

kotrynakuliesiute@gmail.com

Hypoxia is a hallmark of the tumor microenvironment, driving metabolic reprogramming, lipid remodeling, and therapy resistance [1]. It alters plasma membrane composition, potentially affecting protein mobility, receptor clustering, and drug uptake [2]. While hypoxia-induced lipid changes are well documented, their impact on plasma membrane viscosity remains insufficiently characterized.

The aim of this study was to evaluate hypoxia-induced plasma membrane viscosity changes in 2D and 3D cancer cell models (MCF-7 and MDA-MB-231) using fluorescence lifetime imaging microscopy (FLIM) with the viscosity-sensitive probe BODIPY-PM, and to determine whether these alterations influence doxorubicin penetration.

MCF-7 and MDA-MB-231 cells were cultured in 2D and as 3D spheroids. Hypoxia was chemically induced with $CoCl_2$. Membrane viscosity was measured after 24 h using FLIM, and doxorubicin uptake was evaluated under normoxic and hypoxic conditions.

Our study revealed that in 2D cultures, $CoCl_2$ treatment caused a modest increase in membrane viscosity in MCF-7 cells, whereas MDA-MB-231 cells showed decreased viscosity, possibly reflecting their higher metastatic potential. In 3D spheroids, spatial viscosity heterogeneity was observed. $CoCl_2$ did not significantly alter doxorubicin penetration.

In conclusion, hypoxia modulates membrane viscosity in a cell line and model dependent manner. Although chemical hypoxia did not affect drug uptake, changes in membrane viscosity may contribute to tumor adaptation. Further studies under physiological hypoxia are needed.

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TIGR-TAS-MEDIATED EDITING IN *ESCHERICHIA COLI*

D. Palavenis[1], A. Marčenkaitė[1], G. Faure[2], S. Zhu[2], F. Zhang[2], C. Patinios[1]

[1]LSC-EMBL Institute for Genome Editing Technologies, Vilnius University, Lithuania
[2]Broad Institute of MIT and Harvard, Cambridge, MA, USA

domantas.palavenis@gmc.vu.lt

RNA-guided DNA targeting and cleavage systems such as CRISPR-Cas are among the most powerful genome-editing tools in modern molecular biology. However, CRISPR-Cas systems remain limited by protospacer adjacent motif (PAM) requirements that restrict targetable sequence space, and they are large (~1000 aa), thereby complicating vector packaging and delivery. Recently, Faure et al. (2025) described a novel system termed **T**andem **I**nterspaced **G**uide **R**NA (TIGR) array and a **T**IGR-**as**sociated (Tas) protein [1]. This system shares several key features with CRISPR-Cas systems (RNA-guided DNA targeting and cleavage activity) but lacks the PAM requirement, its activity depends on protein dimerization, and it is small (~300 aa). These properties position TIGR-Tas as a promising platform for programmable genome editing.

Although TIGR-Tas has been used to edit the genome of human cells, its efficacy in bacterial cells has not been evaluated [1]. Given the dual-spacer architecture, the lack of PAM requirement, and the observation that mismatches are tolerated [1], we hypothesized that TIGR-Tas exhibits substantial off-target effects that lead to cell toxicity. To investigate this and assess its suitability as a genome editing tool in bacteria, we systematically characterized multiple TIGR-Tas proteins (TaTasR, ParTasR, and FlaTasA), alongside catalytically inactive variants (dTaTasR and dParTasR), and evaluated their toxicity in *Escherichia coli*, quantified genome editing efficiency, and mapped tigRNA specificity.

Our results identify toxicity as a major constraint of TIGR-Tas deployment. Tas protein expression alone (without tigRNA) imposed a measurable growth burden, indicating intrinsic protein-associated toxicity. Co-expression with tigRNAs further increased this burden, with the strongest effect under targeting-guide conditions. Phenotypically, this was reflected by colony miniaturization and stress-associated morphology, including cell elongation and clumping. Among the tested proteins, TaTasR exhibited the most favorable activity-viability balance, both in the absence and presence of a tigRNA, and was therefore selected for downstream editing experiments. In the presence of a targeting tigRNA and the repair template, TaTasR reached ~10% editing efficiency by colony-based quantification and ~7% by nanopore sequencing. In contrast, non-targeting and catalytically inactive controls showed negligible editing (~0.05-0.1%). Although only 7-10% of surviving cells were successfully edited, most surviving cells carried mutations that enabled escape from otherwise lethal DNA cleavage. To determine the basis of this escape, we sequenced the chromosomal target site and the tigRNA-containing plasmid, revealing mutations at either locus consistent with selective pressure imposed by TIGR-Tas.

Lastly, we constructed and tested a random tigRNA library and assessed the depletion or enrichment of guides with full or partial complementarity to genomic *E. coli* sequences. This enabled identification of true non-targeting tigRNAs, which were highly enriched in our assays.

In summary, TIGR-Tas can support programmable genome-editing in *E. coli*, with TaTasR currently providing the most favorable editing efficiency-viability balance. However, major constraints remain, including editor-associated toxicity and poorly defined tigRNA design rules. Current work focuses on defining tigRNA design parameters for reduced off-target activity and enhanced on-target efficiency, and on Tas protein engineering for reduced toxicity and increased editing efficiency.

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I20

INVESTIGATION OF THE CIRCULARLY PERMUTED TAIL SHEATH PROTEIN gp041 OF BACTERIOPHAGE vB_KleM-RaK2

Miglė Vilimaitė[1], Greta Labutytė[1], Rolandas Meškys[1], Vida Časaitė[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center
migle.vilimaite@gmc.stud.vu.lt

Viral nanoparticles are highly ordered, specific biomaterials characterized by icosahedral or helical structures with an internal cavity, therefore posing as an invaluable nanostructure for various applications in nanomedicine and biotechnology, such as small molecule delivery systems, vaccine development, and gene therapy [1,2]. Particular attention is being paid to expanding the application of self-assembling nanosystems as versatile platforms for the attachment and display of functional amino acids, peptides, or enzymes. Since the native termini of phage-derived protein nanoparticles are not always accessible for functionalization, circular permutation can be used as a protein engineering strategy that rearranges the native amino acid sequence, altering the connectivity between secondary structural elements while preserving the overall three-dimensional structure. This approach can increase the number of available modification sites by creating novel N- and C-termini, which could further improve protein properties and broaden the scope of potential applications [3].

Our research focuses on the development of novel circularly permuted viral nanotubes based on the self-assembling tail sheath protein gp041 from the *Klebsiella*-infecting bacteriophage vB_KleM-RaK2 [4]. AlphaFold3 structural modeling of gp041 protein was used to determine suitable positions for the introduction of new N- and C-termini oriented towards the outer space of the tubular nanostructure. Seven circularly permuted proteins were constructed by joining native termini via a polypeptide linker (GSGGTG), and new termini were introduced at positions N56/C55, N119/C118, N237/C236, N279/278C, N382/ C381, N401/ C400, N574/ C573. After the recombinant protein expression, circularly permuted proteins were purified by sucrose density gradient ultracentrifugation. Transmission electron microscopy analysis revealed that out of seven, four circularly permuted proteins maintained the ability to form ordered tubular nanostructures (N119/C118, N382/C381, N401/C400, N574/C573). While the other three formed structures of irregular shapes (N56/C55, N237/C236, N279/C278). In conclusion, our research revealed that vB_KleM-RaK2 bacteriophage tail sheath protein gp041 can be modified using the circular permutation technique, expanding the application of these self-assembling tubular nanostructures.

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I21

OPTIMISATION OF NANOLUCIFERASE CHEMILUMINESCENCE ASSAY FOR THE STUDY OF NEWLY DISCOVERED ORTHOMYKSO VIRUSES TROPISM

Matas Mikulskis[1], Monika Repšytė[1], Milda Norkienė[1], Gytis Dudas[1]

[1]Department of Eukaryote Gene Engineering, Vilnius University, Lithuania
matas.mikulskis@gmc.stud.vu.lt

Orthomyxoviridae family viruses are defined by their envelope and segmented reverse direction RNA genome whose most infamous members are influenza viruses. Decreased sequencing costs provided new opportunities to study viruses in unusual hosts including more exotic members of *Orthomyxoviridae* more closely related to predominantly arthropod-infecting *Thogotovirus* and *Quaranjavirus*.[1] *Thogotovirus* members are known to infect people (for example Bourbon or Dhori viruses) and their main reservoir are ticks with one exception being SINU virus that was detected in mosquitoes, and which is known for its wide tropism.[2] Most members of *Quaranjavirus* are not known to infect humans, but at least one (Quaranfil virus) was found in blood samples of children reporting flu-like symptoms which led to further epidemiology studies.[3] *Quaranjavirus* and *Thogotovirus* share class III viral membrane fusion protein called glycoprotein 64 (gp64).

Due to their unknown zoonotic potential it is safer to use pseudoviruses for the study of tropism of uncharacterized orthomyxoviruses discovered in arthropods. Pseudoviruses are viral particles that have defective genomes and display membrane fusion proteins on their envelope without encoding them in their genome, thus they are only capable of one infection cycle. In this study we use lentiviral II-nd generation vector system which is comprised of three plasmid vectors (packaging, transgene and envelope) [4] to make pseudoviruses in HEK293T cells. Transgene plasmid encodes pseudovirus genome with signal protein that is inserted into host cell genome to be expressed. To determine the number of viruses in a titer we use TaqMan qPCR allowing us to quantify our infections using the same multiplication of infection (MOI).

When infecting mammalian cells as positive control we use SINU virus gp64 for its proven broad tropism.[5] The negative control is quaranjavirus WuMV-6, since its gp64 alone can’t mediate virus entrance into mammalian cells. Infection efficiency is evaluated via nanoluciferase chemiluminescence assay (Promega). Nanoluciferase is an engineered monomeric luciferase enzyme that originates from the deep-sea shrimp (*Oplophorus gracilirostris*) and maintains high enzyme stability both in living cells and in culture medium. During nanoluciferase assays we noticed a very high background signal and decided to also try secreted nanoluciferase and HiBit, to see if their background noise would be any lower. To enable cells to secrete nanoluciferase, the enzyme was modified by adding human interleukin-6 secretion signal peptide to its N-terminus.[6] Chemiluminescence assays conducted with secreted nanoluciferase do not require cell lysis, thus avoiding signal interference from intracellular molecules. HiBit is an 11 amino acid peptide tag which combines with complementary polypeptide LgBit thus reconstituting luminescent enzyme.[7] For now we want to determine which one of these nanoluciferases is the most precise at quantifying infection efficiency.

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I22

PRION-VARIANT–DEPENDENT TRANSCRIPTIONAL RESPONSES TO GUHCL DURING HEAT AND OXIDATIVE STRESS IN *S. CEREVISIAE*

Neda Jonutytė-Trembo[1], Gvidas Katauskas[1], Eglė Lastauskienė[1]

[1]Department of Microbiology and Biotechnology, Vilnius University, Lithuania
neda.jonutyte-trembo@gmc.vu.lt

Yeast prions are protein-based epigenetic elements that generate heritable phenotypic diversity and can intersect with cellular stress responses. Environmental stress increases the frequency of prion appearance in *Saccharomyces cerevisiae* [1]. Guanidine hydrochloride (GuHCl), widely used in yeast prion research, disrupts prion propagation through effects on the Hsp104 chaperone system [2,3]. Here, we evaluated how GuHCl presence modulates transcriptional stress outputs across three prion phenotypes: [*psi*$^{-}$], weak [*PSI*$^{+}$], and strong [*PSI*$^{+}$].

Cells were challenged with medium and extreme heat shock, and with oxidative stress using 1 mM and 3 mM H_2O_2. Transcript levels of the heat-shock marker *UBI4* [4] and oxidative-stress marker *TRX2* [5] were quantified by RT-qPCR. *MEX67* was used as the reference gene based on stable expression across all stress conditions. GuHCl effects were expressed as relative quantification (RQ) compared with matched −GuHCl controls for each phenotype and stress intensity.

Under heat shock, GuHCl decreased *UBI4* in [*psi*$^{-}$] and strong [*PSI*$^{+}$] at both intensities, with mean RQ values of 0.37 and 0.12 in [*psi*$^{-}$], and 0.25 and 0.079 in strong [*PSI*$^{+}$]. The direction of change was consistent across biological replicates, although effect sizes varied. In weak [*PSI*$^{+}$], GuHCl increased *UBI4* with mean RQ values of 3.75 and 2.16, but replicate consistency was lower, indicating greater biological variability. Under oxidative stress, GuHCl increased *TRX2* across all phenotypes at both 1 mM and 3 mM H_2O_2. Mean RQ values at 1 mM were 4.91 in [*psi*$^{-}$], 7.43 in weak [*PSI*$^{+}$], and 14.46 in strong [*PSI*$^{+}$]. At 3 mM, mean RQ values were 6.79 in [*psi*$^{-}$], 8.86 in weak [*PSI*$^{+}$], and 10.11 in strong [*PSI*$^{+}$]. Replicate direction was consistent across oxidative-stress conditions, with the strongest replicate agreement observed for strong [*PSI*$^{+}$] at 1 mM H_2O_2.

Together, these data show that GuHCl can either dampen or enhance stress-gene transcription depending on stress modality and prion variant state, with oxidative-stress induction of *TRX2* showing higher replicate consistency than heat-shock modulation of *UBI4*.

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THE USE OF OUTER MEMBRANE VESICLESIN TRANSFERRING ANTIBIOTICS TO *ACINETOBACTERBAUMANNII*

Jekaterina Porchun[1], Jūratė Skerniškytė[1]

[1]Department of Biochemistry and Molecular Biology, Vilnius University, Lithuania
jekaterina.porchun@gmc.stud.vu.lt

Acinetobacter baumannii is a Gram-negative opportunistic pathogen that has emerged as a global threat associated with high morbidity and mortality [1]. *A. baumannii* can form biofilms on the surfaces of medical devices and is characterized by multidrug resistance, which makes it one of the main pathogens associated with hospital-acquired infections [2]. Gram-negative bacteria produce outer membrane vesicles (OMVs), which transport nutrients and micronutrients to bacteria. This study aims to use OMV produced by *A. baumannii* to transport antimicrobial drugs. The purpose of this study is to evaluate the efficiency of OMV in transferring gentamicin to *A. baumannii* bacteria at the site of infection.

In this study, a deletion mutant of the *A. baumannii ompA* gene is being constructed by homologous recombination, which produces OMVs that do not contain potential antigenic protein OmpA. An *in vitro* infection model of *A. baumannii* was optimized using A549 lung epithelial cell line. The cells were infected with *A. baumannii* bacteria , and after infection, the bacteria were treated with gentamicin or gentamicin incorporated into OMVs by sonication. Bacterial survival was assessed by CFU counting, and eukaryotic cell viability was assessed by the MTT method.

During the optimization of the infection protocol, it was found that the concentration of gentamicin has a greater effect on the survival of *A. baumannii* than the duration of exposure. At 6 µg/ml gentamicin, the CFU/ml of bacteria was consistently lower than at 3 µg/ml, regardless of the duration of infection, so this concentration was selected as the baseline for further studies. Preliminary data also indicate that gentamicin incorporated into OMV results in lower logarithmic values of *A. baumannii* CFU/ml compared to the treatment with gentamicin alone.

The optimized *in vitro* infection model provides a basis for further studies, when OMVs lacking OmpA will be analyzed. Using selected infection conditions, the ability of OMV to transport gentamicin to *A. baumannii* bacteria at the site of infection will be evaluated, and the immunogenicity of OMV purified from wild-type and deletion mutants will be compared. This study is important for evaluating the potential of OMV as antibiotic delivery system in the fight against multidrug-resistant bacteria.

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Expression and characterization of *Candida albicans* Hsp90N for ligand binding studies

Karolina Skurdelytė, Aurelija Mickevičiūtė, Marius Gedgaudas, Lina Baranauskienė

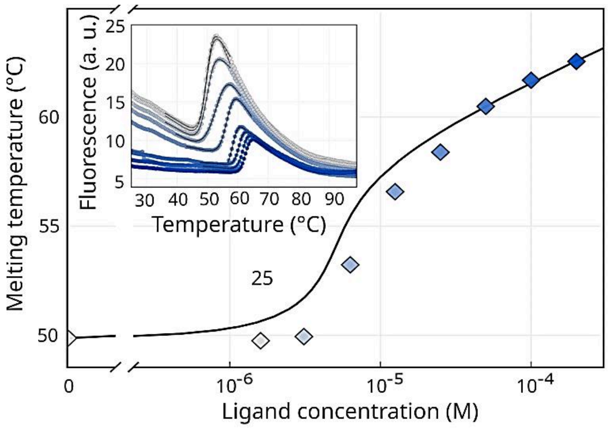
Department of Biothermodynamics and Drug Design, Institute of Biotechnology, Life Sciences Center, Vilnius University
Vilnius, Lithuania
karolina.skurdelyte@gmc.stud.vu.lt

Candida albicans is an opportunistic fungal pathogen in the skin and mucosal microbiome. Despite current treatments, *Candida albicans* infects approximately 400000 people annually and has a mortality rate close to 40% [1]. In humans, this pathogen can cause two types of infections: superficial infections, such as oral or vaginal candidiasis and life-threatening systemic infections [2]. The limited number of antifungal drugs and the rapid emergence of drug resistance highlight the need for new therapeutic strategies [3].

The heat shock protein 90 (Hsp90) is a potential therapeutic target for antifungal drug development. Hsp90 is a molecular chaperone that facilitates the folding of *de novo*-synthesised or misfolded proteins, preventing their aggregation [4]. Hsp90 is the most abundant heat shock protein, constituting 1–2% of the total proteome under non-stress conditions [5]. In the fungal pathogen *C. albicans*, Hsp90 is a key regulator of virulence traits. Moreover, Hsp90 contributes to drug resistance in *C. albicans*. Disruption of Hsp90 inhibits fungal growth or reverses the tolerance of *C. albicans* to traditional antifungal drugs. Targeting Hsp90 is a powerful strategy to combat fungal infections, both as a single-agent therapy and in combination with antifungals to restore their efficacy against drug-resistant pathogens. However, the antifungal utility of current Hsp90 inhibitors is compromised by the toxicity of host Hsp90 inhibition, necessitating the development of fungal-selective compounds [1].

This study aims to produce the recombinant N-terminal domain of *C. albicans* Hsp90 (Hsp90N) for use in fluorescence thermal shift assays (FTSA) to evaluate protein–ligand interactions. Our goal is to identify inhibitors that bind selectively to fungal Hsp90N while showing reduced affinity for the human homolog. To achieve this, we cloned the *C. albicans* Hsp90N fragment (aa 7–218) into a pET15b expression vector containing an N-terminal 6×His tag and transformed it into *E. coli* BL21(DE3) cells for IPTG-induced recombinant protein expression. We purified the protein using Ni^{2+}-immobilised metal affinity chromatography (IMAC) and verified its purity by SDS-PAGE. To characterise the protein, we assessed its thermal stability in different buffers and analysed ligand binding by fluorescence thermal shift assay (FTSA) (Fig. 1).

Figure 1. Recombinant Hsp90N binding to compound ICPD-26 by fluorescent thermal shift assay.



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I25

VALORISATION OF CHEESE WHEY BY PROLONGING FERMENTATION WITH INDUSTRIAL STARTER CULTURES

Dāvis Dūcis[1], Sintija Krastiņa[2], Linda Rozenfelde[1], Kareem Abdulmeguid[1], Dace Pauliņa[2], Indriķis Muižnieks[1], Olga Muter[1]

[1] Faculty of Medicine and Life Sciences, University of Latvia, Jelgavas str.1, Riga LV–1004, Latvia
[2]Joint-stock company "Cesvaines piens" Rūpnīcas str.1, Cesvaine, Madonas county, LV-4871, Latvia
davis.ducis@lu.lv

Around 70-90% of milk used in cheese production is converted into whey. Cheese whey is rich in organic and mineral substances, yet it is only partially used to manufacture high-value-added products [1]. Instead, nearly half of the cheese whey produced in Latvia is discarded, straining wastewater treatment facilities because of its high BOD and increasing waste disposal costs for industry [2, 3]. The capital expenditure (CAPEX) required to establish modern whey-processing lines for permeate, protein, or concentrate production, along with the resulting energy costs, is too high for many SMEs in the dairy business [4].

During cheesemaking, lactic acid bacteria (LAB) are added to milk as a starter culture, along with calcium chloride and a rennet complex [5]. Here, we report an approach to leverage the potential of LAB retained in whey after curd separation, without a substantial increase in processing cost or the need for sophisticated equipment. The approach can be developed to produce new fermented, whey-based beverages.

Non-pasteurized whey was obtained from the first milk coagulation phase of cheese production (JSC “Cesvaines piens”). It contained starter cultures BS-30 and DCC-265 (Chr. Hansen): blends of *Lactococcus, Lactobacillus, Leuconostoc*, and *Streptococcus* strains. After the coagulation, a considerable amount of starter culture 4 × 10^7 CFU/mL remains in the NPW. It can be used for continuing fermentation, provided the necessary physicochemical conditions and nutrients are available. Within a few hours after curd separation, LAB in NPW partially convert available lactose to lactic acid and consume nutrients that limit their proliferation. This results in acidification of the initially sweet cheese whey and hampers further bacterial growth. We investigated the possibility of resuming LAB growth by replenishing critical resources. To prove the concept, we used a complex LAB growth medium, De Man-Rogosa-Sharpe agar (MRS), and K-Na-phosphate buffer to increase the pH of the acidified NPW.

Supplementing acidified NPW with 50% MRS and raising the pH to 6.5 restarted bacterial growth and enzyme production. NPW’s capacity to stimulate enzyme synthesis in Carbon based substrates, especially D-xylose, Itaconic acid, Tween 40, Tween 80 and alpha-cyclodextrin, were demonstrated in EcoPlate™ (Biolog, USA) tests. Fluorescein diacetate hydrolysis, quinone reductase activity, and L-DOPA accumulation indicated antioxidant properties, and synthesis of biologically active compounds within three hours of supplemented NPW cultivation. The temperature - and time - dependence of these features was characterized.

Overall, this first step into the topic suggests a practical and affordable way for dairy producers to make better use of cheese whey. With some optimization, it could lead to simple fermented whey-based beverages and help cut down on waste - all without the need for major investments or complicated equipment.

Acknowledgments: This research was funded by the project “Innovative products containing biologically active peptide fractions derived from fermented cheese whey.”, Contract No. 1.1.1.3/1/24/A/169

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I26

PILOT STUDY OF FACULTATIVE BACTERIAL SYMBIONTS ASSOCIATED WITH *APHIS SPIRAECOLA* (HEMIPTERA, STERNORRHYNCHA: APHIDIDAE) FROM POMOIDEOUS HOSTS (ROSACEAE)

Egidijus Pupius[1], Jekaterina Havelka[2], Rasa Bernotienė[3], Rimantas Rakauskas[2]

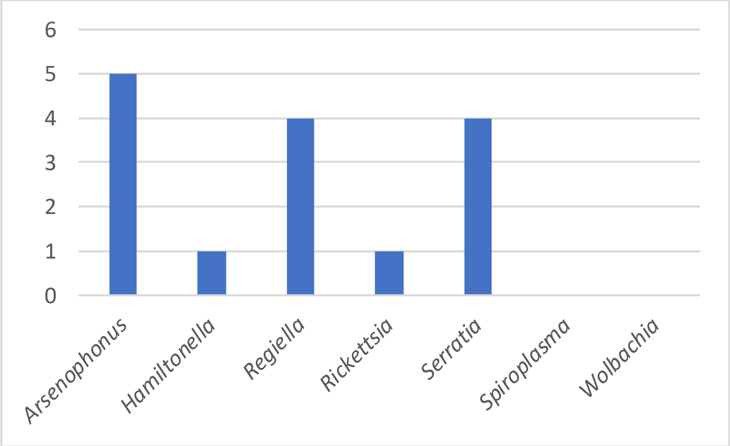
[1]Department of Microbiology and Biotechnology, Vilnius University, Lithuania
[2]Department of Zoology, Vilnius University, Lithuania
[3]Laboratory of Entomology, State Scientific Research Institute Nature Research Centre, Lithuania
egidijus.pupius@gmc.stud.vu.lt

Aphis spiraecola Patch, 1914 is a polyphagous species of aphids alternating between *Spiraea* spp., its primary host, and a wide variety of secondary hosts, also including pomoideous (Rosaceae) species [1]. Symbionts are bacterial or fungal microorganisms that can be obligate or facultative to aphids. Numerous studies showed that facultative (secondary) symbionts have various effects on their aphid host such as resistance to heat shock, parasitoids or fungi [2]. Previous studies of *A. spiraecola* facultative symbionts included samples collected from *Citrus* spp. (Ruthaceae) in Tunisia and analyzed the prevalence of *Arsenophonus* sp., *Hamiltonella defensa*, *Regiella insecticola* and *Serratia symbiotica* [3].

The aim of the present study was to check the prevalence of *Arsenophonus* sp., *H. defensa*, *R. insecticola*, *Rickettsia* sp., *S. symbiotica*, *Spiroplasma* sp. and *Wolbachia* sp. among different populations of *Aphis spiraecola* feeding on plants of the family Rosaceae. Seventeen samples of total genomic DNA extracted from individual aphids collected in Lithuania (n=3), Latvia (n=2), Poland (n=3), Italy (n=2), Turkey (n=2) and China (n=5) were screened for the presence of facultative symbionts using diagnostic PCR with species-specific primers as described by [4-5], and yielding fragments of different length, namely, 1000 bp for *Arsenophonus* sp., 1600 bp for *H. defensa*, 850 bp for *R. insecticola*, 400 bp for *Rickettsia* sp., 500 bp for *S. symbiotica*, 1000 bp for *Spiroplasma* sp., 500 bp for *Wolbachia* sp [4-5].

The most abundant facultative symbiont was *Arsenophonus* sp. detected in 5 samples, followed by *R. insecticola* and *S. symbiotica* found in 4 samples each. There were also 1 sample with *H. defensa* and 1 sample with *Rickettsia* sp.. None of the samples analyzed were found to contain *Spiroplasma* sp. or *Wolbachia* sp. The majority of analyzed *A. spiraecola* DNA samples harboured only one species of facultative symbiont. Coinfection with two species of facultative symbiotic bacteria was observed in 2 samples. Further studies are needed to evaluate the presence and prevalence of these seven facultative symbionts and their proportion in microbiome of *A. spiraecola*.

Figure 1. The distribution of seven facultative symbionts in 17 studied DNA samples of *A. spiraecola*.



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HIGH-THROUGHPUT SINGLE-CELL RT-PCR FROM FIXED BACTERIAL SAMPLES

Laura Kuliešytė, Greta Leonavičienė, Linas Mažutis

Department of Single Cell Analytics, Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania
laura.kuliesyte@gmc.stud.vu.lt

Transcriptomics studies contribute to a better understanding of organisms, their regulatory mechanisms and responses to changing environmental conditions. However, studies at the population level do not take into account the heterogeneity inherent in the population. To probe this heterogeneity, single-cell transcriptomic technology was developed. Despite significant progress in the transcriptomic analysis of eukaryotic cells, the analysis of prokaryotic cells remains relatively constrained [1]. This is due several limitations: most prokaryotes possess a rigid cell wall, complicating the cell lysis, the number of messenger RNA (mRNA) molecules within cells is low and prokaryotic mRNA molecules lack polyadenylation. Additionally, mRNA molecules have a short lifespan, often lasting only a few minutes [2]. Thus, optimizing the fixation of microbiological samples is a crucial step for further transcriptomics research.

In this work, it was tried to optimize the fixation of the microbiological samples with the aim to achieve highest RNA stability. Fixation reagents: formaldehyde, glutaraldehyde and paraformaldehyde (PFA) were evaluated and optimal concentrations and fixation durations determined. After determining the optimal fixation conditions, the goal was to evaluate whether multiple enzymatic reactions could be effectively performed using fixed cells, given that nucleic acid accessibility is reduced. To ensure that, fixed cells were encapsulated into semi-permeable capsules, followed by reverse transcription (RT) and polymerase chain reaction (PCR), which are the primary enzymatic reactions used to analyze cell transcription profiles.

The study found that the most effective cell fixation was achieved using paraformaldehyde and formaldehyde. Higher quality results were obtained with longer fixation durations (16–19 hours). In addition, it was shown that multiple enzymatic reactions could be conducted with fixed cells.

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EXPANDING THE GENUS *XUQUATROVIRUS*: CHARACTERIZATION OF A NOVEL BACTERIOPHAGE

Aušra Semenaitė[1], Lidija Truncaitė[1], Greta Labutytė[1], Rolandas Meškys[1], Aurelija Zajančkauskaitė[1]

[1]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Center, Vilnius University, Lithuania
ausra.semenaite@gmc.stud.vu.lt

Bacteriophages, viruses infecting bacteria, are essential contributors to microbial ecology. Despite decades of phage research, continued hands-on isolation and characterization remain necessary to expand genomic databases and improve taxonomic resolution. Currently, only 26609 out of 95396 *Caudoviricetes* genomes available in the National Center for Biotechnology Information virus database are complete, highlighting the need for further characterization.

This study aimed to isolate and characterize novel bacteriophages infecting laboratory *Escherichia coli* strains.

Environmental samples were collected from the swamp in Stūkai village and screened against laboratory *E. coli* strains using a double-layer agar assay following standard plaque purification procedures. The isolated phage was examined using transmission electron microscopy (TEM). Whole-genome sequencing and automated annotation were performed at SeqVision (Lithuania). The accuracy of annotations was verified using BLASTp, BLASTx, HHpred, and AlphaFold. To ensure accurate taxonomic placement, species delineation was performed according to guidelines provided by the International Committee on Taxonomy of Viruses using VIRIDIC for intergenomic comparison [1].

Here, we present the characterization of a novel *Escherichia* phage vB_EcoP-STUK infecting most of the known *E. coli* laboratory strains. TEM analysis revealed podovirus morphology with a symmetrical icosahedral capsid. Genome analysis revealed a 61435 bp double-stranded DNA genome with 91 predicted coding nucleotide sequences, with no antibiotic resistance, virulence, or anti-CRISPR genes detected. The absence of integrase genes indicates a strictly lytic lifestyle. Despite extensive homology and structure-based analyses, 61 conserved hypothetical proteins remain functionally uncharacterized. Comparative analyses placed this phage within the genus *Xuquatrovirus*, while demonstrating 83.6% intergenomic similarity to its closest relative, bacteriophage Pondi. The genus *Xuquatrovirus* currently contains only two phages (Pondi and PTXU04) and remains poorly studied, limiting our understanding of its genetic and evolutionary diversity. This work expands the known diversity of this genus and provides foundational data for future comparative and functional studies.

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I29

QUANTIFYING SUP35 AGGREGATION WITH GENETICALLY ENCODED SENSOR IN OSMOTIC AND ER STRESS CONDITIONS

Gvidas Katauskas[1], Neda Jonutytė-Trembo[1], Eglė Lastauskienė[1], Audrius Gegeckas[1]

[1]Department of Microbiology and Biotechnology, Institute of Biosciences, Life Sciences Center, Vilnius University, Lithuania
gvidas.katauskas@gmc.vu.lt

Sup35 protein forms amyloid aggregates in yeast cells, called [*PSI*] prion, and affects cell stress response and phenotype [1]. Using flow cytometry and aggregation-sensing genetic construct [2], we track aggregation levels of Sup35 during osmotic and ER stress and the effect of guanidine hydrochloride (GuHCl).

Hsp104 inhibition by guanidine hydrochloride is known to cause Sup35 protein aggregates to grow in size, and formation of these megaDalton-sized aggregates mediates prion curing by disallowing prion fibrils to be inherited by daughter cells. While cellular stress is expected to induce chaperone expression which could fragment [*PSI*] aggregates and propagate prion to daughter cells. In this work, we use yTrap system [2] which works by fusing Sup35 to synthetic transcription factor, the aggregation of this construct “turns on” GFP expression in concentration dependent manner. 74-D694 strain yeast cells were treated with 1 M sorbitol for osmotic stress or 1 mM DTT for ER stress in rich growth media YPD, recovered, fixed and measured on BD FACS A1 flow cytometer. Data was normalized to GFP fluorescence in [*PSI*] prion-lacking cells.

[*PSI*] strong cells treated with osmotic stress and GuHCl alone showed similar GFP+ populations (1.8% vs. 2.7%), but combined treatment increased GFP+ population to 17% of gated cells. Under ER stress, 0.95% of gated cells were GFP positive, while combined GuHCl and DTT treatment increased GFP+ cell population to 10.7% of all gated cells.

In [*PSI*] weak cells, 10.5% GuHCl-treated cells were GFP positive, while under osmotic stress 1.95% of cells were GFP+. Under combined GuHCl treatment and osmotic stress, 27.7% of gated cells were GFP positive. Under ER stress, 3.23% of cells were GFP+, while under combined GuHCl and DTT treatment, 18.9% of gated cells were GFP positive.

Stress increase Sup35 aggregation, as sensed by yTrap system, to a lesser degree than GuHCl treatment. However, combined GuHCl and stress treatment increase aggregation (GFP+ cell population) to a larger degree than either factor alone, with osmotic stress having more pronounced effect on aggregation than ER stress. [*PSI*] prion variant also had effect on aggregation, with weak variant showing more pronounced aggregation than strong variant. The effect of increased aggregation on [*PSI*] prion curing needs to be confirmed by further testing [*PSI*] phenotypic assays and biochemical assays as some of the sensed aggregation could be due to phase separation rather than protein aggregation.

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SCREENING FOR NEW BACTERIAL POLYESTERASES

Greta Namikytė[1], Veronika Mažrimaitė[1], Renata Gudiukaitė[1]

[1]Department of Microbiology and Biotechnology, Institute of Biosciences, Vilnius University, Lithuania
greta.namikyte@gmc.stud.vu.lt

The rising use of plastic results in an appalling amount of waste which is scattered into the environment. Microorganisms and their synthesized enzymes have the potential to reduce this growing problem. These synthesized enzymes, known as polyesterases, are capable of breaking down synthetic polymers such as polyethylene terephthalate (PET). This is one of the most common plastics used to produce bottles, food packaging, clothing, and films. Polyesterases belong to the hydrolase class of enzymes, which can hydrolyze ester functional groups found in polyesters. These enzymes catalyze the breakdown of PET polymer chains into smaller monomers, which can be recycled. Although microbial polyesterases have been identified and increasingly studied, their diversity, catalytic efficiency, and practical applications still remain active areas of research.

In this study, the lipolytic activity of microorganisms isolated from plastic waste was evaluated by hydrolyzing substrates such as PET, tributyrin, and bis(2-hydroxyethyl) terephthalate (BHET). A total of 24 isolates were selected, which were identified by Sanger sequence of the 16S rRNA gene. Based on sequence analysis and hydrolytic activity, two isolates were selected for further study which were assigned to the genera *Ureibacillus sp.* and *Caldifermentibacillus sp*. The selected microorganisms were grown in liquid mM9 media with tributyrin and PET as the only carbon sources. Based on the growth curve, we determined the optimal growth time and the growth phases in which the selected isolates exhibited the highest lipolytic activity. The secreted proteins were precipitated with ammonium sulfate, dialysed, and concentrated. Protein sizes were determined by SDS-PAGE and enzymatic activity was assessed by zymography using tributyrin, PET and BHET as substrates.

The results obtained during the experiment showed that our selected bacterial isolates, *Caldifermentibacillus sp.* and *Ureibacillus sp.*, have the potential to synthesize polyesterases and break down polyesters.

ADVANCED HYBRID NANOPARTICLE PLATFORMS FOR ISCHEMIA

Ugnė Žulpaitė[1], Vilma Petrikaitė[1,2]

[1] Laboratory of Drug Targets Histopathology, Institute of Cardiology, Lithuanian University of Health Sciences, Kaunas, Lithuania
[2] Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania
ugne.zulpaite@lsmu.lt

Ischemia is characterized by insufficient oxygen and nutrient supply, leading to complex biological and physicochemical alterations that can affect molecular transport, stability, and interactions within affected tissues. Among ischemia-related conditions, cardiovascular disease continues to be a leading cause of morbidity and mortality globally [1]. The ability to detect ischemia at an early stage and adjust effective treatment is key to preventing progressive tissue damage and associated complications. Hybrid nanoparticles, which integrate complementary material properties within a single system, offer enhanced functionality, enabling improved interactions with ischemic tissues and supporting both therapeutic and diagnostic approaches within a single platform. Previous studies have demonstrated that nanoparticle-based systems can enhance the visualisation of injured myocardial tissue [2] while simultaneously improving the pharmacokinetic and physicochemical properties of incorporated cardiologic drugs *in vivo* [3]. In this study, hybrid lipid–polymeric nanoparticles (HLPNs) loaded with curcumin (HLPN-CUR) were synthesized by nanoprecipitation using lipid-to-polymer ratios of 1:2 and 1:3.

Poly(lactic-co-glycolic acid), cholesterol, and 1,2-distearoyl-sn-glycero-3-phosphoethanolamine-N-[methoxy(polyethylene glycol)-2000] were used for the synthesis of HLPN. The dynamic light scattering method was used to assess the size, polydispersity index (PdI), and zeta potential of the nanoparticles. Curcumin entrapment in HLPN was analyzed using fluorescence spectroscopy, and the entrapment efficiency was determined from the measured values. Scanning electron microscopy was employed to characterize the morphology, surface structure, and size of the nanoparticles. The *in vitro* curcumin release assay was performed using a dialysis method. The effect of curcumin-loaded nanoparticles on cell viability was analyzed using human fibroblasts under normoxic and hypoxic conditions by MTT assay. Before the MTT assay, cell morphology was examined using a bright-field microscopy.

After curcumin loading, HLPNs exhibited particle sizes of 160 nm (HLPN-CUR 1:2) and 173 nm (HLPN-CUR 1:3), with PdI values of 0.300 and 0.250, respectively. Zeta potentials remained strongly negative at −39.0 mV (HLPN-CUR 1:2) and −40.0 mV (HLPN-CUR 1:3). Curcumin entrapment was slightly higher for the 1:3 formulation (40 %) than for the 1:2 formulation (38.6 %), indicating improved loading with increased polymer content. Scanning electron microscopy confirmed spherical particle morphology with a rough surface. Both formulations exhibited higher curcumin release under acidic conditions compared to physiological pH. *In vitro* cell studies revealed that nanoparticle composition and oxygen availability affected fibroblast morphology and viability, with enhanced cell survival observed for the HLPN-CUR 1:3 formulation under hypoxic conditions at elevated curcumin levels.

In conclusion, the HLPN-CUR 1:3 formulation represents a promising platform for further evaluation as a delivery system for hydrophobic compounds under hypoxic conditions.

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HIGHSCHOOL STUDENTS

NileBright analyzer™ - nanoplastic detector for laboratory and environmental applications

Smilte Vaičiukynė[1,2], Vladimir Orlov[2]

[1]Kauno Jono Jablonskio gimnazija, 10th grade
[2]Kauno tvirtovės VII Fortas,organic chemistry and instrumental analysis education lab.
smilte.vaiciukyne@gmail.com

Plastic pollution is a growing environmental concern, as plastic materials gradually degrade into micro- and nanoplastic particles that disperse in water, soil, and living organisms. These particles can accumulate in aquatic plants and fish and ultimately enter the food chain[1]. Nanoplastics are of particular concern due to their small size and their potential to penetrate biological cells.

At the same time, polymer nanoparticles are increasingly used in scientific research, both as independent nanostructures and as ligands for various molecules, including in environmental pollution studies. Despite the growing relevance of nanoplastics, simple, reliable, and cost-effective methods for nanoplastic synthesis and detection remain limited[2]. This creates challenges for research, environmental monitoring, and the development of practical nanoplastic concentration measurement techniques.

The main objective of this work was to synthesize stable polystyrene nanoplastics under laboratory conditions and to develop a fluorescence-based nanoplastic detection and concentration assay using the hydrophobic dye Nile Red. Polystyrene was selected as a model plastic due to its widespread use, extensive characterization, and frequent occurrence in plastic pollution.

Polystyrene nanoparticles were synthesized via an emulsion polymerization method. Sodium dodecyl sulfate (SDS) was used as a surfactant to stabilize styrene droplets in aqueous solution, while potassium persulfate (KPS) served as the polymerization initiator. The reaction was carried out at elevated temperature with gradual reagent addition to ensure reproducible particle formation. After polymerization, residual monomer was removed, and SDS was replaced with polyvinylpyrrolidone (PVP) to improve nanoparticle stability and suitability for further analysis. Scanning electron microscopy (SEM) and dynamic light scattering (DLS) methods were utilized to enshure quality of performed synthesis. It was shown the mean particle diameter of **80 nm** at concentrations up to 4 mg/mL, demonstrating the effectiveness of the selected synthesis approach as a reliable model system for detection studies.

Based on these results, a nanoplastic detection methodology and system, termed **NileBright™**, was developed. After optimization of the fluorescence conditions (excitation wavelength 535 nm, emission wavelength 635 nm), a near-linear fluorescence response was obtained for 80 nm polystyrene nanoparticles in the concentration range of 5–100 µg/mL. Measurements performed using a commercially available Tecan fluorimeter yielded high signal predictability (R^2 = 0.983), indicating that the method is suitable not only for qualitative detection but also for quantitative analysis and calibration-based applications.

Using the acquired experimental data, a prototype optical bench for the **NileBright™ Analyzer** was constructed employing Thorlabs optical components and an Ocean Optics spectrometer as the detection unit. Initial prototype measurements achieved a reliable signal-to-noise ratio in the concentration range of 20–400 µg/mL, with a coefficient of determination of $R^2 = 0.99$.

Future work will focus on improving detection sensitivity through the application of metal-enhanced fluorescence (MEF) strategies using gold nanoparticles, as well as further refinement of the electronic and optomechanical components of the detection system.

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TYPE G IMMUNOGLOBULIN AND ANIONIC LIGAND COMPLEX ANALYSIS BY ANION EXCHANGE CHROMATOGRAPHY

Kotryna Mieldažytė[1,2], Andrius Jasilionis[3]

[1]Vilniaus jėzuitų gimnazija, Lithuania
[2]Gyvybės mokslų laboratorija, Kauno VII fortas, Lithuania
[3]Division of Biotechnology and Applied Microbiology, Lunds Universitet, Sweden
kotryna.mieldazyte@gmail.com

Immune complexes formed between immunoglobulin G (IgG) and anionic ligands play a central role in the pathogenesis of immune-mediated disorders, most notably heparin-induced thrombocytopenia (HIT) [1,2]. In HIT, IgG-containing complexes arise under specific ligand–protein ratios and trigger downstream cellular activation [2]. In clinical practice, such complexes are predominantly evaluated using indirect immunological or functional assays, including ELISA-based and platelet activation tests, which detect antibody presence or biological effects but provide limited information about the physicochemical properties of the complexes themselves, such as surface charge, heterogeneity, or stability [2]. This creates a gap between biological interpretation and analytical observation of immune complex formation.

The present study addresses this gap by investigating how interaction between IgG and the strongly anionic ligand heparin influences the chromatographic behavior of IgG, using anion-exchange chromatography (AEX) as a charge-sensitive analytical method. The working hypothesis was that formation of IgG–heparin complexes would result in measurable shifts in IgG elution behavior due to redistribution of surface charge, relative to free IgG.

Human IgG was purified from serum by affinity Protein A chromatography, with protocol adaptations aimed at obtaining a structurally intact and homogeneous IgG fraction suitable for analytical studies rather than maximal yield [3,4]. Purity and identity were confirmed by SDS-PAGE, and protein concentration was determined by BCA assay. IgG–heparin mixtures were subsequently prepared under controlled in vitro conditions by varying IgG-to-heparin ratios to generate a spectrum of interaction states, ranging from free IgG to pronounced heparin excess. Prior to AEX analysis, samples were desalted to minimize ionic strength effects that could mask subtle electrostatic interactions [4,6].

Anion-exchange chromatography was performed using a strong anion-exchange column with salt-gradient elution in two buffer systems near physiological pH (Tris pH 7.6 and phosphate pH 7.4) [10,12]. Chromatographic behavior was evaluated using elution profile redistribution and the AUC_ft/AUC_tot ratio as a semi-quantitative descriptor of IgG retention. Increasing heparin content produced reproducible and ratio-dependent shifts in IgG elution behavior, consistent with progressive changes in effective surface charge. Notably, intermediate IgG-to-heparin ratios yielded chromatographic profiles distinct from both free IgG and strong heparin excess, in line with partial charge compensation and heterogeneous complex formation described for pathogenic immune complexes [2,5,6,7]. Buffer composition significantly influenced resolution: the Tris system showed a gradual redistribution of IgG retention across the gradient, whereas the phosphate system revealed discrete chromatographic categories corresponding to free IgG, intermediate interaction states, and heparin excess.

This study demonstrates that anion-exchange chromatography can serve as a controlled experimental platform for detecting affinity changes associated with IgG–heparin complex formation through shifts in chromatographic behavior. While AEX does not permit direct identification or biological classification of immune complexes, it provides a rapid, analytical, and semi-quantitative framework for probing charge-changing interaction trends under well-defined conditions. The observed methodological limitations further support the extension of this approach using mixed-mode chromatographic methods to capture interaction features not solely governed by net surface charge [8].

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EFFECTIVENESS ANALYSIS OF THE EFFECT OF CHEMICAL ANTIMICROBIAL SUBSTANCES ON HOUSEHOLD BACTERIA

Darius Banevičius[1], Greta Gabalytė[2]
[1]Student, VGTU engineering lyceum, Lithuania
[2]Teacher of biology, VGTU engineering lyceum, Lithuania
darius.banevicius@mok.vgtulicejus.lt

Antimicrobial agents are chemical compounds of various origins that inhibit or destroy microorganisms and are widely used in households and medicine due to their effectiveness. The popularity of these substances increased significantly after it was observed that disinfecting surfaces and hands in healthcare facilities reduces morbidity and mortality. During the COVID-19 pandemic, the demand for antimicrobial agents increased even further, and they began to be widely used not only in healthcare institutions but also in public spaces. As a result, the risk of excessive use increased. This study may help address the issue of excessive use of antimicrobial agents. Therefore, the aim of this study was to evaluate the effects of chemical antimicrobial agents on bacteria commonly found in household environments and to determine which of them are the most effective.

During the study, ten samples were collected from different school surfaces: a school desk and a stair handrail, both before and after disinfection with different agents: 3% hydrogen peroxide, an ethanol-based disinfectant, a 0.5% potassium permanganate solution, and a spray containing silver microparticles. The samples were cultured and incubated for four days, after which the number of bacterial colonies was evaluated. The results showed that the ethanol-based disinfectant and 3% hydrogen peroxide were the most effective, while the preparation containing silver microparticles exhibited the lowest effectiveness.

PHARMACEUTICAL CONTAMINATION IN PLANTS: TRANSPORT OF SELECTED COMPOUNDS IN ARUGULA AND RADISH

Dagnė Novikova[1,3] ,Ugnė Jurevičiūtė[2,3], Vladimiras Orlovas[3]

[1]Jonavos Senamiesčio gimnazija, Lithuania
[2]KTU gimnazija, Lithuania
[3]Gyvybės mokslų laboratorija, Kauno VII fortas, Lithuania
dagne.novikova@gmail.com, ugnejur2008@gmail.com

Pharmaceutical products are extensively used worldwide in human medicine, veterinary practice, and livestock production. As a consequence of increasing global population and expanding pharmaceutical consumption, pharmaceutical pollution has emerged as a significant environmental issue [1]. Consequently, analytical methods capable of detecting pharmaceutical compounds at low concentrations are essential. However, many pharmaceutical compounds are not completely removed during conventional wastewater treatment processes, resulting in their continuous release into surface waters, soils, and agricultural environments [2]. Differences in polarity and molecular structure between caffeine and paracetamol may influence their uptake and translocation efficiency.

Among the most frequently detected pharmaceutical contaminants are caffeine and paracetamol, which are widely consumed and commonly identified in environmental matrices [3]. Their presence in agricultural systems raises concerns regarding plant uptake and potential accumulation in edible tissues, creating possible pathways for human exposure through the food chain. Several studies have shown that pharmaceutical compounds can be absorbed by plant roots and translocated to aerial tissues, where they may accumulate depending on plant species and compound properties [4]. Nevertheless, information on the uptake behavior and tissue-specific distribution of such compounds in commonly consumed vegetables remains limited.

Prior to plant experiments, an HPLC–DAD–MS method was developed and optimized for the quantitative determination of caffeine and paracetamol. Quantitative analysis was performed using extracted ion chromatograms (EIC), improving selectivity and reducing matrix interference in plant extracts. Standard solutions of both compounds were prepared to construct calibration curves and to assess method linearity. The method demonstrated excellent analytical performance ($R^2 \approx 0.99$) and enables precise quantification within the established calibration ranges (paracetamol 0.1–10 mg/L; caffeine 0.05–5 mg/L).

Figure 1. Calibration curve of paracetamol.

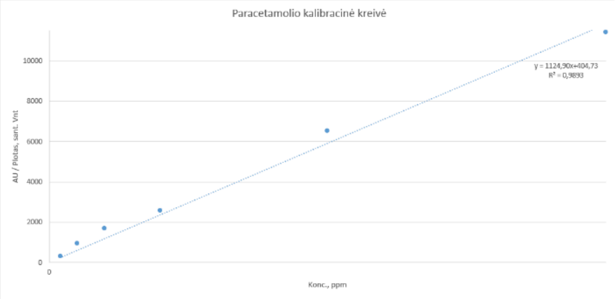
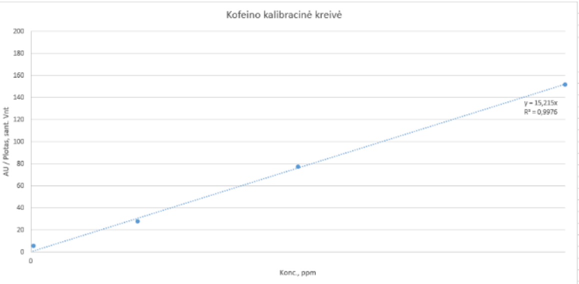


Figure 2. Calibration curve of caffeine.



Arugula (*Eruca sativa*) and radish (*Raphanus sativus*) were cultivated *in vitro* on agar-solidified Murashige and Skoog (MS) medium supplemented with aqueous solutions of caffeine or paracetamol. Control plants were grown under identical conditions on compound-free MS medium to evaluate potential visual growth differences. Both plant species successfully germinated and developed under the applied experimental conditions, and no visible inhibition of early growth was observed in treated plants.

In conclusion, this study establishes a reliable analytical and experimental framework for investigating the uptake of pharmaceutical compounds by plants. The combination of *in vitro* plant cultivation with a validated HPLC–DAD–MS method using EIC provides high accuracy at low concentrations, highlighting the method's ability to enable reliable quantification at low concentration levels and laying a foundation for future studies on pharmaceutical accumulation and tissue-specific distribution in edible crops, contributing to environmental risk assessment and food safety evaluation.

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Synthesis of bioplastic seed coating and analysis by market variables

Ugnė Karečkaitė[1], Marta Marčiulionytė[1]

[1]Kauno Tvirtovės VII fortas, Kaunas, Lithuania
ugne.kareckaite09@gmail.com

Main goal: to synthesise a bioplastic which would heighten the probability of seedling sprouting, and to be on par with the current fertiliser market.

Global plastic pollution and increasingly stringent environmental regulations within the European Union are accelerating the demand for sustainable material solutions in agriculture. This project focuses on the synthesis and performance analysis of a biodegradable bioplastic seed coating derived from renewable biopolymer compositions. The developed coating is designed to enhance seed protection against adverse environmental conditions while minimizing ecological impact. Key challenges in seed germination - including low soil temperature, nutrient limitations, and seed ageing - were addressed through optimising the mechanical integrity and functional properties of the coating. The study evaluates the coating's structural stability, environmental degradability, and its effectiveness in supporting early plant development under varied growth conditions. Starch can be kept in the diverse tissues of plants. This organic starch can then be used in order to make biodegradable plastics by going through various complicated processes [1].

4 grams of starch (potato, corn, tapioca), 2 milliliters of glycerol, and 22 milliliters of distilled water are mixed and heated. 3 milliliters of 0.1M HCl solution is added higher concentration acid would destroy the plastics structure Figure 1. The mixture is heated until thick paste formation and is neutralized to a pH of 7 using 0.1M NaOH, which is tested with a universal pH testing paper. The mixture is spread into a mold and left to dry.

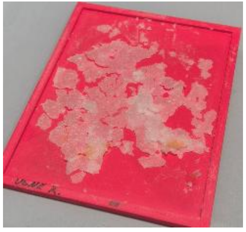

Figure 1. 1M HCl bioplastic structure failure.

After drying, the bioplastics' characteristics are tested. The testing is made up of several different parts to identify the bioplastics quality. Water solubility test: the plastic film is cut into five 2cm^2 pieces and are weighed. The pieces are submerged in 100 millilitres of distilled water and are mixed with a magnetic stirrer at 180rpm, 25°C until the plastic has dissolved (about a few hours). Each plastic film square is dissolved and filtered separately. After filtering and drying they are weighed and their solubility percentage is calculated with Figure 2.

$$W_s\ (\%) = \frac{(W_0 - W_f)}{W_0} \times 100$$

Figure 2. Water solubility formula, where W_s is solubility in water; W_0 is the weight at the beginning of the bioplastics; and W_f is the final weight of the bioplastics [2].

Biodegradability Test - Soil Burial Test: For Biodegradability test specimen is cut into pieces of 4.0 cm^2 . Here soil that is near the roots of plants considered rich in nitrogenous bacteria is used, 500 grams of soil that is bit moist was collected and stored in a flask. Bioplastic sample was submerged inside the soil at a deepness of 2 cm and other buried at a depth of 3 cm for 15 days in atmospheric conditions. The weight of the bioplastic was measured before and after the testing. The biodegradability test was measured by Equation Figure 3.

$$\text{Weight Loss } (\%) = \frac{(W_0 - W)}{W_0} \times 100$$

Figure 3. Where W_0 the weights of samples before and W are after the test [3].

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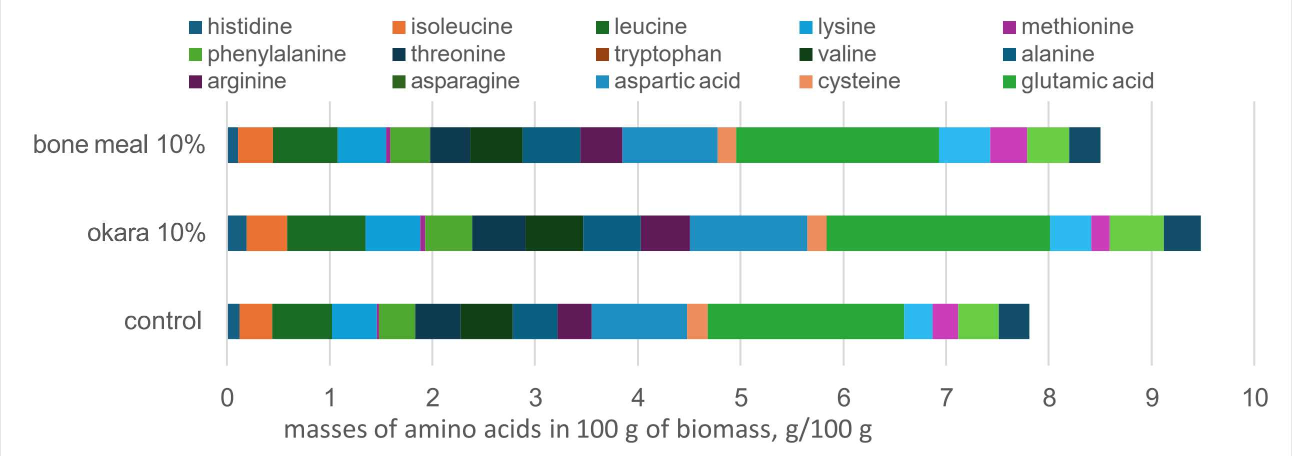
M6

SUBSTRATE-DEPENDENT MODULATION OF AMINO ACID COMPOSITION IN *FUSARIUM VENENATUM A3/5*: AN EXPERIMENTAL AND GENOME LEVEL ANALYSIS

Arielė Rupšė[1], dr. Mikas Sadauskas[2], dr. Vilma Baužienė[3]
[1]Grade 11, Meridiano licėjus, Vilnius, Lithuania
[2]Department of Molecular Microbiology and Biotechnology, Institute of Biochemistry, Life Sciences Centre, Vilnius University, Lithuania
Senior Teacher of Biology, Meridiano licėjus, Vilnius, Lithuania
ariele.rupse@gmail.com

Fusarium venenatum, a filamentous, protein-rich fungus, is a promising microorganism utilised in production of mycoprotein, a sustainable protein source, although the essential amino acid (EAA) profile modulation depending on substrate composition and how this variation relates to genomic characteristics remains understudied. This study presents a novel analysis of the environmental stress-induced effect of okara (soybean residue) and bone meal on *F. venenatum* biochemical EAA composition and its genomic nature. The experimental part consisted of solid-state fermentation, soluble protein and phenol content analyses by spectrophotometry and amino acid profiling by high performance liquid chromatography (HPLC). Bioinformatic genome analysis of 13,946 coding sequences and their encoded proteins was performed to evaluate codon frequency and EAA distribution across the proteome. Results showed that okara 10% did not increase the soluble protein fraction, whereas the EAA concentrations, especially of leucine and phenylalanine were enhanced by up to 150%. Bone meal 10% samples had a minor impact of soluble protein concentration and lower EAA concentrations, likely due to its high mineral content (Fig. 1).

Figure 1. Mass of each essential and nonessential amino acid in 100 g of *Fusarium venenatum* sample.



Preliminary results of genomic analysis suggest that structural variation in EAA distribution across coding sequences may provide data for interpreting substrate dependent EAA variation and poses a possibility of implemented environmental stress influence in EAA variation likely due metabolic regulation. In summary, through substrate modification, the biochemical properties, specifically amino acid composition of *Fusarium venenatum* can be modified and enhanced, displaying its potential of an environmental stress induced protein quality enhancement. As negative effects of highly polluting industries such as livestock manufacture pose significant contribution to climate change, such studies providing optimisation of sustainable mycoprotein production in biotechnological industries may contribute to supporting alternative solutions for mitigating pollution and waste through support of circular food economy.

CENTRIFUGAL SPIN COLUMN REUSIBILITY ASSESSMENT FOR HUMAN IgG PURIFICATION USING PROTEIN A HIGH PERFORMANCE SPIN TRAP

Aukse Armoškaitė, Saulė Gaudėšiūtė, Vladimir Orlov
Kauno tvirtovės VII fortas, Lithuania
armaukse@gmail.com

Immunoglobulin G (IgG) purification is widely applied in biomedical research, diagnostics, and biotechnology. Protein A–based centrifugal spin columns are typically designed for single use; however, their cost may limit accessibility in small-scale or educational laboratories. Evaluating whether such columns can be reused without significant performance loss could reduce expenses and improve laboratory sustainability.

This study aimed to assess the reusability of Protein A High Performance SpinTrap centrifugal columns for repeated IgG purification from the same human plasma sample.

Human plasma was isolated from whole blood by centrifugation. IgG purification was performed using three independent Protein A spin columns. Each column underwent four consecutive purification cycles consisting of binding, washing, elution, and neutralization steps according to the manufacturer's protocol.

Protein composition and purity of eluates were analyzed by SDS-PAGE under reducing conditions. IgG concentrations were quantified using the Bradford assay at 595 nm with bovine serum albumin calibration standards ($R^2 = 0.9969$).

SDS-PAGE analysis revealed dominant protein bands corresponding to IgG subunits under reducing conditions. No visible bands corresponding to Protein A (45–57 kDa) were detected within the sensitivity limits of the method. IgG concentrations across four purification cycles ranged from 0.43 to 1.97 mg/mL. Although variability between columns and cycles was observed, no consistent decrease in IgG yield was detected over four consecutive uses.

The results suggest that Protein A High Performance SpinTrap columns retain functional binding capacity after multiple purification cycles under the tested conditions. Reuse of columns up to four times did not result in substantial reduction of IgG yield or observable purity changes. This approach may contribute to cost reduction and resource efficiency in educational and small-scale laboratory environments. Further validation with larger sample size and statistical analysis is recommended.

ORAL PRESENTATIONS

O1

New *Haemoproteus* lineage (hNECMON01) detected from Hooded Vulture (*Necrosyrtes monachus*) highlights primer selection challenges

Misa Shimizu[1], Oliver J. L. Fox[2], Jez Blackburn[2], Michael Colley[2], Colin Cross[2], Wendy James[2], John Jarju[2], Emmanuel Jatta[2], Naffie Sarr[2], Roger Walsh[2], Stephanie Key[3], Claudio Navarrete[1], Carolina R. F. Chagas[1], Jenny C. Dunn[4]

[1] P. B. Šivickis Laboratory of Parasitology, State Scientific Research Institute Nature Research Centre, Vilnius, Lithuania
[2] Kartong Bird Observatory, Kartong, Kombo South, The Gambia
[3] Faculty of Epidemiology and Population Health, London School of Hygiene and Tropical Medicine, London, United Kingdom
[4] School of Life Sciences, Keele University, Newcastle-under-Lyme, Staffordshire, United Kingdom
misa.shimizu@gamtc.lt

Haemoproteus (Haemosporida: Haemoproteiidae) is an avian blood parasite and is known for its genetic diversity and avian host-specificity [1]. *Haemoproteus* can cause internal organ damage to its vertebrate hosts, which raises concerns about their health impacts [2]. However, their distribution remains largely unexplored in Africa, particularly among non-passerine birds. The impact of *Haemoproteus* infections on avian hosts is also poorly understood, posing a potential threat to endangered species. The sub-Saharan area, including The Gambia, is a biodiversity hotspot with several critically endangered vulture species [3], yet information on their haemosporidian parasites is scarce. This study aimed to identify an effective primer set for detecting Haemoproteus parasites in Hooded Vultures (*Necrosyrtes monachus*) and to provide the first molecular confirmation of infection in this species and country. From 2019 to 2025, Hooded Vultures in The Gambia were captured using cannon nets, and blood samples were taken via venipuncture of the brachial vein. All the samples underwent polymerase chain reaction (PCR) targeting parasite mitochondrial DNA cytochrome b gene (CytB), Among the tested primers, only one set, UNIVF/ UNIVR3, successfully amplified *Haemoproteus* DNA from the Hooded Vulture samples. Sequencing and phylogenetic analyses revealed a novel *Haemoproteus* lineage, named hNECMON01. This represents the first molecular record of avian haemosporidian parasites in Hooded Vultures and in The Gambia. Our findings highlight the importance of optimizing molecular tools for detecting haemosporidian parasites in non-passerine hosts and contribute to our understanding of the distribution and diversity of *Haemoproteus* in sub-Saharan Africa. Further studies on parasite diversity in scavenging birds are essential for integrating parasite monitoring into avian conservation efforts.

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O2

EVALUATION OF EPIGENETIC BIOMARKERS FOR RENAL TUMOUR DIAGNOSIS

Dalia Jurkėnaitė[1], Raimonda Kubiliūtė-Mikalauskienė[1], Eidvilė Masterkovaitė[1], Viktorija Palšytė[1], Karolina Matekonytė[1], Reda Kybartaitė[1], Austėja Lesnickaitė[1], Algirdas Žalimas[2], Sonata Jarmalaitė[1]

[1]Life Sciences Center, Vilnius university, Lithuania
[2]Lithuanian National Cancer Institute, Vilnius, Lithuania
dalia.jurkenaite@gmc.vu.lt

According to the International Agency for Research on Cancer (IARC) data for 2022, Lithuania ranked seventh worldwide in renal cancer incidence and second in mortality [1]. Renal cancer is often asymptomatic in its early stages, and when symptoms do occur, they are typically non-specific; therefore, many cases are diagnosed incidentally, limiting timely intervention [2]. In cases of localized disease, the most common treatment is surgical removal of the tumour by partial or radical nephrectomy, often performed without prior biopsy [3]. This may result in overtreatment, as kidneys are sometimes removed due to benign tumors, significantly impairing patients' quality of life. Despite ongoing research, no molecular biomarkers have yet been approved for routine clinical use to support early diagnosis, risk stratification, or treatment selection. Epigenetic alterations, particularly DNA methylation changes, are considered promising diagnostic and prognostic biomarkers because they can be detected non-invasively in liquid biopsies [4]. The aim of this study is to investigate the methylation levels of six selected genes in urinary sediments of patients with renal cancer and to evaluate their diagnostic and prognostic potential.

In total, 149 urine sediment samples were analyzed, including 104 from patients with malignant renal tumours, 18 from patients with benign renal tumours, and 27 from healthy individuals. DNA was purified using the standard phenol-chloroform method and subsequently modified with sodium bisulfite. Methylation levels of the regulatory regions of selected genes were assessed using quantitative methylation-sensitive PCR.

All six analyzed genes exhibited higher methylation levels in urine sediment samples from renal cancer patients compared to both healthy individuals and patients with benign renal tumours. The sensitivity of individual biomarkers ranged from 50% to 84.6%, while specificity ranged from 75% to 100%. Among the evaluated markers, one demonstrated the highest diagnostic accuracy (AUC = 0.89), with a sensitivity of 64.6% and specificity of 100%. Given the limited performance of single biomarkers, combinations of multiple genes were further assessed. The best-performing combination achieved an AUC of 0.96, with 82.9% sensitivity and 100% specificity. No significant associations were observed between gene methylation levels and clinical or demographic parameters, suggesting limited prognostic value. However, this stability across patient subgroups may support their potential utility as diagnostic biomarkers, particularly for early disease detection.

Overall, methylation alterations in the studied genes demonstrate promising diagnostic potential for renal cancer.

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O3

IMMUNOMODULATORY EFFECTS OF DIOSPYROS PEREGRINA FRUIT PREPARATION IN NSCLC-MEDIATED ANTIGEN PRESENTATION AND T_H CELL DIFFERENTIATION

Nawaneetan Sriraman [1] ·Ankita Sarkar [1] ·Sohom Naskar [1] Nitika Mahajan [1] ·Oishi Mukherjee [1] R. Pradeep [1] ·Melvin George [2] ·Koustav Sarkar [1]

[1]Department of Biotechnology, SRMInstitute of Science and Technology, Kattankulathur, Chennai, Tamil Nadu 603203, India
[2]Department of Clinical Pharmacology, SRMMedical College Hospital and Research Centre, Kattankulathur, Tamil Nadu 603203
nitika.mahajan@gmc.stud.vu.lt

Diospyros peregrina is a dioecious plant which is native to India. It belongs to the family of Ebenaceae and is extensively used to treat various ailments, such as leucorrhoea and other uterine-related problems. Though few studies have been on D. peregrina for their anti-tumour response, little is known. Therefore, this intrigued us to understand its immunomodulatory capabilities on various types of cancer extensively. Our primary focus is on NSCLC (NonSmall Cell Lung Cancer), which is ranked as the second largest form of cancer in the world, and the treatments demand non-invasive agents to target NSCLC effectively. In an objective to generate an efficient Lung Cancer Associated Antigen (LCA) specific anti-tumour immune response, LCA was presented using dendritic cells (DCs) in the presence of D. peregrina fruit preparation (DFP). Moreover, we also investigated DFP's role in the differentiation of Thelper (TH) cells. Therefore, this study aimed at better LCA presentation mediated by DFP by activating the LCA pulsed DCs and T helper cell differentiation for better immune response. DCs were pulsed with LCA for tumour antigen presentation in vitro, with and without DFP. Differentially pulsed DCs were irradiated to co-culture with autologous and allogeneic lymphocytes. Extracellular supernatants were collected for the estimation of cytokine levels by ELISA. LDH release assay was performed to test Cytotoxic T lymphocytes (CTLs) mediated lung tumour cell cytotoxicity. Thus, DFP may be a potential vaccine to generate anti-LCA immune responses to restrict NSCLC.

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O4

WHO GETS HURT AND WHY? INJURY PATTERNS IN SCOOTER RIDERS

Eva Staponkutė[1], Diana Vasiljevaitė[2], Sigitas Chmieliauskas[2], Sigitas Laima[2]

[1]Faculty of Medicine, Vilnius University, Lithuania
[2]Department of Pathology, Forensic Medicine, Institute of Biomedical Sciences, Faculty of Medicine, Vilnius University, Lithuania
eva.staponkute@gmail.com

Since the introduction of electric scooters in early 2019 [1], Lithuania has faced a mounting public health issue. To date, emergency departments continue to report surging scooter-related injuries [2]. Scooter riders operate in several environments, including roadways, bicycle lanes, and pedestrian areas, which may lead to unique injury patterns. However, the gap in understanding the characteristics and injury mechanisms of scooter-related accidents leaves policymakers without complete data to develop effective safety interventions. Recognising distinct injury patterns in scooter riders has become critical, as current guidelines struggle to regulate this proliferating mode of transport.

The objective of this study was to examine injury patterns in scooter-related accidents. The study aims to determine how different mechanisms of injury influence the type of trauma and its anatomical distribution, while further identifying factors determining trauma severity.

The medical records of patients were obtained from the Lithuanian State Forensic Medicine Service database from 2019 to October 2025 for retrospective analysis. The statistical analysis was performed with R Commander (version 4.5.2). The following variables were analysed: age, sex, month, use of protective equipment (helmet), alcohol use, trauma mechanism, type of injury, trauma region and anatomical location.

This study included 58 scooter riders, of which 74.1% were men. The median age was 30 years (Q1 15.50, Q3 40.75), although individuals aged 10 to 19 and 30 to 39 years were predominant in this group. Accidents were higher during August and September. At the time of the accident, 20.69% of riders were under the influence of alcohol. No statistical significance in risk factors, such as alcohol consumption or helmet use, was found (all $p > 0.05$).

Almost half of the external injuries were abrasions (49.68%) and mostly affected the head (35.87%), specifically the forehead and eye region (10% each). There were significant differences in trauma mechanisms among injury types ($p = 0.003$). Vehicle collisions resulted in a higher incidence of lacerations, while falls from scooters led to more abrasions. The head was significantly more likely to sustain lacerations, whereas limbs were more frequently affected by abrasions ($p < 0.0001$). Gender distribution showed that significantly more pelvic injuries occurred in women than men ($p = 0.014$). Regarding injury severity by region, minor injuries were more likely to occur in the back and less frequently in the pelvis, while the upper limb sustained more moderate than severe injuries ($p = 0.0164$). Further analysis of the specific anatomic location showed that minor injuries involved the back and lip area, moderate injuries affected the upper hand and palm region, and severe injuries involved unspecified sites of the lower limb ($p = 0.042$).

Regarding internal traumas, the most common injury type was fractures (68.14%). The mechanism of injury showed no significant association with internal trauma type ($p > 0.05$). Riders frequently sustained lower limb injuries (23.33%), primarily from vehicle collisions ($p < 0.0001$), with the majority being moderate injuries ($p = 0.0003$). The head represented the most injured anatomical site (33.63%), with men sustaining significantly more head injuries than women ($p = 0.032$). Notably, only 2 riders were documented as wearing a helmet. Analysing the specific anatomic location further, there were significant differences between trauma mechanisms ($p = 0.003$). Surprisingly, dental trauma, injuries to the base of the skull and the maxilla occurred more frequently from collisions with bicycles than with vehicles. Calvarial and brain injuries are most often associated with falls from scooters, while collisions with pedestrians result in ulnar and scapula injuries. Severity of injury was also significant ($p < 0.0001$), with dental traumas classified as minor, the radius as moderate, and severe injuries occurring in the brain and lungs.

Scooter-related injuries present distinct patterns between the mechanism of injury, trauma type, region, anatomical location, and trauma severity. Internal traumas primarily consisted of moderate lower limb injuries and severe injuries to the head. The head region also accounted for most external injuries, emphasising the importance of wearing a helmet. Most of these injuries occurred due to collisions with vehicles, although collisions with bicycles also posed a risk of head trauma. This demonstrates the need for improved urban infrastructure and clearer traffic regulations for scooter use.

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O5

EXPLORING GUT MICROBIOME AND BACTERIAL METABOLITES IN BREAST CANCER PATIENTS UNDERGOING NEOADJUVANT CHEMOTHERAPY

Elza Elizabete Liepina[1], Elina Sivina[1,2], Lelde Jurkane[1,3], Zanda Daneberga[1,4]

[1]Institute of Oncology and Molecular Genetics, Riga Stradins University, Latvia
[2]Solid Tumor Systemic Treatment Clinic, Riga East University Hospital, Latvia
[3]Clinic of Oncology, Pauls Stradins Clinical University Hospital, Latvia
[4]Department of Biology and Microbiology, Riga Stradins University, Latvia
ElzaElizabete.Liepina@rsu.lv

Responses to neoadjuvant chemotherapy (NAC) remain highly variable among breast cancer patients [1, 2]. In current clinical practice NAC efficacy prediction relies predominantly on tumor-intrinsic and tissue-based biomarkers, however they only partially explain observed inter-individual variability in treatment response, underscoring the need for additional research [3]. In recent years, the gut microbiome has emerged as a potential noninvasive biomarker and a therapeutic target, representing a new frontier for personalizing chemotherapy and immunotherapy strategies and improving patient outcomes across several malignancies, while its role in breast cancer remains underexplored.

This study aimed to characterize microbiome composition, functional potential, and microbially derived metabolites in breast cancer patients undergoing NAC. Baseline stool samples from 39 chemotherapy-naive breast cancer patients undergoing NAC were analysed using shotgun metagenomic sequencing and targeted metabolomics. Patients were stratified by pathological complete response (pCR, *n* = 17; no pCR, *n* = 22). Microbial taxonomic and functional profiles, short-chain fatty acids (SCFAs) and bile acids were assessed, with subgroup analysis performed in triple-negative breast cancer (TNBC).

Patients achieving pCR exhibited significantly higher baseline microbial richness compared to non-responders ($p = 0.040$). Differential abundance analysis revealed higher abundance of 3 known genera in responders, while 7 genera

deoxycholic acid (DCA) levels in non-responders and elevated caproic acid (C6) levels in responders, although these differences did not remain statistically significant after correction for multiple comparisons. Similar trends were observed in TNBC subgroup (n = 15).

Our results show exploratory associations between gut microbial diversity, specific taxa, and metabolite signatures, and response to neoadjuvant chemotherapy in breast cancer. Despite limited size, trends in the TNBC sub-cohort support potential biological relevance. Larger studies in more homogeneous, independent cohorts and functional validation are needed to confirm these findings and evaluate the microbiome and its produced metabolites as non-invasive predictive biomarkers.

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IMPACT OF POLYSACCHARIDE CAPSULE ON ANTIBIOTIC RESISTANCE IN *ACINETOBACTER BAUMANNII*

Dominykas Grigorjevas[1], Jūratė Skerniškytė[1]

[1]Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
dominykas.grigorjevas@gmc.stud.vu.lt

Multidrug resistance is one of the most pressing global health crises today. Infections once easily treatable are now more difficult to manage, routine medical procedures have become high-risk operations, and recovery times have substantially increased [1]. The World Health Organization (WHO) compiled a list of priority multidrug-resistant pathogens, classifying *Acinetobacter baumannii* as a critical pathogen [2]. *A. baumannii* is an opportunistic pathogen capable of forming biofilms and a polysaccharide capsule, both of which contribute to its ability to cause infections and withstand treatment [3]. Although this pathogen is clinically relevant, there is a lack of understanding of its antibiotic resistance mechanisms and virulence factors.

This study aimed to evaluate the impact of the polysaccharide capsule on antibiotic resistance in both planktonic cells and biofilms, as well as to assess capsule gene expression in the presence of antibiotics. The effect of the polysaccharide capsule on planktonic cell antibiotic resistance was evaluated by comparing the minimal inhibitory concentrations (MICs) of 12 antibiotics (kanamycin, streptomycin, gentamicin, chloramphenicol, tetracycline, colistin, ampicillin, cefazolin, imipenem, meropenem, and a combination of sulfamethoxazole/trimethoprim) between the wild-type isolate and its capsule-deficient mutant (Δ*galU*). The effect of the capsule on antibiotic resistance in biofilms was assessed by forming biofilms of the wild-type isolate and its capsule-deficient mutant, exposing them to antibiotics, and evaluating colony-forming units (CFUs). The expression of the *galU* gene, which is critical for polysaccharide capsule formation, was measured in response to antibiotics using RT-qPCR.

The results showed that planktonic cells of capsule-deficient *A. baumannii* mutant were more vulnerable to gentamicin, tetracycline, and colistin. Biofilms of the capsule-deficient mutant exhibited reduced CFU counts when exposed to tetracycline. However, colistin had a greater effect on the capsule-forming wild-type isolate than the Δ*galU* mutant, which could be explained by the mutant's ability to form thicker biofilms. In response to tetracycline and colistin, bacteria increased *galU* gene expression in both planktonic cells and biofilms, suggesting increased polysaccharide capsule production. These findings highlight the multilayered dynamics among the polysaccharide capsule, biofilms, and antibiotic resistance, which warrant further investigation in future research.

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O7

IDENTIFICATION OF A NOVEL 2′-*O*-METHYL NUCLEOSIDE DEMETHYLATION REACTION AND ITS ASSIGNMENT TO HUMAN PHYHD1 AND OTHER PHYH-LIKE OXYGENASES

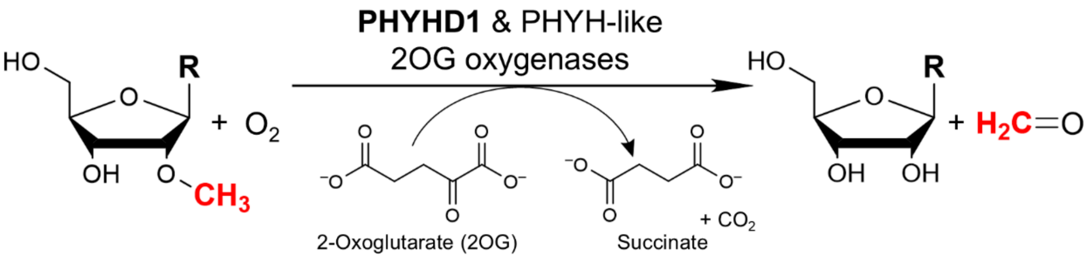
Justas Stonkus[1], Rasa Rutkienė[1], Rita Meškienė[1], Agota Aučynaitė[1], Justas Lazutka[2], Darius Balčiūnas[2,3], Giedrius Vilkaitis[2], Rolandas Meškys[1]

[1]Institute of Biochemistry, Life Sciences Center, Vilnius University, Vilnius LT-10257, Lithuania
[2]Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius LT-10257, Lithuania
[3]Department of Biology, Temple University, Philadelphia, PA 19122, United States
justas.stonkus@gmc.stud.vu.lt

RNA chemical modifications are post-transcriptional alterations to RNA base or ribose components that serve as a crucial regulatory layer, influencing RNA stability, folding, and translation. Among the plethora of known modifications, 2′-*O*-methylation (Nm) is one of the most common and is found across diverse RNA species in all known domains of life, as well as in RNA viruses. In recent years, considerable progress has been made in mapping Nm sites and elucidating their biological roles [1]. However, the fate of 2′-*O*-methylated nucleosides, metabolites produced by RNA hydrolysis, has been studied to a lesser extent. Crucially, the enzymatic pathways responsible for the catabolism of 2′-*O*-methylated nucleosides remain largely unexplored, as until our study, only a few bacterial nucleoside hydrolases have been characterized.

Therefore, we have been trying to identify novel enzymes active towards 2′-*O*-methyl uridine/cytidine, using the auxotrophic *Escherichia coli* DH10B Δ*pyrFEC* strain. During one of these searches, we identified a novel 2′-O-methylated nucleoside demethylation reaction catalyzed by a bacterial 2-oxoglutarate- and Fe(II)-dependent dioxygenase, which we named FJS. Following this, we assigned this activity to FJS-related enzymes annotated as members of the phytanoyl-CoA dioxygenase (PHYH) family or as phytanoyl-CoA dioxygenase domain-containing protein 1 (PHYHD1). Whilst human PHYH has a clearly defined function, previous studies had not identified the primary substrate of human PHYHD1 [2].

Figure 1. Reaction scheme for the PHYHD1-catalyzed demethylation of 2′-O-methylated nucleosides.



R - purine or pyrimidine base

We found that, like FJS, eukaryotic PHYHD1 dioxygenases demethylate a broad range of ribose-methylated nucleosides (Fig. 1) but do not demethylate modified nucleotides or RNA *in vitro*. To determine whether the loss of PHYHD1 function affects 2′-*O*-methylation levels of RNA-incorporated or free nucleosides, we generated a *phyhd1* knockout (*phyhd1*-KO) zebrafish line. While we observed no visible phenotype changes and the RNA modification levels remained unchanged, the amounts of free 2′-*O*-methylated inosine, guanosine, cytidine, and uridine were significantly elevated in both *phyhd1*-KO embryo and adult zebrafish extracts. These findings indicate that PHYHD1 does not directly demethylate RNA but instead functions in the turnover of free 2′-*O*-methylated nucleosides [3]. Therefore, our study identifies a previously unrecognised metabolic pathway for 2′-*O*-methylated nucleosides and defines PHYHD1 as the key dioxygenase involved in their demethylation, providing new insights into the catabolism of modified nucleosides.

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GRASSLAND FUNGI IN LITHUANIA: ECOLOGY AND INDICATORY SYSTEMS

Rūta Urbonaitė[1], Reda Iršėnaitė[2]
[1]Vilnius University, Life Sciences Center, Bioscience Institute, Botany and Genetics Department
[2]State Scientific Research Institute Nature Research Centre, Laboratory of Mycology
ruta.urbonaite1978@gmail.com

Fungi are fundamental components of terrestrial ecosystems, functioning as primary decomposers of organic matter, key participants in nutrient cycling, and important symbiotic partners of plants [1]. Certain fungi have long been recognised as valuable bioindicators of grassland quality, reflecting long-term continuity, nutrient-poor conditions, and low-intensity management of semi-natural grasslands [2]. In Europe, particular attention has been given to a group of macrofungi collectively known as CHEGD (*Clavariaceae*, *Hygrocybe*, *Entoloma*, *Geoglossaceae*, *Dermoloma*), whose presence and species richness are used to assess grassland ecological value and habitat quality [2,3]. Indicator-based approaches relying on these fungi have been applied for several decades in various European countries, however, comparable studies focusing specifically on grassland mycobiota have not previously been carried out in Lithuania.

The aim of this study was to investigate the fungal mycobiota of steppe (6210) and xeric sand calcareous (6120) grasslands representing habitat types defined under the EU Habitats Directive in Lithuania [4], in order to improve understanding of their ecological patterns and to establish a baseline for the development of a fungal indicator methodology adapted to Lithuanian conditions. The study combined herbarium data analysis with field surveys and environmental measurements.

Fungal specimens from the herbarium of the Nature Research Centre (BILAS) were reviewed, digitised, and analysed using RStudio. Field data were collected from nine grassland sites using a standardized transect method [5]. In parallel, grassland quality was assessed using the CHEGD-based indicator approach commonly applied in the United Kingdom, which relies on threshold numbers of characteristic species recorded per site [2]. Soil samples were taken for chemical analysis (pH and C:N ratio), and percentage cover of grasses, mosses, and lichens was estimated. Fungi were identified using microscopic examination and standard keys, with selected taxa confirmed by rDNA ITS sequencing. Statistical analyses were performed using PAST 5.3.

In total, more than 500 herbarium specimens from almost all regions of Lithuania were examined, of which 346 were confirmed as grassland-associated CHEGD taxa. These represented 93 species from 13 genera, indicating that the currently documented diversity of grassland fungi in Lithuania is lower than that reported from countries where fungal indicator methodologies are well established [2]. Field surveys recorded representatives of all CHEGD groups alongside other grassland-associated fungi, including gasteromycetes such as *Bovista* and *Lycoperdon*. However, none of the surveyed grasslands reached the CHEGD species thresholds required by the indicator system used in the UK [2], even at sites independently recognised as being in good ecological condition based on vegetation structure and management history. Fungal species richness showed statistically significant relationships with vegetation cover, as indicated by Spearman rank correlation analysis. Moss cover showed the strongest positive associations with the number of species per site, particularly for the genera *Entoloma* ($r = 0.83$, $p < 0.05$) and *Cuphophyllus* ($r = 0.67$, $p < 0.05$). In contrast, significant negative correlations were observed between grass cover and *Hodophilus* ($r = -0.62$, $p < 0.05$), as well as between lichen cover and *Ramariopsis* ($r = -0.62$, $p < 0.05$). These relationships were evaluated using Spearman rank correlation analysis. Soil pH showed only weak to moderate correlations that were not statistically significant ($p > 0.05$). Indicator Species Analysis (IndVal) identified *Hygrocybe acutoconica* as the only statistically significant indicator species ($p < 0.05$) for the steppe grassland habitat type among the studied sites.

In conclusion, grassland fungal species richness in the studied habitats was most strongly associated with moss cover, while only a single species was statistically confirmed as an indicator of a specific grassland type. The application of CHEGD-based grassland assessment criteria developed in other European regions proved to be less suitable for the Lithuanian context, suggesting that direct transfer of these approaches requires regional calibration. These findings suggest that current data are insufficient for robust application of fungal indicator systems in Lithuania and highlight the need for expanded surveys, repeated sampling, and further methodological development to support reliable grassland assessment using fungi.

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CURRENT STATUS OF GLACIAL RELICT CALANOID CRUSTACEANS IN LITHUANIAN LAKES

Viktorija Arcerytė[1,2], Kęstutis Arbačiauskas[2]

[1]Life Science Centre, Vilnius University, Lithuania
[2]Laboratory of Evolutionary Ecology of Hydrobionts, Nature Research Centre, Lithuania
varceryte@gmail.com

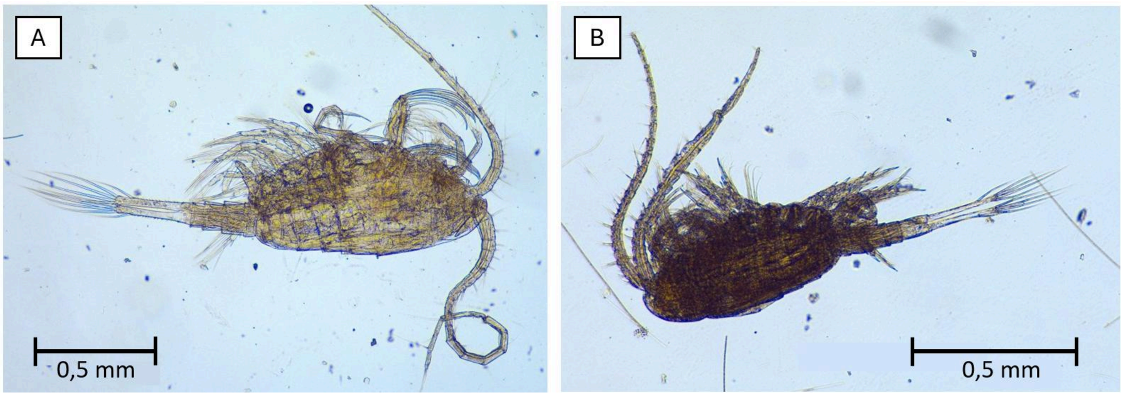
Two species of glacial relict calanoid crustaceans, *Limnocalanus macrurus* (Sars G.O., 1863) and *Eurytemora lacustris* (Poppe, 1887) (Fig. 1), are still found in Lithuanian lakes of glacial origin [1]. These species are of considerable scientific interest. The status of their populations may provide valuable information about the ecological status of lakes. In addition, relict crustaceans constitute an important part of the trophic web, serving as food for fish and significantly contributing to fish production [2]. Unfortunately, the survival of these crustaceans is threatened by eutrophication and global warming [3].

The aim of this work was to summarize available information and to investigate the distribution and abundance of *L. macrurus* and *E. lacustris* in Lithuania lakes.

The research material was collected in 2022 and 2025 using two plankton nets of different diameters and analyzed under a stereomicroscope. Analysis of the material collected in 2022 revealed that relict calanoids inhabited all 22 studied lakes: *L. macrurus* was found in 14 lakes, while *E. lacustris* was recorded in 8 lakes. Relict calanoid crustaceans were detected in all their historically known habitats. In addition, new habitats were identified: *L. macrurus* was recorded in lakes Juodieji Lakajai and Žeimenys, whereas *E. lacustris* was found in lakes Alaušas, Avilys, and Galstas. In 2025, previously unstudied lakes — Virintai, Smalvos, Sartai and Drūkšiai — were examined to determine the presence of relict calanoids crustaceans. In addition, two previously studied lakes, Avilys and Žeimenys, were re-examined to confirm the presence of relict calanoids and to assess the abundance of their populations.

The results indicate that Lithuanian lakes remain insufficiently investigated, as new habitats of relict calanoid crustaceans continue to be discovered. Consequently, more comprehensive and systematic research is required to accurately assess the status of these species' populations in Lithuania.

Figure 1. A - *Limnocalanus macrurus*; B - *Eurytemora lacustris* (photo by V. Arcerytė).



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ELUSIVE PARASITES: THE CHALLENGE OF STUDYING *LANKESTERELLA* SPP. IN THEIR NATURAL HOSTS AND VECTORS

Germán Alfredo Gutiérrez Liberato[1], Kristina Valavičiūtė-Pocienė[1], Alžbeta Šujanová[1,2], Tanja Himmel[3,4], Carolina Romeiro Fernandes Chagas[1]

[1]State Scientific Research Institute Nature Research Centre.
[2]Institute of Zoology, Slovak Academy of Sciences, Bratislava, Slovakia.
[3]Department of Pathology, Medical University Vienna, Waehringer Guertel 18-20, 1090 Vienna, Austria.
[4]Institute of Pathology, Department for Biological Sciences and Pathobiology, University of Veterinary Medicine Vienna, Veterinaerplatz 1, 1210 Vienna, Austria.
german.liberato@gamtc.lt

The number of described *Lankesterella* species infecting birds has increased substantially with the application of molecular tools, revealing that several taxa previously identified based solely on morphology were misclassified. Despite this progress, the genus *Lankesterella* remains poorly studied, and its true diversity, life cycle, and transmission routes are still insufficiently understood. We investigated the occurrence and diversity of *Lankesterella* in wild birds and mosquitoes using an integrative approach combining blood smear microscopy, histology, chromogenic in situ hybridization, PCR screening, and phylogenetic analyses. DNA from nearly 2,000 mosquitoes representing multiple species was screened by PCR to evaluate potential vector involvement.

Lankesterella sp. prevalence was 33%, with 18% detected by microscopy and 25% by molecular methods. Phylogenetic analyses revealed a high genetic diversity among avian *Lankesterella* lineages, with patterns indicating strong host specificity. However, evidence of exoerythrocytic development was not found in tissues from seven dissected birds (Paridae and Acrocephalidae), despite frequent detection of parasite blood stages in the spleen. All screened mosquitoes tested negative.

Overall, our results document substantial genetic diversity in avian *Lankesterella* while demonstrating the difficulty of detecting tissue stages and identifying potential vectors in naturally infected hosts. These findings highlight important gaps in current knowledge and provide a basis for future investigations to resolve the life cycle and transmission of this parasite genus.

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WHAT IS FACILITATING A GLOBAL VIRUS PANDEMIC IN MOSQUITOES?

Aistė Židonytė[1], Linas Gasparavičius[1], Gytis Dudas[1]

[1]Institute of Biotechnology, Vilnius University Life Sciences Center, Lithuania
aiste.zidonyte@gmc.vu.lt

Wuhan Mosquito Virus 6 (WuMV-6) is a virus belonging to the *Orthomyxoviridae* family whose other members cause diseases like influenza and infectious salmon anaemia, amongst others. By now, WuMV-6 was found in multiple metagenomic studies in every inhabited continent. Although viruses closely related to WuMV-6 have been found to infect vertebrates and cause disease, so far WuMV-6 has only been found to infect *Culex* mosquitoes. That being said, the virus has spread extremely large distances in a short period of time [1]. Since mosquitoes are unable to fly more than a few kilometers during their lifetime, such rapid virus migration is unusual.

A previous study has shown that WuMV-6's surface protein gp64 is evolving faster at the amino acid level than the rest of its' proteome [1]. Such rapid evolution and diversification of gp64 is unlikely to be driven by invertebrate immune systems. It resembles antigenic drift similar to that in Influenza viruses and suggests the presence of a vertebrate host. As related viruses have been discovered during outbreaks of disease in birds, the periodic long distance migration birds undertake as well as known preference of Culex mosquitoes for avian bloodmeal hosts, we speculate that the vertebrate host of WuMV-6 could be a bird [2].

These early findings were based on a dataset of 27 genomes. In this study, we are focusing on analyzing the spread of WuMV-6 using nearly 5-times larger dataset of sequences, obtained from publicly available databases. We use Bayesian modelling to reconstruct the pathways of virus' migration in China and Europe using continuous phylogeography, which incorporates precise sampling coordinates. This approach enables the reconstruction of the virus' directional spread in continuous geographic space. In-depth analyses using SERAPHIM were conducted to estimate the effects of environmental factors in influencing viral dispersal dynamics [3]. We then combine it with discrete phylogeographic analyses, where we model migration as occurring between discrete states. We extend the Bayesian model with generalized linear models (GLMs) representing geographic, climate, demographic, and economic data as explicit predictors of migration. This framework allows to evaluate which factors contribute to viral transition rates between regions.

We found no factors significantly influencing the rate of WuMV-6 transmission using continuous phylogeography. However, using discrete phylogeography we observe distinct distance-based effects – positive associations between migration and sharing land borders or negative associations with more distant region centroids. Although predictors related to bird migration and trade were evaluated, we found no evidence of bird movement influencing the spread of WuMV-6.

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O12

COMBINING BIOINFORMATICS AND WEARABLE HEALTH DATA FOR PERSON-CENTERED DISEASE RISK PREDICTION

Faustinus Chukwuma Onyima[1], Emmanuel Arinze Onuorah[2], Third Author[3]

Department of Nursing, Vilnius University Faculty of Medicine Vilnius Lithuania
chukwumaonyima@gmail.com

The quest for precision medicine is motivating healthcare stakeholders towards the development of new disease risk prediction strategies that strengthen person-centered care. However, the overreliance on single data sources for predictive analysis may jeopardize the required level of predictive accuracy, considering the dynamics of human biology and functioning. Bioinformatics, like the polygenic risk scores (PRS), empirically estimates the risk of chronic illnesses, such as cardiovascular diseases (CVD) [1]. Due to its focus on genetic information, PRS cannot identify risk factors associated with challenges related to physiology and lifestyle. Conversely, wearable health data captures real-time data on human physiology, such as heart rate variability, which is essential in determining cardiovascular health [2]. Still, data from the medical device cannot inform clinicians about genetic predisposition to illness. The shortcomings of both data streams justify a case for the development of an integrative model.

This study proposes a multi-modal predictive framework that combines bioinformatics and wearable health data to improve individualized CVD risk prediction. A retrospective cohort design will be used, drawing data from platforms like the UK Biobank, which are public platforms with both genomic and wearable device data. Using an A/B framework, the predictive capabilities of the two systems will be developed, whereby Model A is the genetic baseline that utilizes PRS, while Model B combines PRS data with longitudinal data from the wearable devices. The analytical process will use a late-fusion ensemble approach, whereby each model is assessed separately before they are combined to create a single predictive framework.

When evaluating model performance, data will be divided into training and testing groups. Each model will be assessed based on its accuracy in disease prediction, the reliability of the outcomes, and whether the integrated model is better in prediction compared to using genetic data alone. Preliminary findings are expected to show that Model B outperforms Model A due to its ability to capture genetic risk, which is static, and physiological changes, which are dynamic.

The expected findings will show that wearable health data strengthens the predictive analysis of bioinformatics in individualized risk prediction. Clinicians and researchers will benefit from this multi-modal approach due to the capability of early identification of high-risk persons. Tailored preventive measures can then be developed in primary care settings.

In summary, this study addresses a critical gap in person-centered preventive medicine by uniting bioinformatics and wearable health data into a single predictive framework. Findings will help stakeholders, including doctors, nurses, and researchers, to design accurate, proactive, and person-centered disease prevention strategies against CVD. More importantly, the outcomes provide a clear roadmap for the integration of digital health and genomics in clinical practice.

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O13

Anthropozoonotic spillovers reveal sustained long-term cryptic circulation of SARS-CoV-2 within and between Lithuanian mink farms

Martynas Smičius[1], Gytis Dudas[1]

[1]Institute of Biotechnology, Vilnius University, Lithuania
martynas.smicius@bti.stud.vu.lt

Several studies have documented reverse zoonotic transmission of SARS-CoV-2, including in farmed mink which are susceptible to human respiratory viruses and are known for serving as a reservoir capable of generating new virus variants in densely populated farms. Here, we present the genomic investigation launched in response to detection of human infections with mink-origin SARS-CoV-2 lineages, and show evidence of at least 14 high-confidence introductions of SARS-CoV-2 from humans into farmed mink in Lithuania where sustained transmission in farmed mink lasted up to a year. We estimated the most likely timeframes for these introductions encompassing at least six SARS-CoV-2 lineages, some of which were already extinct in humans, with Bayesian phylogenetic and molecular clock analyses. The inferred human-to-mink transmission events are presented in Figure 1. This study highlights the public health risks posed by fur farms and underscores that passive genomic surveillance systems are ineffective without the active involvement and expertise of responsible institutions.

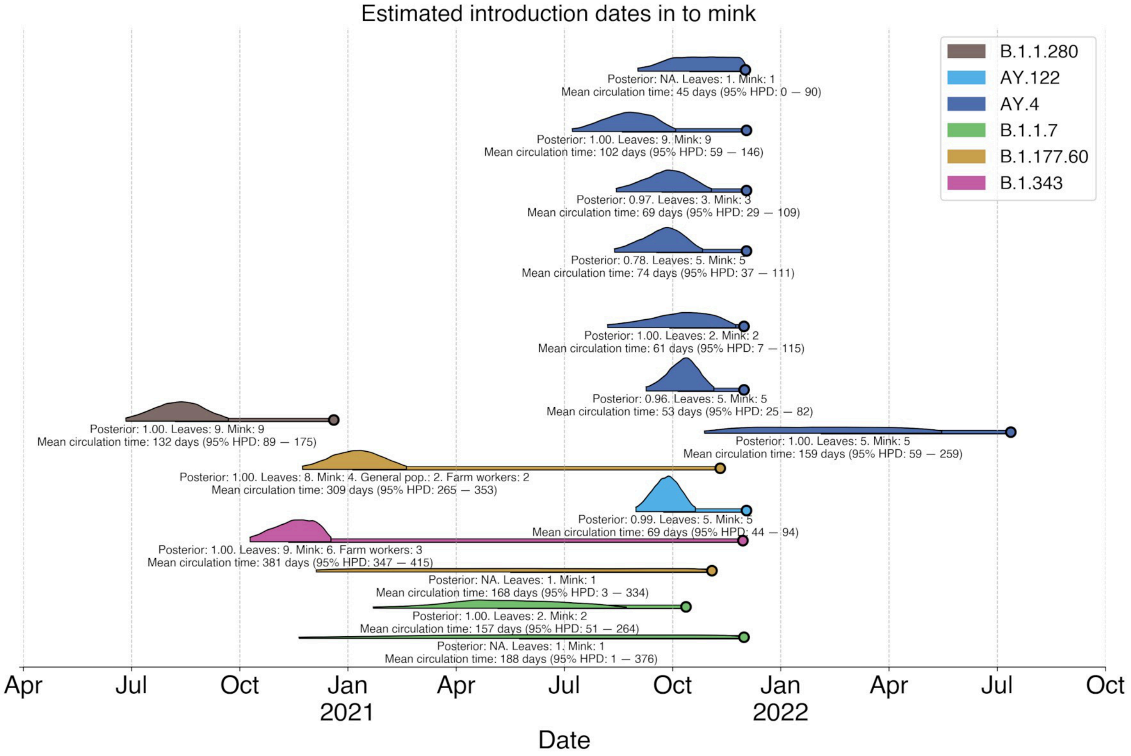

Figure 1. The 95% highest posterior density (HPD) intervals for estimated dates of SARS-CoV-2 introductions into mink, conditioned on events detected in maximum credibility clade trees. The coloured line continuing after the 95% HPD signifies the continued circulation period until the last detected sample, which is marked by the dot at the end of the line. The text below each line marks the mean circulation period in days with 95% HPD range, number of leaves and the posterior probability for a given node (does not apply if there was only a single sample in mink for a transmission event).

THE EFFECT OF TETRAHYDROCANNABIVARIN (THCV) ON VOLUNTARILY ALCOHOLDRINKING IN MALE RATS

Ceyda Türkcan, Ieva Poceviciute, Valentina Vengelienė

Department of Neurobiology and Biophysics, Institute of Biosciences, Life Sciences Center, Vilnius University, Vilnius, Lithuania
ceyda.turkcan@gmc.stud.vu.lt

Alcohol use disorder (AUD) represents a significant public health challenge, and current pharmacological interventions remain limited. Mounting evidence suggests that antagonists and inverse agonists of the cannabinoid CB1 receptor are effective in reducing alcohol consumption, but their use is limited due to adverse effects [1,2].

Δ^9-Tetrahydrocannabivarin (THCV), a phytocannabinoid acting as a neutral CB1 antagonist, may provide a safer alternative for treating alcohol use disorder (AUD) by blocking CB1 receptors without eliciting the negative psychiatric side effects associated with inverse agonists [3].

Despite promising preliminary findings, THCV's influence on the behavioral patterns and underlying mechanisms of alcohol consumption remains poorly understood. Thus, in this study, we examined the effects of repeated THCV administration on voluntary alcohol intake, drinking microstructure, and locomotor activity in long-term drinking male Wistar rats.

Our data demonstrated that treatment with THCV significantly reduced voluntary alcohol intake without affecting water consumption, decreased alcohol bout size and licking rate, indicating reduced hedonic value of alcohol ("liking"), and reduced the number of alcohol bouts, suggesting diminished motivational drive to consume alcohol ("wanting"). Locomotor activity was not altered by THCV treatment, indicating that the effects on alcohol intake were not caused by sedation.

Overall, our results demonstrate that THCV reduces both the hedonic and motivational components of alcohol consumption through neutral CB1 antagonism. These findings suggest that THCV may be a promising novel compound for AUD treatment with potentially fewer adverse effects compared to traditional CB1 inverse agonists.

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CAN ELECTRICAL SIGNALS MODIFY INTERCELLULAR CONNECTIVITY IN PLANTS? BIOPHYSICAL PROPERTIES OF THE NODE IN *NITELLOPSIS OBTUSA*: CHANGES UNDER GLUTAMATE

Tekla Svanidze, Vilmantas Pupkis, Vilma Kisnieriene, Aušrinė Navickaitė,Indrė Lapeikaitė

Department of Neurobiology and Biophysics, Life Sciences Center, Vilnius University, Lithuania
tekla.svanidze@gmc.stud.vu.lt

All multicellular life requires intercellular signalling. The generation of electrical signals - action potentials (AP) - is not unique to animal cells, as plant cells also require ion channels to transmit information over long distances and coordinate responses [1]. Intercellular communication in the macroalga Nitellopsis obtusa is mediated by plasmodesmata; analogous to gap junctions, these pores between adjacent cells ensure electrical continuity in plants [2]. Fine-tuning of plant physiology encompasses the interactions between the physiological properties of these junctions, ion transport systems, and signaling molecules such as glutamate.

Glutamate (Glu) is a well-studied intercellular signalling molecule that can affect AP parameters in plants and is suggested to be a universal signalling molecule involved in the transmission and regulation of electrical signals across diverse forms of life. While Glu is widely known to participate in stress responses and metabolic functions, it can also influence intercellular communication [3] and, specifically, AP transmission between cells [4]. It does so by increasing the propagation velocity of AP within a single cell while reducing the probability of AP transmission between adjacent cells [4,5]. Glu can affect AP parameters by inducing systemic increases in cytosolic Ca^{2+} via glutamate-like receptors [6]. Glu hindering AP transmission, rather than promoting it, may be due to the elevation of nodal resistance. It is unknown whether and how Glu affects the biophysical properties of the node. The present work, therefore, examines the node's role in the regulation of AP transmission. This research aims to investigate the electrophysiological parameters of the node under a control solution (APW'), and how they change over time with exposure to Glu.

The research employed the two-electrode technique, with one intercellular glass electrode inserted into both cells of each tandem (two internodal cells connected by a multicellular node), with one electrode serving as the reference. Three APs were elicited in each cell by a cold solution. The transnodal potential was consistently recorded in both cells, and current pulses were applied to measure the resistance. The protocol was repeated bidirectionally, first, the whole tandem was exposed to APW' and then to Glu dissolved in APW'.

First, it was established that transnodal resistance is a stable electrophysiological parameter in APW', exhibiting no temporal drift in the absence of stimulation. However, upon electrical signal generation, a single AP, as well as repeated AP elicitation, markedly increases the node's resistance, indicating that APs can modulate intercellular connectivity. Nevertheless, such AP effects are observed only in the control solution. Conversely, when the surface of the cell is exposed to Glu, nodal resistance remains unchanged following both single and repeated AP elicitation. In fact, a single AP elicited under Glu was associated with a slight decrease in transnodal resistance, indicating a downward trend opposite to that seen in the control condition. Temporal changes under Glu revealed further complexity: after 30 minutes, resistance showed an upward trend, while after 1.5 hours it increased significantly, indicating a delayed yet pronounced effect. Throughout this period, APs had little to no effect on resistance, underscoring that Glu alters the node's biophysical properties.

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INTRACELLULAR ACTIVITY VIZUALIZATION USING DYNAMIC FULL-FIELD OPTICAL COHERENCE MICROSCOPY

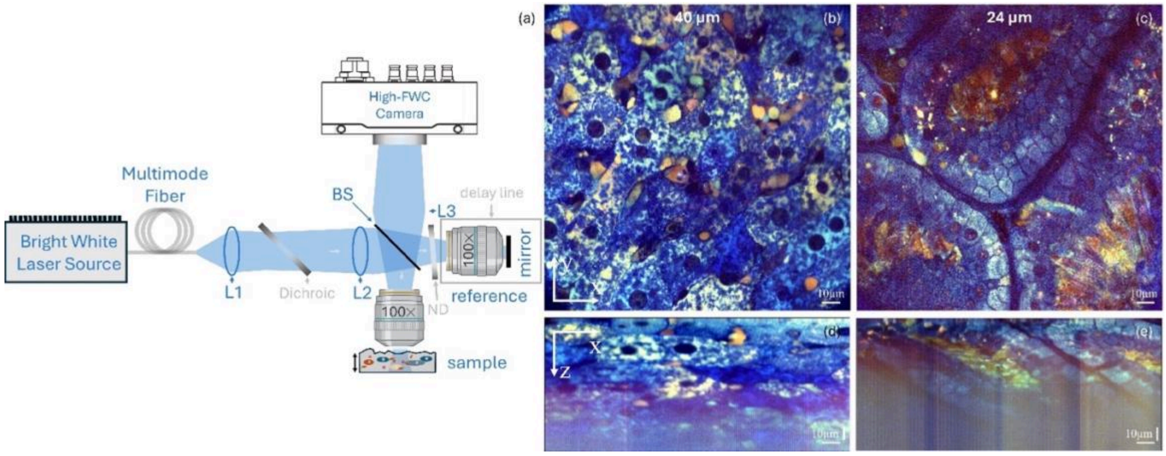
Erikas Tarvydas[1,2], Austėja Trečiokaitė[1], Egidijus Auksorius[1]

[1]Center for Physical Sciences and Technology, Saulėtekio av. 3, LT-10257 Vilnius
[2]Faculty of Medicine, Vilnius University, M. K. Čiurlionio st. 21, LT-03101 Vilnius
email: erikas.tarvydas@ftmc.lt

Dynamic full-field optical coherence microscopy (d-FF-OCM) is a label-free, interferometric imaging technique that enables visualization of cells and their intracellular dynamics deep in freshly excised biomedical tissues [1,2]. By recording fluctuating signal arising from organelle motion and metabolic activity, d-FF-OCM provides functional contrast linked to underlying cellular biomechanics and physiological processes.

In this work, we push the limits of resolution and imaging depth in d-FF-OCM by utilizing 100× magnification objective lenses and a high-brightness incoherent white light source [3], as illustrated in Fig. 1a. A high full-well-capacity (FWC) camera is used in conjunction to capture interferometric images with a high signal-to-noise ratio. The system achieves a lateral resolution of 270 nm and an axial resolution of 500 nm, reaching imaging depths of up to 120 µm [3]. For tissue imaging, we utilized freshly excised organs from C57BL/6 mice. Intrinsic cellular vibration images were derived by recording 512 interferometric images at a specific imaging plane and performing a Fourier analysis of the time-lapse signal captured by each pixel [3]. The resulting vibration spectrum for each pixel was categorized into three frequency bands: blue (1–3 Hz), green (3–100 Hz), and red (100–250 Hz). A dynamic RGB image was then reconstructed by assembling these pixels, as shown in Fig. 1b–e. Images at different depth in a sample were recorded by translating a sample axially. Figure 1b display image of fresh mouse liver, where hepatocyte bodies exhibit low-range frequencies (appearing bluish), while sinusoids—the capillary-like vessels of the liver—map to a broader, higher frequency range (green-red). Notably, this label-free approach preserves native tissue physiology and eliminates the need for exogenous fluorescent markers or contrast agents, thereby maintaining the biochemical and structural integrity of the biological specimen [3]. Figure 1c show mouse gut image acquired from the mucosal side; here, the villi and epithelial cells display lower frequencies, while the lamina propria exhibits higher frequency components. Axial images of both sample are also shown in Fig. 1d and 1e.

Figure 1. (a) Schematic diagram of the high-resolution FF-OCM system. (b) Image of liver tissue highlighting hepatocyte and sinusoid morphology. (c) Image of mouse intestinal tissue captured from the mucosal (villus) side of the intestinal wall featuring epithelial cells and lamina propria. (d) and (e) Mathematically reconstructed axial views providing cross-sectional depth information.



Dynamic contrast, driven by intrinsic intracellular activity, revealed microstructures not visible with conventional OCT—including sinusoidal networks and organized hepatocyte layers in the liver, as well as epithelial layers, neural plexuses, crypts, and microvilli in the intestine—all visualized label-free [3].

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O17

THE MECHANISMS OF TYPE I RESTRICTION-ENDONUCLEASES AT SINGLE-MOLECULE RESOLUTION USING MAGNETIC TWEEZERS

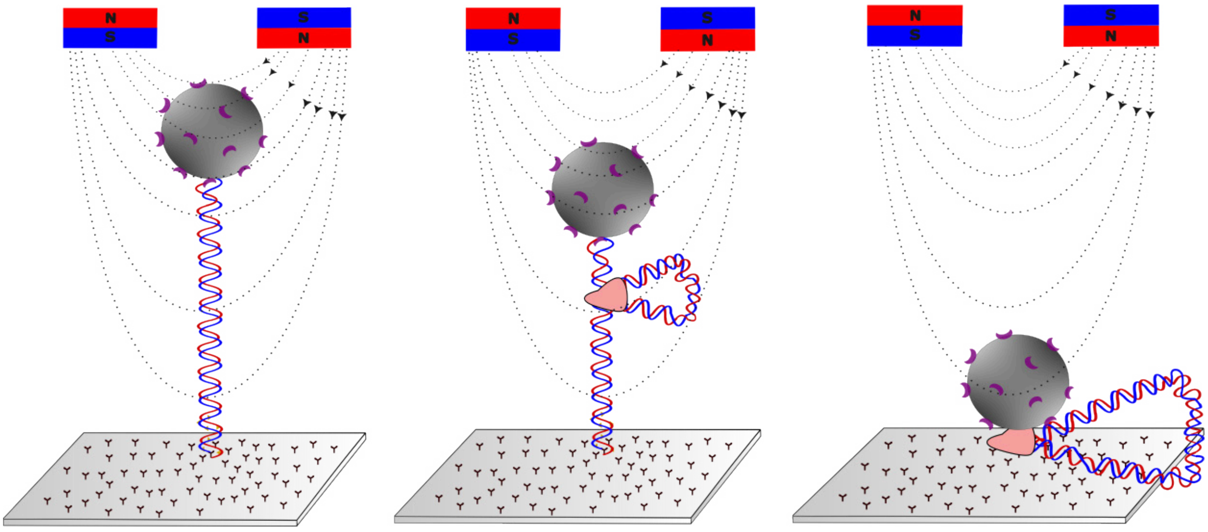
Farzaneh Alipoor Azizollahi[1], Paulius Toliušis[1], Mindaugas Zaremba[1], Algirdas Toleikis[1]

[1]Institute of Biotechnology, Life Sciences Center, Vilnius University, Vilnius, Lithuania
farzaneh.alipoor@bti.stud.vu.lt

Restriction–endonuclease (RE) enzymes play a key role in the bacterial defense against foreign DNA invasion. NgoAVII is a type I RE enzyme composed of the restriction subunit with PLD nuclease and B3-like DNA-binding domains, and the helicase-like subunit, containing DEAD-box and Z1 domains.

Unlike traditional RE systems, it not only cuts the DNA but also translocates on the dsDNA using ATP. The translocation dynamics of NgoAVII is not fully understood, particularly how it is coordinated with DNA sequence recognition and cutting. To investigate the translocation, we use a custom-built magnetic tweezers microscope to study it at single-molecule level. We find that the translocation occurs via looping and unlooping events. We further investigate how translocation depends on force and the interaction between restriction and translocation subunits. We anticipate that the mechanical properties obtained in this study will provide valuable insight into the mechanisms of type I RE systems.

Figure1.A schematic of DNA and restriction-endonuclease enzyme in single-molecule resolution



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