FEBS3+ Baltics:
Biochemistry at the Fore Line

ABSTRACT BOOK

Vilnius, Lithuania
April 23-25, 2025

Welcome word

Dear Colleagues,

I am pleased and honored to welcome you to the joint conference FEBS3+ Baltics: Biochemistry at the Fore Line! Building on the success of previous FEBS3+ meetings organized in Riga and Tallinn, we are excited to foster the tradition of excellence in Vilnius University's Life Sciences Center.

The fruitful collaboration of the Lithuanian Biochemical Society with Latvian, Estonian, and Finnish Biochemical Societies allows for a unique opportunity to bring together scientists at the forefront of biochemistry and molecular biology. The FEBS3+ Meeting format supports regional collaboration and provides a platform for inspiring the next generation of researchers.

The conference program covers an ample variety of topics in contemporary biochemistry – from Fundamental Biochemistry to Systems Biology, from Gene Editing to 3D Cellular Models. We strive to gather knowledge and cure disease, taking front rows in a battle for a healthier future for people. None of this would be possible without sharing ideas, efforts, and passion for responsible Science.

Welcome to Vilnius to explore cutting-edge biochemistry, build lasting connections, and shape the future of scientific research in the Baltics!

Prof. Saulius Serva
Chairman of the Lithuanian Biochemical Society

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Prof. Saulius Serva, president of the LBS
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Dr. Rasa Žūkienė (Vytautas Magnus University)

Sponsors

PROGRAMME

April 23

	Common area	Grand Auditorium (R106)	Room R101	Room R102
8:30 - 10:00	Registration			
10:00 - 10:50		Welcome address KEYNOTE 1 **Virginijus Šikšnys**		
10:55 - 12:15			**Gene editing** Session chair **Inga Songailienė** 10:55 - 11:30 **Guillermo Montoya** cryoEM reveals that Retron-Eco1 assembles NAD+-hydrolyzing filaments providing immunity against bacteriophages 11:30 - 11:50 **Giedrius Sasnauskas** Structural basis for Cas9-mediated prespacer selection in Type II-A CRISPR-Cas adaptation 11:50 - 12:10 **Inga Songailienė** Molecular details of the interference stage of type I-F CRISPR-Cas multiprotein effector	
12:15 - 13:15	Lunch			
13:15 - 15:00			**Gene editing** Session chair **Mindaugas Zaremba** 13:15 - 13:40 **Stephen Jones** How precise is precise? Pursuing specific editing outcomes with CRISPR nucleases 13:40 - 14:05 **Patrick Pausch** A bacterial immune system that makes DNA and breaks RNA 14:05 - 14:30 **Gintautas Tamulaitis** Different enzymatic activities contributing to type III CRISPR-Cas immunity 14:30 - 14:50 **Rafael Pinilla-Redondo** Functional Insights into Type IV CRISPR-Cas	**Fundamental and applied biochemistry** Session chair **Ly Villo** 13:15 - 13:50 **Emilio Parisini** Engineering PETases for enzymatic plastic degradation 13:50 - 14:10 **Nicholas Taylor** 5:2 molecular motors: from bacterial motility to anti-phage defense 14:10 - 14:30 **Sebastian Glatt** tRNAslational control of eukaryotic gene expression 14:30 - 14:40 Selected **Džiugas Sabonis** Mechanisms of bacterial antiphage defense by NAD+ based intracellular signals 14:40 - 14:50 Selected **Saloua Biyada** Enhanced biodegradability of textile waste through a microbial consortium and its enzymatic activities achieved by bioaugmentation
15:00 - 15:30	Coffee break			
15:30 - 17:30			**Environmental and plant biochemistry** Session chair **Elena Servienė** 15:30 - 16:00 **Nils Rostoks** New Genomic Techniques for sustainable agriculture in the EU 16:00 - 16:20 **Alons Lends** Structural studies of intact fungal cell walls using solid-state NMR spectroscopy 16:20 - 16:40 Selected **Liudmyla Kozeko** Heat shock protein response as an indicator of the action of exogenous proline and GABA on plant proteostasis under drought 16:40 - 17:00 **Eglė Lastauskienė** Colorectal carcinogenesis – lessons from the microbiota shift 17:00 - 17:15 Selected **Bazilė Ravoitytė** Microbial safety of industrially reared *Hermetia illucens* larvae and frass	**Fundamental and applied biochemistry** Session chair **Hannu Koistinen** 15:30 - 16:00 **Ly Villo** Mechanisms of endothelial lipolysis 16:00 - 16:20 **Baiba Jansone** The Long-Term Impact of Ischemic Stroke: Insights from Experimental Research 16:20 - 16:40 **Hannu Koistinen** Prostatic proteases 16:40 - 17:00 **Juozas Gordevičius** Understanding and Applying Epigenetic Aging Clocks in Aging Research and Interventions 17:00 - 17:20 **Mindaugas Zaremba** DNA cleavage mechanism of the short prokaryotic Argonaute-containing SPARDA system 17:10 - 17:20 Selected **Danielius Umbrasas** Viral inflamation, bioenergetics, and neurodegeneration 17:20 - 17:30 Selected **Mantas Žiaunys** The Role of Liquid-Liquid Phase Separation in Alpha-Synuclein Amyloid Aggregation
17:30 - 19:00	Poster session 1	Environmental and plant biochemistry	Fundamental and applied biochemistry	Gene editing
19:00 - 21:00	Welcome reception			

April 24				
	Common area	**Grand Auditorium (R106)**	**Room R101**	**Room R102**
8:30 - 9:00	**Registration**			
9:05 - 9:45		**Opening remarks** **KEYNOTE 2** **Peter D. Nagy** **Deep insights into biogenesis of tombusvirus replication organelles**		
9:50 - 11:00			**Drug discovery and therapy approaches** Session chair **Vilmantė Borutaitė** 9:50 - 10:20 **Jukka Westermarck** PP2A inhibitor protein CIP2A is a prime target for TNBC therapy 10:20 - 10:40 **Anni Allikalt** *In vitro* testing of genetic mutations of G protein-coupled receptors 10:40 - 11:00 Selected **Kotryna Vaidžiulytė-Simutienė** Atypical nucleus phenotypes in confined cancer cells	**Fundamental and applied biochemistry** Session chair **Dukas Jurėnas** 9:50 - 10:20 **Rolandas Meškys** From field to fork: does a functional screening still seem a reasonable approach to identify and develop novel enzymes? 10:20 - 10:40 **Gert Bange** Emerging function of the mysterious Di-adenosine tetraphosphate 10:40 - 11:00 **Dalius Ratautas** Point-of-care biosensors for astronauts' muscle health monitoring in space
11:00 - 11:30	**Coffee break**			
11:30 - 13:00			**Drug discovery and therapy approaches** Session chair **Miglė Tomkuvienė** 11:30 - 12:00 **Jānis Kloviņš** Decoding the molecular landscape of acute and Long COVID through metabolomic and transcriptomic analysis 12:00 - 12:20 **Miglė Tomkuvienė** Epigenetic avalanche: the effects of human DNA methyltransferase DNMT3A variants 12:20 - 12:40 **Aurelijus Burokas** Targeting the microbiota-gut-brain axis in aging 12:40 - 12:50 Selected **Saurabh Singh Dhakar** High-throughput screening assay for PARP-HPF1 interaction inhibitors to affect DNA damage repair	**Environmental and plant biochemistry** Session chair **Vida Mildažienė** 11:30 - 12:00 **Yrjö Helariutta** On a two-way street between Arabidopsis and tree research 12:00 - 12:20 **Zigmunds Orlovskis** Underground Biological Internet: Common Mycelial Networks in Inter-Plant Signalling and Resistance to Pathogens 12:20 - 12:40 **Vida Mildažienė** Phytohormone story: how the response of seeds to stress is translated to changes in plant biochemical and physiological performance 12:40 - 13:00 **Ilse Kranner** Infrared thermography enables unlocking metabolic signatures of seed viability and ageing in *Pisum sativum* seeds
13:00 - 14:00	**Lunch**			
14:00 - 15:30			**Drug discovery and therapy approaches** Session chair **Daumantas Matulis** 14:00 - 14:30 **Maija Dambrova** Fatty Acid-derived Energy Metabolites Acylcarnitines: Going with the Flow? 14:30 - 14:50 **Augustas Pivoriūnas** Extracellular vesicles: are we lost in translation? 14:50 - 15:10 **Daumantas Matulis** Anticancer drug design via thermodynamics and the protein-ligand binding database 15:10 - 15:20 Selected **Francisco Alejandro Lagunas Rangel** Repurposing Statins for Colorectal Cancer 15:20 - 15:30 Selected **Anastasija Kudrevceva** Protein-ligand binding affinity prediction using descriptors derived from Voronoi tesselation	**Biochemistry of infectious disease** Session chair **Kaspars Tars** 14:00 - 14:30 **Vyacheslav Yurchenko** RNA editing in Trypanosomatidae: lessons from the "obscure" species 14:30 - 14:50 **Kaspars Tars** Biology of ssRNA phages in metagenome sequencing era 14:50 - 15:10 **Gytis Dudas** The global pursuit of Wuhan mosquito virus 6, a metagenomic RNA virus implicated in vertebrate infection 15:10 - 15:20 Selected **Gerda Jasinevičienė** Finding yeast virus: localization of L-A capsids in host cell
15:30 - 16:00	**Coffee break**			
16:00 - 16:30	**Excursion in Life Sciences Center**	**LBS meeting**		
16:30 - 17:30		**Partners' session** **GoVilnius** **Future Biomedicine Foundation** **Biomatter designs** **SeqVision**		
17:30 - 19:00	**Poster session 2**	**Biochemistry of infectious disease** **3D cellular models**	**Drug discovery and therapy approaches**	**Omics and systems biology**
19:30 - 22:00	**Gala Dinner**			

April 25				
	Common area	**Grand Auditorium (R106)**	**Room R101**	**Room R102**
8:30 - 9:00	**Registration**			
9:00 - 9:45		**Opening remarks** KEYNOTE 3 **Andres Metspalu** **From biobanking to personalised medicine**		
9:50 - 11:00			**Omics and systems biology** Session chair **Vilmantė Borutaitė** 9:50 - 10:20 **Marko Vendelin** Decoding molecular properties from seemingly chaotic fluorescence intensity traces using FITSA 10:20 - 10:40 **Agne Velthut-Meikas** Endocrine disruptive chemicals change ovarian metabolism and sub-cellular composition leading to reduced ovarian response to FSH stimulation 10:40 - 11:00 **Vita Rovīte** Exploring landscape of pancreatic neuroendocrine tumour microenvironment by transcriptomics	**Biochemistry of infectious disease** Session chair **Dukas Jurėnas** 9:50 - 10:10 **Peter Sarin** Code and recode – the multifaceted RNA-based tactics employed by Shewanella phage 1/4 for productive infection 10:10 - 10:30 **Dukas Jurėnas** Golden death bacteria – equipped to kill them all 10:30 - 10:50 **Julija Armalytė** The Evolving Threat of Antibiotic-Resistant Opportunistic Pathogens: Diverse Strategies to Achieve the Same Goal
11:00 - 11:30	**Coffee break**			
11:30 - 13:00			**Omics and systems biology** Session chair **Saulius Serva** 11:30 - 11:50 **Tõnis Laasfeld** Deep learning based high content live-cell microscopy image analysis: Focus on quality beyond blind metrics 11:50 - 12:10 **Sampsa Hautaniemi** Analysis and integration of multi-omics data to overcome chemotherapy resistance in ovarian cancer 12:10 - 12:30 **Linas Mažutis** Semi-permeable microcapsules: emerging technology for single-cell -omics studies 12:30 - 12:50 **Kaarel Adamberg** Cultivation of microbial consortia for future probiotics 12:50 - 13:00 Selected **Alina Rekena** Quantitative Systems biology Analysis of lipid-producing yeast *Rhodotorula toruloides* cultivation	**3D cellular models** Session chair **Vytautė Starkuvienė-Erfle** 11:30 - 12:00 **Karen Biebak** Experimental Cell Therapy and Advanced Cellular Models to Replace Animal Testing 12:00 - 12:20 **Darja Lavõgina** Adding third dimension: changes in response to cytotoxic agents, radiation or endocrine disruptors in 3D vs 2D cell culture 12:20 - 12:40 **Aki Manninen** Losing adhesions to promote prostate cancer 12:40 - 12:50 **Daiva Baltriukienė** Impact of the Microenvironment on Cell Fate Determination 12:50 - 13:00 Selected **Ismaïl Hermelo** Patient-derived glioma organoids real time identification with differential ion mobility spectrometry
13:00 - 14:00	**Lunch**			
14:00 - 16:30		**Education and science policy** Session chair **Liudvika Leišytė** 14:00 - 14:25 **Gintaras Valinčius** TBA 14:25 - 14:45 **Liudvika Leišytė** Conditions fostering and impeding academic freedom in Lithuanian academia 14:45 - 15:00 **Anja Hartewig** Why establish a junior section 15:00 - 16:00 **Panel Discussion** **16:00 - 16:30** **Awards and closing**		

ORAL PRESENTATIONS

KEYNOTE LECTURE

DEEP INSIGHTS INTO BIOGENESIS OF TOMBUSVIRUS REPLICATION ORGANELLES

Peter D. Nagy

Department of Plant Pathology, University of Kentucky, USA

Tomato bushy stunt virus (TBSV) is a plus-strand RNA virus, which replicates on co-opted host peroxisomal membranes with the help of numerous co-opted host factors as well as subverted membrane resources. Previous genome- and proteome-wide screens in yeast surrogate host identified hundreds of host genes that affected TBSV replication and recombination. The roles of host and viral proteins in biogenesis and function of TBSV replication organelle (VROs) will be presented. TBSV replication proteins induce major modifications of subcellular membranes, alterations of lipid synthesis and lipid transport during VRO formation in infected tobacco (*Nicotiana benthamiana*). However, TBSV also hijacks cytosolic and nuclear proteins via direct interactions with the viral replication protein into VROs. We find that several of the co-opted proteins partition into biomolecular condensates, called vir-condensates, induced by the membrane-bound TBSV replication proteins. The vir-condensate is associated with the membranous VRO structures. We also found that co-opted actin filaments and ER network are part of VROs, likely stabilizing vir-condensate. The vir-condensate is found to trap various host proteins to inhibit their antiviral functions. The trapped proteins include core selective autophagy proteins, such as ATG8 and NBR1 autophagy cargo receptor in the VROs. This shows an example how TBSV inhibits the autophagy flux into the vacuole and evades the antiviral function of autophagy. Overall, the molecular dance on lipid surfaces between TBSV and host factor components is riveting new chapter in TBSV replication. Based on our discoveries on antiviral host proteins, we used Alphafold2 structure predictions to improve the antiviral activities of co-opted host protein.

FUNDAMENTAL AND APPLIED BIOCHEMISTRY

ENGINEERING PETASES FOR ENZYMATIC PLASTIC DEGRADATION

Emilio Parisini

Latvian Institute of Organic Synthesis, Latvia

The fast and uncontrolled accumulation of plastic waste in the environment has long begun to impact on the natural ecosystems and to pose an existential threat to all forms of life on our planet. Advanced technical solutions to the plastic waste management problem are therefore in urgent demand. To this end, enzymatic approaches to plastic degradation hold great promises as novel and more efficient enzymes are constantly being discovered. The first report of an efficient PET hydrolase from *Thermobifida fusca* was published in 2005.1 Since then, numerous thermally stable PET hydrolases and their related enzymes from the cutinase group have been identified in different organisms. The search for thermostable PET hydrolases is driven by the fact that PET can be more effectively hydrolyzed at temperatures close to its glass transition temperature (≈70-80°C), where the polymer chains become more flexible, enabling PET hydrolases to function optimally. In 2016, Yoshida et al.2 reported on a mesophilic bacterium (Ideonella sakaiensis) that can thrive on an amorphous PET film as its primary carbon source already at 30°C, making the enzyme responsible for PET hydrolysis (IsPETase) the best option for PET waste decomposition. However, IsPETase is heat-labile and loses its activity at temperatures > 40°C. Therefore, more suitable scaffolds have also been explored. Leaf-branch Compost Cutinase (LCC), is a naturally occurring PETase that has been reported to outperform all other known PET-degrading enzymes and to present a melting temperature (Tm) of 84.7°C. This enzyme has been noticeably engineered in 20203, leading to the so-called ICCG variant (Tm = 94.0°C), which has become the gold standard. Recently, we have engineered a Leaf-branch Compost Cutinase (LCC) that features enhanced PETase activity and thermal stability relative to gold standard ICCG.4 This mutant shows a Tm > 98 °C and remarkable activity on amorphous PET films beyond 6 days at 68°C. Moreover, we have recently characterized a novel PETase-like enzyme from Streptomyces sp. SM14 and tested it on post-consumer plastic substrates. PETase SM14 demonstrated high salt tolerance, good heat resistance, and optimal activity at pH 9, highlighting its potential for PET waste bioremediation.

The enzymatic degradation of PET holds immense promises as a sustainable solution to the escalating crisis of plastic waste accumulation, offering efficient and eco-friendly alternatives to current waste management practices.

References:
1. Müller *et al. Macromol. Rapid Commun*. 2005, 26 (17), 1400
2. Yoshida *et al. Science*. 2016, 351 (6278), 1196
3. Tournier *et al. Nature* 2020, 580 (7802), 216
4. Bhattacharya *et al. bioRxiv* 2024.07.04.602061

MECHANISMS OF ENDOTHELIAL LIPOLYSIS

Ly Villo

Tallinn University of Technology, Estonia

Endothelial lipolysis refers to the enzymatic breakdown of triglyceride-rich lipoproteins in the vascular endothelium, primarily catalyzed by lipoprotein lipase (LPL). This process plays a crucial role in regulating blood plasma triglyceride levels. The fatty acids released from lipolysis are either oxidized for energy (in the heart and skeletal muscles) or re-esterified in adipose tissue for storage. Impaired LPL function can lead to hypertriglyceridemia—an abnormal elevation of triglyceride-rich lipoproteins—posing a significant risk for atherosclerosis, pancreatitis, and fatty liver disease. The transport, activity, and stability of LPL are tightly regulated by a complex network of apolipoproteins, GPIHBP1, angiopoietin-like proteins, proteoglycans, and various lipids. Recent studies have provided deeper insights into this intricate system, paving the way for novel treatments for hypertriglyceridemia. In my presentation, I will summarize the current understanding of endothelial lipolysis and share our contributions to advancing this field.

FROM FIELD TO FORK: DOES A FUNCTIONAL SCREENING STILL SEEM A REASONABLE APPROACH TO IDENTIFY AND DEVELOP NOVEL ENZYMES?

Rolandas Meškys

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

The main scientific activities lay in the field of biochemical and genetic diversity of microorganisms and bacteriophages, biocatalysis and enzyme biotechnology including development of high-throughput systems for screening and selection of novel biocatalysts, application of enzymes for organic synthesis and biosensors.

5:2 MOLECULAR MOTORS: FROM BACTERIAL MOTILITY TO ANTI-PHAGE DEFENSE

Nicholas Taylor

Center for Protein Research, University of Copenhagen, Denmark

Bacteria move through the rotation of large filaments know as flagella. Flagellar rotary motion is powered by a flagellar motor, driven by stator units (MotAB). The MotAB proteins convert the ion motive force across the bacterial inner membrane into rotation of the filament, but it was not understood how this occurred.

Using cryo-EM we have determined structures of the MotAB complex, which we show has a 5:2 stoichiometry shared across different species. By visualizing MotAB in its plugged, inactive state, as well as mimics of its active state, we come up with models for how torque is generated in the flagellar motors, as well as how direction switching in the flagellar motor occurs. We also reveal our recent progress on how ion specificity is obtained and propose a mechanism for how stator units become active upon motor incorporation.

I will also present unpublished results on a newly discovered bacteriophage defense system, Zorya, that uses a 5:2 motor complex to sense bacteriophage infection. Using a combination of structural biology, functional assays, light microscopy and mass spectrometry, we provide novel insight into the unique Zorya mechanism of action. We provide data indicating that Zorya detects phage infection by monitoring integrity of the peptidoglycan layer. Upon phage infection, the ZorAB motor proteins get activated and through a 700 Å long tail locally recruit and activate ZorD nuclease that can degrade the phage genome, halting the infection.

tRNASLATIONAL CONTROL OF EUKARYOTIC GENE EXPRESSION

Sebastian Glatt

Jagiellonian University Krakow, Poland
University of Veterinary Medicine Vienna, Austria

My Research Group studies different translation control mechanisms, which regulate the production of specific sets of proteins by chemical modifications of tRNA molecules. Every protein in the cell is produced by the ribosome, which uses transfer RNA (tRNA) molecules to translate the sequence information coded in mRNAs into correctly assembled poly-peptide chains. The lab is focusing on understanding the molecular mechanisms that lead to the specific base modifications in anticodons of tRNAs. These modifications have a strong influence on the efficiency and accuracy of the codon-anticodon pairing and therefore regulate the translational rates and folding dynamics of protein synthesis. Recent findings have shown that alterations of these modification pathways play important roles in the onset of certain neurodegenerative diseases and cancer. We mainly use X-ray crystallography (MX) and cryogenic electron microscopy (cryo-EM) to obtain snapshots of the involved macromolecular machines and analyse their reaction intermediates at atomic resolution. Subsequently, we employ different complementary in vitro and in vivo approaches to validate and challenge our structural observations. Furthermore, we have started working on other (t)RNA modification pathways and elucidate the structure of folded RNA molecules directly by cryo-EM. Furthermore, we aim to understand how these post-transcriptional modifications affect ribosomal decoding and translation elongation by directly imaging translating ribosomes at atomic resolution. Last but not least, we develop novel structural, biochemical and biophysical approaches to study structured RNA domains. In summary, our work contributes to the fundamental understanding of eukaryotic gene expression and its complex regulatory mechanisms.

THE LONG-TERM IMPACT OF ISCHEMIC STROKE: INSIGHTS FROM EXPERIMENTAL RESEARCH

Baiba Jansone

Faculty of Medicine and Life Sciences, University of Latvia, Latvia

Ischemic stroke is a leading cause of disability and mortality worldwide. This presentation highlights recent findings on the acute and chronic effects of cerebral ischemia/reperfusion (I/R) injury, with a focus on mitochondrial dysfunction, behavioral alterations, and neurogenesis in experimental stroke model. The results obtained using high-resolution fluorespirometry, demonstrated significant hemispheric differences in mitochondrial respiration and reactive oxygen species (ROS) production, particularly in the early hours and up to six months post-stroke. The findings indicate that mitochondrial respiration initially increases in the contralateral hemisphere as a compensatory mechanism, a long-term impairment persist, particularly in complex I and II activities, with aging further exacerbating these deficits. Beyond mitochondrial dysfunction, behavioral assessments have shown severe neurological deficits, impaired motor performance, and disrupted circadian activity persisting for months after stroke. These findings underline the necessity of long-term monitoring to better understand post-stroke recovery. Furthermore, neurogenesis plays a crucial role in brain repair, with increased proliferation of neuroblasts in the subventricular zone (SVZ) and dentate gyrus (DG) observed in the ischemic hemisphere. However, these regenerative processes are accompanied by a reduction in differentiated neurons and decreased expression of pro-angiogenic factors, such as VEGF, in both hemispheres, indicating widespread ischemic impact. The obtained data from the rodent studies emphasize the importance of studying ischemic stroke beyond the acute phase, considering both cerebral hemispheres and their compensatory mechanisms. The long-term effects on mitochondrial function, behavior, and neurogenesis underscore the complexity of post-stroke recovery and a need developing effective therapeutic strategies for stroke rehabilitation and improving patient long-term outcomes.

PROSTATIC PROTEASES

Hannu Koistinen

Faculty of Medicine, University of Helsinki, Finland

Proteases have been shown to play a significant role in various stages of malignant progression going well beyond just degrading extracellular matrix. The human degradome, i.e. the complete set of known human proteases, consists of about 600 proteases, half of which are expressed in prostate. We have performed a degradome-wide RNAi screen and identified several proteases, including calpain-2, that are associated with growth and invasion of prostate cancer cells grown as cellular spheroids inside extracellular basement preparation. In validation studies, siRNAs and modified antisense oligonucleotides targeting calpain-2 and calpain inhibitors all reduced formation of invasive prostate cancer cell spheroids. Our preliminary results with patient-derived tissue models support these results. Furthermore, we found that high calpain-2 protein levels are associated with development of metastatic disease after radical prostatectomy. I will shortly describe these studies and our ongoing efforts to develop methods better recapitulating prostate cancer cell invasion.

UNDERSTANDING AND APPLYING EPIGENETIC AGING CLOCKS IN AGING RESEARCH AND INTERVENTIONS

Juozas Gordevičius

VUGENE, Lithuania

Epigenetic aging clocks have emerged as transformative tools for quantifying biological age and evaluating interventions designed to slow or reverse the aging process. By measuring patterns of epigenetic DNA modifications, these clocks provide a bridge between chronological age and biological aging, offering a more in-depth understanding of the aging process and its modulation by lifestyle and therapeutic interventions.

This talk will explore the development and application of epigenetic aging clocks, focusing on the machine learning methodologies underpinning their construction and the biological insights they reveal. We will discuss critical considerations for designing studies that utilize these tools to evaluate the efficacy of anti-aging therapies, addressing challenges such as variability in human populations and the integration of multi-omics data. We will review findings from recent research, including studies in mouse models demonstrating how small extracellular vesicles from young adipose-derived stem cells can reduce epigenetic age and improve healthspan. Epidemiological studies comparing groups, such as Olympic champions versus non-athletes, further highlight the real-world implications of slowed epigenetic aging. Together, these advances are reshaping our understanding of aging mechanisms, informing the design of targeted interventions, and paving the way for clinical applications that could revolutionize healthcare and longevity.

DNA CLEAVAGE MECHANISM OF THE SHORT PROKARYOTIC ARGONAUTE-CONTAINING SPARDA SYSTEM

Mindaugas Zaremba

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

According to the domain organization, prokaryotic Argonautes (pAgos) are divided into long and short pAgos. Long pAgos, similar to eukaryotic Agos (eAgos), comprise four major functional domains (N, PAZ, MID, and (in)active PIWI). Short pAgos contain only the MID and inactive PIWI domains and are typically associated with various effector domain-containing proteins in their operons. Recently, it has been demonstrated that short prokaryotic Argonautes, DNase/RNase associated (SPARDA) using RNA guides recognize their DNA targets, leading to nonspecific collateral cleavage of DNA and RNA(1,2). Such activation of SPARDA by plasmids or phages results in the degradation of cellular DNA and cell death or dormancy, conferring population immunity against invading nucleic acids. However, the detailed structural SPARDA activation mechanism remains unknown. Here, we combine in vivo, in vitro, single-molecule experiments, X-ray, and cryo-EM structural studies to provide the detailed DNA cleavage mechanism of the previously uncharacterized SPARDA system.

References:
1. Prostova *et al. Nat Microbiol*. 2024, 9(5):1368-1381.
2. Lu *et al. Nucleic Acids Res*. 2024, 52(2):844-855.

EMERGING FUNCTION OF THE MYSTERIOUS DI-ADENOSINETETRAPHOSPHATE

Gert Bange

Center for Synthetic Microbiology (SYNMIKRO), University of Marburg and Max-Planck Institute for terrestrial Microbiology, Marburg, Germany
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Diadenosine tetraphosphate (Ap4A) and its relatives are multifunctional signaling molecules involved in diverse biological processes, including stress response. These dinucleotides act as metabolic messengers, linking cellular energy status to various physiological pathways. Despite their ubiquity and importance, many aspects of their synthesis, degradation, and signaling mechanisms remain poorly understood. I will share our latest progress in unraveling the roles of Ap4A and related molecules, shedding light on their molecular targets, regulatory pathways, and emerging biological functions. Our findings open new perspectives on their conserved significance in cellular homeostasis.

POINT-OF-CARE BIOSENSORS FOR ASTRONAUTS' MUSCLE HEALTH MONITORING IN SPACE

Dalius Ratautas

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

Space poses significant health challenges for astronauts, particularly on long-duration missions where microgravity induces muscle atrophy, bone density loss, anemia, and vestibular disorders. Current diagnostic capabilities in space are limited; standard tests like blood panels are unavailable on spacecraft, and transporting blood samples to Earth is costly, with results delayed by weeks or months. One critical concern is muscle mass loss—reports from ESA indicate substantial muscle decline during long missions despite countermeasures, with decreases reaching 20-30% in some cases. Plasma free L-amino acids (L-AA), essential for muscle protein synthesis, change significantly before and after missions, likely reflecting muscle breakdown or metabolic shifts, though data during missions remain limited. To address this gap, we present results from a project funded by the ESA, aimed at developing a portable, easy-to-use biosensor to measure total L-AA levels directly from human blood in space. This device could enable real-time monitoring of muscle status and metabolic health, providing critical insights into muscle degradation and nutritional needs without requiring Earth-based testing. In-situ L-AA monitoring could enhance astronaut health management and offer new diagnostic capabilities for space medicine, supporting future missions to Mars and beyond. This project has broad implications, potentially aiding remote and resource-limited environments on Earth. By advancing real-time diagnostic technology, we aim to contribute to space health and promote the development of portable biosensors for both space and terrestrial applications.

MECHANISMS OF BACTERIAL ANTIPHAGE DEFENSE BY NAD^+ BASED INTRACELLULAR SIGNALS

Dziugas Sabonis

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

In nature, bacteria are in constant danger posed by viruses that infect them, named bacteriophages (phages). This caused bacteria to evolve genetic elements called defense systems, to protect them against phage predation.In addition to the well-known gene and genome "scissors" - restriction-modification and CRISPR-Cas systems - more than 100 different bacterial antiviral defense systems have recently been discovered[1]. Many of these systems are based on two module composition: a sensor which recognizes phage infection and an effector which halts the phage cycle, commonly by exerting a toxic effect to the bacterial cell. Thoeris is a two module bacterial defense system consisting of a sensor ThsB homologous to the TIR (Toll/interleukin-1 receptor)-domain and an effector, ThsA protein[2]. ThsB recognizes a phage infection and synthesizes a unique signaling molecule which is then bound by the ThsA effector, exerting a toxic effect[3]. Based on the domain composition of ThsA proteins Thoeris systems are classified into types: type I (SIR2-STALD)[4] and type II (TM-Macro)[5]. Structural and functional studies revealed the chemical structure of signaling molecules and toxicity mechanisms of ThsA proteins.

References:
[1] Georjon & Bernheim, *Nat Rev Microbiol*. 2023, 21(10):686-700.
[2] S. Doron *et al. Science* 2018, 359(6379):eaar4120.
[3] G. Ofir *et al. Nature* 2021, 600(7887):116-120
[4] G. Tamulaitiene *et al. Nature* 2024, 627(8003):431-436
[5] D. Sabonis *et al*. *bioRxiv* 2024, https://doi.org/10.1101/2024.01.03.573942

ENHANCED BIODEGRADABILITY OF TEXTILE WASTE THROUGH A MICROBIAL CONSORTIUM AND ITS ENZYMATIC ACTIVITIES ACHIEVED BY BIOAUGMENTATION

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In the current study, the effect of the three bacterial consortia on composting time was investigated using textile waste as the primary substrate, mixed with paper, cardboard and green waste. The performance of these micro-organisms in boosting the degradation of organic matter was thoroughly investigated. To achieve this, three concentrations (4%, 6% and 8%, v/w) of bacterial consortium were applied and tested through bioaugmentation. As a result, the consortium showed a high organic matter degradation potential, reaching a total organic carbon content of 19%, a total Kjeldahl nitrogen of 1.56%, a C/N ratio of 13 and a temperature above 55 ◦C. Mature compost in the ongoing study was completed in 10 weeks, instead of the 44 weeks required for standard composting. Identification of the consortium strains16S rRNA sequencing indicated that they belonged respectively to *Bacillus* sp., *Paenibacillus* sp. and *Enterobacter aerogenes*. These strains are known for their outstanding ability to decompose a broad range of organic matter, including lignocellulosic molecules, through their enzymatic machineries, which was confirmed throughout this study. Several enzymatic activities were identified, including cellulase, β-xylanase, endoglucanase, acid phosphatase, alkaline phosphatase, etc. Moreover, addition of a bacterial consortium to the waste mixture lengthened the thermophilic phase by two weeks, thereby considerably shortening the compost production time. Our results demonstrate that the use of bioaugmentation by endogenous microbial strains consortium significantly accelerates thecomposting process. Ultimately, the bioaugmentation approach could be an environmentally friendly waste management strategy that overcomes the challenges of by-product biodegradation and produces high added-value products.

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VIRAL INFLAMMATION, BIOENERGETICS, AND NEURODEGENERATION

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The causes of most common neurodegenerative diseases (NDDs), like Alzheimer's and Parkinson's, remain largely unknown, however, neuroinflammation is considered an important pathophysiological factor that contributes to neuronal loss during NDDs. Emerging evidence suggests an association between prior viral infections and the onset of NDDs [1]which highlights the potential causative link between viral neuroinflammation and neurodegeneration. Viral infections lead to the activation of innate immune cells of the brain – microglia, and prolonged activation results in a toxic environment which exacerbates neuronal damage and, in turn leads to further microglial activation fueling a vicious cycle of neuroinflammation. During bacterial inflammation changes in microglial inflammatory response are accompanied by changes in their energy metabolism involving the glycolytic system, TCA cycle and the OxPhos system[2], however not much is known about the effects of viral infection on the energy metabolism in microglia. Since the bioenergetic profile of immune cells is linked to their functional phenotype, it is important to know the impact of viral inflammation on the metabolism of microglial cells. We found that viral mimetics poly-(I:C) and loxoribine applied to primary neuronal-glial cultures induces loss of living neurons without increases in apoptosis or necrosis. Moreover, this loss of neurons was accompanied by microglial activation and increased phagocytic activity. In addition, we measured the impact of these viral mimetics on the mitochondrial respiration, glycolytic activity and amounts of certain TCA cycle-derived metabolites in a murine microglia cell line (BV-2) and found varying effects caused by these viral mimetics. In conclusion, we showed that viral inflammation can induce loss of neurons with concomitant microglial activation and it can also affect microglial energy metabolism.

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THE ROLE OF LIQUID-LIQUID PHASE SEPARATION IN ALPHA-SYNUCLEIN AMYLOID AGGREGATION

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Liquid-liquid phase separation (LLPS) of proteins and nucleic acids is a rapidly emerging field of study, aimed at understanding the process of biomolecular condensate formation and its role in cellular functions. LLPS has been shown to be responsible for the generation of promyelocytic leukemia protein bodies (involved in genome stability and programmed cell death), stress granules (modulation of stress response), and intrinsically disordered protein condensates (onset of neurodegenerative disorders). Additionally, it has been demonstrated that LLPS of amyloid proteins, such as alpha-synuclein (related to Parkinson's disease), Tau (Alzheimer's disease) and TAR DNA-binding protein 43 (TDP-43, amyotrophic lateral sclerosis) may be an intermediate step during the transition from their native states to cytotoxic fibrillar aggregates.

Recent studies have reported that a number of neurodegenerative disease-related proteins are also capable of forming heterotypic condensates, including prion protein with Tau and alpha-synuclein, as well as alpha-synuclein with Tau and TDP-43. Amyloid-beta (related to Alzheimer's disease) has also been shown to associate into heterotypic droplets with proteins containing low complexity domains. Similarly, cross-interactions of multiple such amyloid proteins were demonstrated to influence their aggregation propensity and the characteristics of the resulting structures. Apart from proteins, which are considered to be directly involved in amyloid-plaque formation, a growing number of evidence also points to S100A9 as being a crucial component in the onset of neurodegenerative disorders via cross-interactions with its monomeric and fibrillar forms.

In this work, we examine how LLPS influences the cross-interactions and aggregation reactions of amyloidogenic proteins. We show that during homotypic alpha-synuclein phase separation, the aggregates formed within protein condensates possess a number of distinct structural characteristics when compared to their non-LLPS variants. On the other hand, alpha-synuclein cross-interaction with S100A9 during heterotypic condensate formation stabilises a single amyloid fibril conformation. In both cases, the formed aggregates pertain a higher self-replication potential, as well as increased cytotoxicity. These results suggest that the occurrence of LLPS as an intermediate step in amyloid aggregation may play a critical role in the onset and progression of neurodegenerative disorders.

BIOCHEMISTRY OF INFECTIOUS DISEASE

RNA EDITING IN TRYPANOSOMATIDAE: LESSONS FROM THE "OBSCURE" SPECIES

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The trypanosomatid flagellates possess in their single mitochondrion a highly complex kinetoplast (k) DNA, which is composed of interlocked circular molecules of two types. Dozens of maxicircles represent a classical mitochondrial genome, and thousands of minicircles encode guide (g)RNAs, which direct the processive and essential uridine insertion/deletion mRNA editing of maxicircle transcripts. While the details of kDNA structure and this type of RNA editing are well established, our knowledge mostly relies on a narrow foray of intensely studied human parasites of the genera *Leishmania* and *Trypanosoma*.

My talk will be focused on non-model representatives of Trypanosomatidae. We analyzed kDNA, its expression, and RNA editing of two members of the poorly characterized genus *Vickermania* with very different cultivation histories. In both *Vickermania* species, the gRNA containing HL circles are atypically large with multiple gRNAs each. Examination of *V. spadyakhi* HL circle loci revealed a massive redundancy of gRNAs relative to the editing needs. In comparison, the HL circle repertoire of extensively cultivated *V. ingenoplastis* is greatly reduced. It correlates with *V. ingenoplastis*-specific loss of productive editing of transcripts encoding subunits of respiratory chain complex I and corresponding lack of complex I activity. This loss in a parasite already lacking genes for subunits of complexes III and IV suggests an apparent requirement for its mitochondrial ATP synthase to work "in reverse" to maintain membrane potential. In contrast, *V. spadyakhi* retains a functional complex I that allows ATP synthase to work in its standard direction.

BIOLOGY OF ssRNA PHAGES IN METAGENOME SEQUENCING ERA

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Bacteriophages belonging to family Leviviricetes are among the simplest known viruses with short ssRNA genomes typically encoding just four proteins. For a long time it was assumed that due to severe restrictions in genome size, presence of other ORFs in any similar phages is unlikely. However, due to advances in metagenome sequencing there is accumulating evidence that some ssRNA phage genomes actually encode further proteins of unknown function. We performed bioinformatics analysis of about 94,000 Leviviricetes sequence entries, which in combination with expression and structural studies of several candidate hypothetical proteins indeed supported presence of further ORFs in genomes of some ssRNA phages. We have also searched for ssRNA phages with similar genomes in locally available environmental samples and confirmed presence of similar ORFs. Currently, we are isolating the ssRNA phages in order to establish the biological function of discovered ORFs.

THE GLOBAL PURSUIT OF WUHAN MOSQUITO VIRUS 6, A METAGENOMIC RNA VIRUS IMPLICATED IN VERTEBRATE INFECTION

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Metagenomic studies over the last decade have identified thousands of novel RNA viruses that remain poorly characterised. One of these viruses, called Wuhan mosquito virus 6 (WuMV-6), was first discovered in 2013 in China but is now frequently encountered by metagenomic studies exploring mosquito viromes. By now WuMV-6 has been detected and sequenced on every inhabited continent in sufficient numbers to enable genomic epidemiology analyses more frequently applied to outbreaks and epidemics of human or livestock diseases. In my presentation I will outline the multiple lines of evidence implicating WuMV-6 as a likely pathogen of vertebrate animals despite its detection to date in mosquitoes alone.

CODE AND RECODE – THE MULTIFACETED RNA-BASED TACTICS EMPLOYED BY SHEWANELLA PHAGE 1/4 FOR PRODUCTIVE INFECTION

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Viruses feature an evolutionary shaped minimal genome that is obligately dependent on the host cell's transcription and translation machinery for propagation. To ensure efficient replication and suppress host cell immune responses, viruses utilize numerous virus- and host-encoded components to commandeer gene expression and protein synthesis [1]. Lately, ribonucleic acid (RNA)-based regulatory strategies, such as post-transcriptional ribonucleoside modification dynamics, infection-induced transfer RNA (tRNA)-derived fragments, and virus-encoded tRNAs alike have been reported to interfere with host cell translation and promote viral infection [2]. We have recently characterized a host-virus model where all three approaches come to play. Indeed, we have shown that Shewanella phage 1/4 – a tailed bacteriophage with a dsDNA genome belonging to the family myoviridae and infecting the psychrophilic bacterium Shewanella glacialimarina TZS-4 [3] – overcomes codon availability restrictions by altering queuosine modification levels on host tRNAs, thus enabling codon expansion and successful translation of the virus major capsid protein [4]. Shewanella phage 1/4 infection also triggers the formation of specific host tRNA-derived fragments, possibly as a result of Gabija anti-phage host defences being triggered, although at no apparent cost to viral replication. Intriguingly, we have also demonstrated that virus-encoded tRNAArg and tRNAGly are abundantly expressed during Shewanella phage 1/4 infection, although their exact role remains to be determined. The convergence of these three distinct tRNA-related phenomena in Shewanella phage 1/4 makes it a unique model for studying the intricate relationship between tRNA biology and viral replication. This research not only advances our fundamental understanding of virus-host interactions, but also opens new avenues for therapeutic applications to combat both bacterial and viral infections.

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GOLDEN DEATH BACTERIA – EQUIPPED TO KILL THEM ALL

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Chryseobacterium are environmental bacteria from phylum Bacteroidota, that unites microorganisms capable of degrading complex sugar and protein polymers. Strikingly, different species of *Chryseobacterium* adopt various lifestyle – from friendly association with plants, to pathogenic behavior towards animals. Our working model is *C. nematophagum* – bacteria that efficiently kills and completely digests nematodes. We have identified three different secretion systems and unusual toxins employed by this bacterium and demonstrated that each of them is involved in elimination of different prey – nematodes and amoeba and other species of bacteria.

THE EVOLVING THREAT OF ANTIBIOTIC-RESISTANT OPPORTUNISTIC PATHOGENS: DIVERSE STRATEGIES TO ACHIEVE THE SAME GOAL

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The introduction of antibiotics was initially expected to significantly reduce the prevalence of infectious diseases. However, at the end of the 20th century, infectious diseases were already making a return due to the emergence of antibiotic-resistant strains e.g. *Streptococcus pneumoniae*, *Mycobacterium tuberculosis* and others. Another concerning trend of growing antibiotic resistance is the rise of a novel group of pathogens known as opportunistic pathogens: microorganisms, which are relatively harmless to a healthy person, but still able to cause hard-to-treat infections in immunocompromised patients. Some opportunistic pathogens, like *Acinetobacter baumannii*, have been recognized for decades as multi-drug resistant pathogens causing nosocomial infections. Others, though less well-known, similarly pose significant treatment challenges and can be life-threatening. The growing list of microorganisms that can cause infections in vulnerable populations highlights the urgent need for alternative treatment strategies. To address this challenge, it is essential to investigate the tools that opportunistic pathogens useto survive and adapt. A key factor contributing to their survivalis their high innate resistance to antibiotics. Environmental bacteria possess various mechanisms to compete for resources among themselves, which allows them to both produce antibiotics and develop resistance to them; these mechanisms can be speedily adapted by a pathogen to counteract treatment. The ability of opportunistic pathogens to survive in clinical settings and evade the host's immune system further complicates treatment. Another common trait ofthese pathogens is their quick adaptation to new antibacterial therapies. Interestingly, there is no universal strategy among opportunistic pathogens to fulfil these requirements. For instance, *A. baumannii* tends to follow a clonal tactic, with isolates from different parts of the world displaying high genetic similarity. In contrast, another rising opportunist, *Stenotrophomonas maltophilia*, demonstrates significant genetic diversity among its isolates. Our goal is to understand what strategies these opportunistic pathogens undertake and how we can predict future changes in their behaviour and prevent their spread.

FINDING YEAST VIRUS: LOCALIZATION OF L-A CAPSIDS IN HOST CELL

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Baker's yeast *Saccharomyces cerevisiae* is exploited in food industry, biotechnology and is one of the best understood eukaryotic model organisms. It is used for research of various biological processes like gene expression, cell cycle, metabolism, and virus research. Most natural and laboratory *Saccharomyces* yeast isolates harbor endogenous dsRNA totiviruses. Recent findings suggest that dsRNA viruses may play a major role in shaping host response to the environment and even the evolution of genome. However, yeast viruses are commonly overlooked in research as they don't noticeably change yeast growth during standard laboratory cultivation.

The object of this study is an endogenous 4,6 kb dsRNA totivirus ScV-LA. ScV-LA replicates in the cytoplasm of its host and is only transmitted during cell division, sporogenesis and cell fusion. However, it was revealed in our laboratory that ScV-LA capsids localize in the nucleus of the host. In this work we further investigate the localization of ScV-LA capsid and its determinants.

We show that viral proteins, tagged with GFP, are capable of forming capsids and encapsidating viral RNA within them. Using fluorescent microscopy, we established nuclear localization of ScV-LA capsid in different states of yeast cycle: haploid, diploid, and spores. Likewise, it was determined that native viruses do not affect this localization. This work highlights insights of ScV-LA capsid localization in model organism *S. cerevisiae* and suggests yet unknown interactions with the host.

This study was funded by the Research Council of Lithuania (project no. S-MIP-23-28).

ENVIRONMENTAL AND PLANT BIOCHEMISTRY

NEW GENOMIC TECHNIQUES FOR SUSTAINABLE AGRICULTURE IN THE EU

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EU introduced the Green Deal aiming at no net emissions of greenhouse gases by 2050. Agriculture is a net producer of GHG due to agronomic practices and use of fertilizers. Crop plants are also affected by the biotic and abiotic stresses due to climate change. New Genomic Techniques can introduce targeted changes in crop plant genomes allowing for rapid crop improvement to address climate change challenges. Some NGT plants are non-transgenic, however, they are GMOs according to the European Court of Justice ruling. Targeted edits in crop plant genomes can achieve new traits contributing to more sustainable agriculture. Some third countries are already commercializing NGT plants with potential adverse impact on EU food safety and competitiveness of our agricultural innovations. EC produced a legal proposal for plants obtained by certain NGTs and developed criteria to distinguish NGT1 and NGT2 plants which is under consideration by the European Parliament and the Council. Several options for regulating NGT plants in the EU have been proposed and will be discussed. The legal proposal on NGT plants is essential for competitiveness of European agriculture, and to achieve goals of the Green Deal and the Farm2Fork strategy.

ON A TWO-WAY STREET BETWEEN ARABIDOPSIS AND TREE RESEARCH

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Initially my lab has been using Arabidopsis thaliana as a model for tree biology with the focus on vascular development [1, 2]. We have discovered several concepts, some of which (such as the role of cytokinin phytohormone signaling in controlling cambial development [1] or callose, b-1,3-glucan as a spacer in cell wall [4]) have been subsequently tested in a tree system, Populus. More recently we have started to work on a genetic approach in Betula trees, with the focus on branch development [3, 5]. We have uncovered new concepts that have been subsequently validated in Arabidopsis. I will give an update on both approaches.

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STRUCTURAL STUDIES OF INTACT FUNGAL CELL WALLS USING SOLID-STATE NMR SPECTROSCOPY

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The structural studies at atomic level of pathogenic fungal and yeast intact cell walls are crucial to understand the fungal life cycle and bring new antifungal discovery. In recent years solid-state NMR (ssNMR) spectroscopy has emerged as a non-destructive technique to analyse molecular architecture, hydration, dynamics and drug interactions of intact cell walls of fungi. Here we will demonstrate a new ssNMR approaches to study intact fungal cell walls using novel proton detection methods. We will present a detailed study of spectral resolution, coherent life-times, chemical shift assignments and dynamics of cell walls of intact Candida albicans fungus at 60-150 kHz MAS range. The proton line-width and transverse relaxation times for rigid components of the cell wall can be dramatically improved by increasing MAS rates from 60 up to 150 kHz. As a result, we manage to acquired a well-resolved 2D and 3D assignment spectra, leading to the near-complete chemical shift assignment and dynamics data of the of the main polysaccharides. Additionally, we also tested different deuteration techniques to improve proton line-widths.

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COLORECTAL CARCINOGENESIS – LESSONS FROM THE MICROBIOTA SHIFT

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Colorectal cancer (CRC) is the third most common cancer and the second-leading cause of cancer-related diseasesworldwide (1, 2). Since the genetic background has a low relationship to the development of the disease, lifestyles such as smoking, obesity, high-fat content diet, etc., are primarily associated with colorectal cancer progression (2-4). The recent findings also show significant alteration in microbiota in the gut of CRC patients. The reduction of the butyrate-producing bacteria, such as *Bifidobacteria, Roseburia, Faecalibacterium prausnitzii* (5-8), and an increase in opportunistic pathogens Enterococcaceae, Campylobacteras (5, 6) is observed in several studies.

In this study, the analysis of the local colon microbiota was performed. The tissue samples were collected from the patients during colonoscopy or surgery – right and left side of the normal-appearing colon and terminal ileum mucosa. The total DNA was extracted by enzymatic cell lysis. V3-V4 regions of the colon samples and V1-V2 regions of the fecal samples were amplified by PCR, and sequencing was conducted on an Illumina platform.

Microbiota analysis was conducted using the QIIME2 framework (7) to compare the microbiota composition and diversity across different neoplasm groups, such as adenoma and carcinoma. However, no statistically significant differences were observed in alpha diversity (Shannon, Fisher, and Faith PD indices) when analyzing the microbiota compared to the control group. Redundancy analysis (RDA) further revealed no clustering patterns based on the neoplasm type. Differential abundance analysis using the ANCOM-BC2 method (8) failed to identify any statistically significant genera for the neoplasm when controlling for the gut site, patients' sex, and age.

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UNDERGROUND BIOLOGICAL INTERNET: COMMON MYCELIAL NETWORKS IN INTER-PLANT SIGNALLING AND RESISTANCE TO PATHOGENS

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Arbuscular mycorrhizal fungi (AMF) like Rhizophagus irregularis form common mycelial networks (CMNs) that interconnect multiple plant hosts, potentially serving as a conduit for inter-plant communication that influences stress responses. We explored how CMNs affect pathogen tolerance in Medicago truncatula plants connected by AMF and investigated the impact of a known plant defence elicitor on the pathogen tolerance of plants receiving signals via CMN by analysing leaf metabolites, emitted volatiles, and transcriptome data. We found that the integrity of the CMN significantly shaped the responses of signal-receiving plants, with distinct changes in defence-related transcripts and plant isoprenoids, including volatile monoterpenes and triterpene saponins. Additionally, plants receiving signals through intact CMNs from stressed donor plants exhibited increased resistance to Fusarium sporotrichoides and heightened susceptibility to Botrytis cinerea. Our findings emphasize the role of CMNs in shaping plant pathogen responses and suggest that the mechanisms of inter-plant signalling may influence genetic regulation of defence priming, offering new insights into plant pathogen interactions.

PHYTOHORMONE STORY: HOW THE RESPONSE OF SEEDS TO STRESS IS TRANSLATED TO CHANGES IN PLANT BIOCHEMICAL AND PHYSIOLOGICAL PERFORMANCE

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Numerous studies on various crop species have reported that seed irradiation with cold plasma CP or vacuum can positively affect the photosynthetic machinery and plant growth, secondary plant metabolism and stress resistance,yields and quality of production.The effects of CP treatments on seed germination are explained by the rapid changes induced in the balance of seed phytohormones, a diverse group of highly active substances that function as key regulators of virtually all plant processes.The aim of this study was to estimate how the effects of CP on the amount of the active forms of seed phytohormones (abscisic acid, ABA; auxines, IAA; cytokinins, CK; jasmonates, JA, etc.) in red clover (*Trifolium pratense* cv. 'Arimaičiai') seeds of two colors (yellow, Y and purple, P) are related to changes in phytohormone amounts in leaves of 15 day-old seedlings. Seeds were treated with CP for 3 and 7 min, and vacuum for 7 min (further denoted as CP3, CP7 and V7 groups) using low pressure CP equipment. Phytohormones were detected by LC/MS after separation with a reverse-phase–cation exchange SPE column into the acid and basic fractions.The results indicated differences in phytohormone content between Y and P seeds in thecontrol: e.g., the content of IAA and JA-Ile was larger, while the content of 4Cl-IAA, DZ, and MeJA (3.4 times) was smaller in Y compared to P seeds.The effects of seed treatments on phytohormone content were also dependent on seed color. Seed treatment with CP7 and V7 reduced ABA amount twice stronger in P compared to Y seeds. All treatments reduced the content of IAA in Y seeds, while in P seeds the amount of 4-Cl-IAA was decreased. Seedlings grown from P seeds contained several-fold higher amount of ABA-GE, IAA, JA and MeJA compared to Y seedlings. The data on the impact of seed treatments on seedling leaf phytohormones were scattered but the effects were observed. CP7 decreased ABA-GE content, V7 – increased IAA in Y seedlings; CP3 increased DZ in Y seedlings but doubled tZ in P seedlings; CP7 and V7 reduced DZ in P seedlings. V7 reduced JA content in Y seedlings, while in P seedlings JA content was strongly decreased by CP3 and V7.

The results reveal that changes induced by treatments in seed phytohormone composition are followed by modified patterns in the amounts of phytohormones (key regulators of growth and development) in leaves of the growing seedlings.

INFRARED THERMOGRAPHY ENABLES UNLOCKING METABOLIC SIGNATURES OF SEED VIABILITY AND AGEING IN *PISUM SATIVUM* SEEDS

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Understanding why stored seeds age is imperative for the seed trade, agriculture, and conservation, particularly when developing molecular markers of ageing. Analyte partitioning in aged lots containing a mixture of viable and dead seeds can confound results, but distinguishing aged-viable from aged-dead seeds prior to germination is hindered by the lack of effective separation tools. Here, we used infrared thermography (IRT) during seven hours of seed imbibition, to separate pea (*Pisum sativum* L.) seeds from an aged, "unsorted" lot (UnS) into "sorted viable" (S_{viable}) and "sorted dead" (S_{dead}) sub-lots, re-dried and stored them. Changes in the seed metabolome were assessed using a GC-MS-based metabolite profiling approach in UnS, S_{viable} and S_{dead} seeds, with "non-aged" (NA; all viable seeds) and "dead" seeds (D; no viable seeds after extensive ageing) as positive and negative controls. Upon germination, the abundances of distinct metabolite groups increased in NA but decreased in D seeds, or *vice versa*, whereas UnS seeds showed intermediate values. S_{viable} and S_{dead} seeds exhibited the same diverging patterns as NA and D seeds. Thus, levels of ageing-responsive molecules in UnS seeds remained ambiguous, masking clear patterns of seed ageing metabolism. However, IRT-based sorting revealed that the metabolism of S_{viabl} and S_{dead} seeds mirrors the distinct profiles of NA and D seeds, enabling novel insights into the mechanisms underlying seed ageing.

HEAT SHOCK PROTEIN RESPONSE AS AN INDICATOR OF THE ACTION OF EXOGENOUS PROLINE AND GABA ON PLANT PROTEOSTASIS UNDER DROUGHT

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Our preliminary study showed that foliar application of proline (Pro) and gamma-aminobutyric acid (GABA) increased plant drought tolerance. These amino acids accumulate in plant cells under unfavorable conditions and perform many functions, including acting as osmolytes. Moreover, Pro is thought to function as a molecular chaperone, and GABA action may be associated with the regulation of protein degradation. However, the main system that maintains protein structure in cells is heat shock proteins (HSP)/chaperones. Induction of HSPs is a universal response to disruption of plant cellular proteostasis under the influence of stressful factors including water deficit. Its regulation is carried out by heat shock transcription factors (HSF). To understand the effects of exogenous Pro and GABA, individually and in combination, on HSP induction, we studied expression of genes encoding 4 HSPs of different families, 7 HSFs and bZIP60 in *Arabidopsis thaliana* (Col-0). Four-week-old plants grown in soil were sprayed with 0.1 mM Pro, 0.1 mM GABA, and Pro + GABA, after which watering was stopped for half of the plants. After severe wilting, the plants were rehydrated. Leaf samples were collected to characterize key points in this process. RT-qPCR analysis showed that use of both Pro and GABA changed the dynamics and expression level of*AtHSP70-4*, *AtHSP17.4, AtHSP18.2, AtHSP101, AtHsfA2, AtHsfA7a, AtHsfB1, AtHsfB2a* and *bZIP60s* under drought and had no effect on*AtHsfA3* and *AtHsfC1*. It was also noted that some of these genes responded to amino acids under normal conditions, and expression of*AtHsfB1* and *bZIP60s*changed during rehydration. Differences in the effects of Pro, GABA and Pro+GABA were revealed. In particular, the mutual influence of these amino acids (from positive to negative), observed when they were used together, indicates a complex relationship between their metabolism and functioning. Thus, the obtained results demonstrated that exogenous Pro and GABA (each via its own pathway) can modulate the HSP response and thereby cellular proteostasis under drought.

MICROBIAL SAFETY OF INDUSTRIALLY REARED *HERMETIA ILLUCENS* LARVAE AND FRASS

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The black soldier fly larvae (BSFL) efficiently convert food waste into valuable biomass and serve as an alternative source of fat, protein, and chitin. However, limited information is available on the total microbiota of whole BSFL and its implications for the microbiological safety of food and feed. This study aimed to assess bacterial microbiota dynamics in whole BSFL and frass residues during industrial rearing, examining the effects of thermal treatment, total bacterial microbiota (by sequencing V3–V4 region of 16S rRNA), identification of cultivable bacteria, and the presence of antibiotic resistance genes (ARGs).

Second and fourth instar larvae, frozen and dried fourth instar larvae, and frass samples were analyzed. The bacterial microbiota composition in BSFL samples was predominantly Proteobacteria, whereas Firmicutes dominated in frass. The lowest bacterial diversity was observed in the second instar larvae, while frass exhibited the highest diversity. Overall, bacterial community diversity was relatively low. Identified cultivable bacterial strains were primarily from the genera *Proteus, Providencia, Morganella, Staphylococcus, Klebsiella, Enterococcus,* and *Bacillus*. Bacterial counts increased as larvae grew and were comparable in the fourth instar larvae and frass residues, while dried larvae had the lowest viable counts and were primarily colonized by spore-forming bacteria. The viable aerobic counts met the standards for edible insects.

PCR analysis detected ARGs conferring resistance to aminoglycosides, β-lactams, erythromycin, tetracycline, and vancomycin, with the highest diversity and detection rate in frass. The *tetM*, providing resistance to tetracycline, was the most prevalent gene, identified in all tested sample groups. These findings contribute to the limited knowledge of microbial dynamics and ARGs distribution in the industrial-scale upcycling of food waste using BSFL.

This project has received funding from the Research Council of Lithuania (LMTLT), agreement No S-PD-22-93.

GENE EDITING

CRYOEM REVEALS THAT RETRON-ECO1 ASSEMBLES NAD+-HYDROLYZING FILAMENTS PROVIDING IMMUNITY AGAINST BACTERIOPHAGES

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Bacterial immune systems are sophisticated defense mechanisms evolved to protect bacteria from invading genetic elements such as bacteriophages and plasmids. These systems include a diverse array of strategies, ranging from innate defenses, like restriction-modification systems, to adaptive systems, such as CRISPR-Cas, which provide immunity by detecting, targeting, and neutralizing foreign DNA or RNA. These immune systems play a critical role in bacterial survival, highlighting the complexity and innovation of bacterial immunity, with significant implications for biotechnology and medicine. Retrons are part of these defense mechanism. They are toxin-antitoxin systems protecting bacteria against bacteriophages via abortive infection. The Retron-Eco1 antitoxin is formed by a reverse transcriptase (RT) and a non-coding RNA (ncRNA)/multi-copy single-stranded DNA (msDNA) hybrid that neutralizes an uncharacterized toxic effector. In this work we unveil the molecular mechanisms underlying phage defense. We show that the N-glycosidase effector hydrolyzes NAD^+ during infection. Cryoelectron microscopy (cryo-EM) analysis reveals that the msDNA stabilizes a filament that cages the effector in a low-activity state in which ADPr, a NAD^+ hydrolysis product, is covalently linked to the catalytic E106 residue. Mutations shortening the msDNA induce filament disassembly and the effector's toxicity, underscoring the msDNA role in immunity. Furthermore, we discovered a phage-encoded Retron-Eco1 inhibitor (U56) that binds ADPr, highlighting the intricate interplay between retron systems and phage evolution. This work outlines the structural basis of Retron-Eco1 defense, uncovering ADPr's pivotal role in immunity.

STRUCTURAL BASIS FOR CAS9-MEDIATED PRESPACER SELECTION IN TYPE II-A CRISPR-CAS ADAPTATION

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During CRISPR-Cas adaptation, prokaryotic cells become immunized by the insertion of foreign DNA fragments, termed spacers, into the host genome to serve as templates for RNA-guided immunity. Spacer acquisition relies on the Cas1-Cas2 integrase and accessory proteins, which select DNA sequences flanked by the protospacer adjacent motif (PAM) and insert them into the CRISPR array. It has been shown that in type II-A systems selection of PAM-proximal prespacers is mediated by the effector nuclease Cas9, which forms a complex with the Cas1-Cas2 integrase and the Csn2 protein. Here, we employ cryo-electron microscopy to characterize structures of type II-A prespacer selection and integration complexes. Our study uncovers the mechanism of Cas9-mediated prespacer selection in type II-A CRISPR-Cas systems, and reveals the role of the ring-shaped accessory protein Csn2. Repurposing of Cas9 by the CRISPR adaptation machinery for prespacer selection characterized here demonstrates Cas9 plasticity and expands our knowledge of the Cas9 biology.

MOLECULAR DETAILS OF THE INTERFERENCE STAGE OF TYPE I-F CRISPR-CAS MULTIPROTEIN EFFECTOR

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Type I CRISPR-Cas action involves large ribonucleoprotein effectors (>400-500 kDa). Type I-F multiprotein CRISPR-Cas effectors bind DNA targets and serve as docking sites for the in trans target-degrading HD-nuclease and DEAD/H-box helicase. While I-F can be used for genome editing in eukaryotic cells, data on their action is limited for the effector complex together with the nuclease. Here, we use single-particle cryo-EM to address the question of how the nuclease is loaded onto the R-loop formed by the effector complex to ensure the DNA interference.

HOW PRECISE IS PRECISE? PURSUING SPECIFIC EDITING OUTCOMES WITH CRISPR NUCLEASES

Stephen Jones

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The last decade has produced a massive expansion in the genome editing toolset, paving the way for new therapies, diagnostics and technologies. Cas9, Cas12a, and related CRISPR nucleases form the foundation, offering precise genome editing at target sites that complement their RNA guide (gRNA). Yet, two major phenomena limit their utility: 1) Occasional "off-target" cutting activity at sites with just partial gRNA complementarity, and 2) heterogeneous editing outcomes at the intended target site. My team develops high-throughput strategies that benchmark and quantify the off-target propensity of leading CRISPR nucleases, uncovering their precise rates and sites of cutting with nucleotide resolution. Armed with this data, we pursue re-engineering gRNAs to make editing outcomes less variable and more predictable. Our efforts help provide CRISPR users with greater genome editing success without increasing cost or complexity.

A BACTERIAL IMMUNE SYSTEM THAT MAKES DNA AND BREAKS RNA

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How Eco2 mediates anti-phage defense by reverse transcribing non-coding RNA into multicopy single-stranded DNA (msDNA) is unknown. Unlike most other retrons, Eco2 relies on a single protein for defense. Our study reveals the unique trimeric nucleoprotein structure of Eco2, which supports an msDNA-dependent regulation of Eco2's reverse-transcriptase and nuclease fusion protein. We also show that Eco2's broad defense against various phages is triggered by a phage-encoded endonuclease that degrades the msDNA. msDNA decay in turn activates Eco2 for tRNA cleavage, resulting in shutdown of gene expression for abortive infection. Our findings not only advance the understanding of Eco2’s defense mechanism but also offer insights into the biogenesis of this widespread innate immune system.

DIFFERENT ENZYMATIC ACTIVITIES CONTRIBUTING TO TYPE III CRISPR-CAS IMMUNITY

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Type III CRISPR-Cas system provides prokaryotes with adaptive immunity by targeting foreign RNA, triggering a downstream signaling cascade to counteract infections. Upon binding to a complementary transcript, the effector complex catalyzes the synthesis of cyclic oligoadenylates (cOA) from ATP, which in turn activate various auxiliary proteins. These proteins, typically containing cOA-sensing CARF or SAVED domains coupled with diverse effector domains – such as nucleases, proteases, and deaminases–mediate antiviral defense. Structural and functional studies have elucidated the roles of Csm6/Csx1 and Can1/Can2 nucleases, which degrade RNA and/or DNA, thereby suppressing gene expression and potentially inducing programmed cell death. However, the functions of many CARF/SAVED-domain proteins remain unknown.

Here, I present structural and biochemical analyses of several auxiliary proteins, shedding light on their contributions to type III CRISPR-Cas-mediated immunity and proposing an antiviral defense model in bacterial cells.

FUNCTIONAL INSIGHTS INTO TYPE IV CRISPR-CAS

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Type IV-A CRISPR-Cas systems are encoded by conjugative plasmids that often carry antibiotic-resistance genes. Unlike other CRISPR-Cas systems, they lack a Cas1-2 spacer acquisition module, feature a DinG helicase instead of a nuclease, and form ribonucleoprotein complexes with unclear biological functions. In my talk, I will discuss how a Klebsiella pneumoniae Type IV-A system compensates for its lack of an adaptation module to update its CRISPR array, and how these systems mediate plasmid-plasmid conflicts through DinG-assisted crRNA-guided transcriptional interference. Our findings reveal the molecular mechanisms and ecological role of Type IV-A systems and demonstrate their promising biotechnological applications.

DRUG DISCOVERY AND THERAPY APPROACHES

DECODING THE MOLECULAR LANDSCAPE OF ACUTE AND LONG COVID THROUGH METABOLOMIC AND TRANSCRIPTOMIC ANALYSIS

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Long COVID, also known as post-acute sequelae of SARS-CoV-2 infection (PASC), presents significant challenges in understanding its heterogeneous mechanisms, diverse outcomes and developing effective therapeutic interventions. This talk will explore findings from comprehensive longitudinal studies utilizing transcriptomics and metabolomics to elucidate the biological underpinnings of Long COVID. Leveraging datasets and performing omics analysis from hospitalized COVID-19 patients collected across acute and recovery phases, we identified key differentially expressed genes (DEGs), enriched pathways, and metabolic changes associated with acute disease as well as persistent symptoms. These include disrupted amino acid metabolism, immune dysregulation, and mitochondrial dysfunction. Additionally, we discuss how these findings integrate with metabolomic evidence of dyslipidemia and energy metabolism dysregulation to uncover pathways central to Long COVID pathophysiology. This research provides a foundation for identifying biomarkers and therapeutic targets, offering hope for improved diagnostics and precision treatments.

FATTY ACID-DERIVED ENERGY METABOLITES ACYLCARNITINES: GOING WITH THE FLOW?

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Acylcarnitines, esters of fatty acids and L-carnitine, are synthesized and metabolized through fatty acid metabolism pathways. Based on the length of the acyl group and the specificity of associated enzymes and transporters, acylcarnitines are classified as short-chain (2–5 carbons), medium-chain (6–12 carbons), long-chain (13–20 carbons), and very-long-chain (>20 carbons) species. Advances in mass spectrometry have enabled detailed profiling of acylcarnitines in biological samples, opening new opportunities for their use as biomarkers. However, the physiological roles of specific acylcarnitines and the functional significance of their chain-length diversity remain incompletely understood.

Metabolomic studies have identified strong associations between altered acylcarnitine levels and various conditions, including metabolic disorders, diabetes, insulin resistance, cardiovascular diseases, neurological disorders, and certain cancers. A critical knowledge gap remains in differentiating normal acylcarnitine levels from those indicative of pathological states.

Short-chain acylcarnitines, such as acetyl-carnitine, show therapeutic potential. Acetyl-carnitine is widely used as a dietary supplement and is under investigation for its role in treating neurological disorders. Conversely, long-chain acylcarnitines serve as essential energy metabolites in mitochondria under healthy conditions but can negatively impact metabolic health when accumulated in excess. Elevated levels of long-chain acylcarnitines have been shown to impair insulin signaling and mitochondrial function, effects that are reversible upon reducing their concentrations.

Notably, drugs, dietary supplements, and nutritional interventions can modulate acylcarnitine profiles. HMG-CoA reductase (HMGCR) is a key enzyme in cholesterol and isoprenoid biosynthesis and the molecular target of statins.Our research demonstrates that long-chain acylcarnitines accumulate at toxic levels in the liver of Hmgcr knockout mice, establishing a critical link between acylcarnitine metabolism, lipid homeostasis, and potential statin-induced adverse effects. Furthermore, OCTN2 inhibitors such as meldonium and methyl-GBB effectively lower long-chain acylcarnitine levels, offering protection against ischemia-induced brain damage in experimental stroke and isolated heart models.

The emerging evidence underscores the importance of defining physiological and pathological thresholds for acylcarnitines. Understanding their diverse roles in cellular processes offers promising avenues for developing novel biomarkers and therapeutic strategies across metabolic and non-metabolic diseases.

IN VITRO TESTING OF GENETIC MUTATIONS OF G PROTEIN-COUPLED RECEPTORS

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G protein-coupled receptors (GPCRs) are seven transmembrane receptors crucial in transmitting signals across the cell membrane. GPCRs are one of the most important drug targets, with more than 30% of all approved drugs targeting these proteins. Our workgroup has been focusing on developing assays to identify novel drug candidates that can modulate GPCR signaling, leading to potential new therapies. As a result, we have developed several fluorescence-based assays that enable us to describe various aspects of GPCR signaling. On the one hand, fluorescence anisotropy assay allows monitoring ligand binding in real-time in a simplified model system by using receptors expressed on viral particles. On the other hand, we use fluorescence microscopy with live cells to follow ligand binding in more native-like expression systems, enabling quantification of kinetic parameters and monitoring ligand-receptor complex trafficking. For this assay, we have developed automatic machine learning-based workflows that allow us to quantify whole cell and cell membrane fluorescence intensity. The receptors' activation can be measured using FRET-based biosensors by monitoring the second messenger cAMP level. All the assays have been validated with several different wild-type GPCRs and corresponding fluorescent ligands.

Our current study focuses on the genetic variation of GPCRs, specifically dopaminergic receptors. For these receptors, we have several different fluorescent ligands available. Therefore, the tools we have developed can potentially be used to test the impact of missense mutations on ligand binding and receptor activation of specific GPCRs in vitro. Moreover, by testing already approved drugs on various genetic mutants and comparing the results with the wild-type receptors we can obtain information about differences of receptor signaling at the molecular level. In vitro testing allows for the prediction of drug responses, potentially helping to identify drugs that would be optimal for a particular patient in terms of better response or reduced side effects.

EPIGENETIC AVALANCHE: THE EFFECTS OF HUMAN DNA METHYLTRANSFERASE DNMT3A VARIANTS

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DNA methyltransferase 3Ais involved in formation and maintenance of genomic DNA methylation, which is one of the most important epigenetic processes in mammalian development, health and disease. The activity of this conserved multidomain protein depends on autoregulation and multiple interactions with other proteins. Moreover, as a key epigenetic regulator in cells, the enzyme itself affects the activity of many other genes. Altogether, this explains the wide range of clinical features caused by pathogenic variants of DNMT3A and their variable expressivity.

Tatton-Brown-Rahman syndrome (TBRS) is a rare congenital genetic disorder caused by autosomal dominant, usually de novo, pathogenic variants in the DNA methyltransferase DNMT3A gene. Typical TBRS clinical features are overgrowth, intellectual disability, and minor facial anomalies. However, since the syndrome was first described in 2014, a widening spectrum of abnormalities is being described. Cardiovascular abnormalities are less commonly reported but can be a major complication of the syndrome.

We have described a family of three individuals diagnosed with TBRS in adulthood, with a variable expression of cardiovascular features. Exome sequencing and computational protein analysis suggested that the novel familial DNMT3A mutation Ser775Tyr is located in the methyltransferase domain, however, distant from the active site, DNA binding loops or regulatory sites. Nevertheless, as shown by the molecular dynamics analysis, this bulky substitution has a significant effect on DNMT3A protein structure, dynamics, and function. Analysis of patient peripheral blood cfDNA and transcriptome showed shortened mononucleosome fragments and altered gene expression in a number of genes related to cardiovascular health and of yet undescribed function, including several lncRNAs. Our study highlights the importance of the overall protein structure integrity for enzymatic performance, and the importance of epigenetic regulation by DNMT3A on cardiovascular system development and function.

TARGETING THE MICROBIOTA-GUT-BRAIN AXIS IN AGING

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It is estimated that one in three people in Europe are or will suffer from a brain disorder, and this number is expected to increase as a consequence of the general aging of the European population. Brain disorders include neurodegenerative diseases, like Alzheimer's and Parkinson's diseases, but also schizophrenia, epilepsy, autism, depression, stroke, migraine, sleep disorders, traumatic brain injury, pain syndromes, and addiction. Currently, many of them lack an effective treatment or do not have a cure at all. Neuroinflammation, characterized by the activation of microglia cells, is involved in various brain disorders and could be one of the main targets for treating them. Meanwhile, aging increases neuroinflammation, while some microbiota-derived metabolites can reduce it.

Therefore, the microbiota-gut-brain axis seems an interesting candidate for the modulation of neuroinflammation for treating various brain disorders and diminishing negative aging-related consequences. Accordingly, we aim to identify the possible targets in the gut microbiota for the aging brain and to create the tools that allow us to manipulate those targets. The obtained results could provide a deeper understanding of the role of the gut microbiota in microglia activation and a possibility of obtaining a safer and more economical treatment intervention for aging-related brain disorders.

EXTRACELLULAR VESICLES: ARE WE LOST IN TRANSLATION?

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Extracellular vesicles (EVs), secreted by all cell types, serve as carriers for biologically active molecules. Although numerous pre-clinical studies over the past decade have demonstrated their therapeutic potential, broad clinical application remains a distant goal. This is primarily due to two major challenges. First, large-scale manufacturing is required to produce the substantial quantities of EVs necessary for clinical applications, which may impact their composition and therapeutic properties. Second, developers of EV therapeutic products are expected to provide regulatory authorities with detailed information about the therapeutically active substances contained within EVs and their mode of action (MoA). Given the immense molecular complexity of EVs, determining their specific MoA often poses a significant challenge.

I will present our recent advancements in developing new technology for the large-scale production of EVs suitable for preclinical and early-phase clinical trials targeting Parkinson's disease (PD). I will then discuss our findings in the context of the challenges presented above.

ANTICANCER DRUG DESIGN VIA THERMODYNAMICS AND THE PROTEIN-LIGAND BINDING DATABASE

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Human hypoxic solid tumors overexpress carbonic anhydrase IX (CAIX), a transmembrane protein that promotes metastasis and invasiveness, helps cancer cells survive under hypoxia by acidifying the tumor microenvironment, and is a promising anticancer target. We designed, synthesized, and measured affinities (1) of over a thousand small-molecule compounds for the CA family of enzymes and determined the principles of selectivity by inhibiting the CAIX enzymatic activity. The CAIX-selective compounds helped visualize CAIX expression in cancer cells, measure dissociation constants, visualize tumors, and showed efficacy in mice xenograft models.

In search ofdisease-target protein inhibitors, we designed, synthesized, and measuredinteraction thermodynamics and kinetics of over a thousand small molecules. This large amount of datawas assembled into the Protein-Ligand Binding Database (PLBD),available at https://plbd.org (2). The database contains 7677 binding datasets of 623 sulfonamides and other compounds binding with the 12 catalytically active human CA isozymes, Hsp90, COVID proteases, and other proteins, determined by the fluorescent thermal shift assay, ITC, inhibition of enzymatic activity, and SPR. In the PLBD, we emphasize the intrinsic thermodynamic parameters that account for the binding-linked protonation reactions. The database includes calorimetrically measured binding enthalpies, enhancing the understanding of reaction mechanisms. The binding data are linked to 139 X-ray crystal structures of protein complexes with ligands shedding light on the protein-ligand recognition principles that determine affinity and selectivity. The database has been built using the FAIR data principles and the database schema and deposited data have revision and versioning systems providing historical traces of database evolution. The PLBD is useful for the application of AI-ML approaches for small molecule drug design.

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ATYPICAL NUCLEUS PHENOTYPES IN CONFINED CANCER CELLS

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Strong confinement that metastasizing cancer cells encounter can challenge the integrity of the cell nucleus. Nevertheless, the damage is rapidly repaired, ensuring its overall stability. Submitting a panel of cell lines to mechanical confinement, we have identified a novel nuclear phenotype suggesting a strong instability - these nuclei fold into a "sickle-shape", displaying large openings in the nuclear envelope and strong chromatin condensation. However, the cells remain alive, are devoid of apoptotic markers and can even divide once released from confinement. This phenotype was found to be vastly predominant in a cell line derived from circulating tumor cells (CTCs). Analysis of nuclear envelope composition and perturbation experiments point to the conjunction of multiple factors required for occurrence of these atypical nuclei: low lamin A/C and low vimentin levels together with a high level of contractility. This suggests a working model in which strong acto-myosin contractility induced by confinement would tear apart the envelope of mechanically fragile nuclei. It is still unclear whether the "sickle-shape" phenotype is purely detrimental to cell survival under strong confinement conditions, explaining the known short life span of CTCs; or whether it could have been selected as an advantage for a specific set of mechanical constrains in blood circulation. Our work might provide novel insights on the least researched step of the metastatic cascade – circulation in the blood. It also reveals a particular cellular state in which the nuclear envelope becomes highly unstable, potentially suggesting novel therapeutic approaches.

HIGH-THROUGHPUT SCREENING ASSAY FOR PARP-HPF1 INTERACTION INHIBITORS TO AFFECT DNA DAMAGE REPAIR

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ADP-ribosyltransferases PARP1 and PARP2 play a major role in DNA repair mechanism by detecting the DNA damage and inducing poly-ADP-ribosylation dependent chromatin relaxation an recruitment of repair proteins. Catalytic PARP inhibitors are used as anticancer drugs especially in the case of tumors arising from sensitizing mutations. Recently, a study showed that Histone PARylation Factor (HPF1) forms a joint active site with PARP1/2. The interaction of HPF1 with PARP1/2 alters the modifcation site from Aspartate/Glutamate to Serine, which has been shown to be a key ADP-ribosylation event in the context of DNA damage. Therefore, disruption of PARP1/ HPF1 interaction could be an alternative strategy for drug development to block the PARP1/2 activity. In this study, we have developed a FRET based high-throughput screening assay to screen inhibitor libraries against PARP-HPF1 interaction. We optimized the conditions for FRET signal and verifed the interaction by competing the FRET pair in multiple ways. The assay is robust and easy to automate. Validatory screening showed the robust performance of the assay, and we discovered two compounds Dimethylacrylshikonin and Alkannin, with μM inhibition potency against PARP1/2-HPF1 interaction. The assay will facilitate the discovery of inhibitors against HPF1-PARP1/2 complex and to develop potentially new effective anticancer agents.

REPURPOSING STATINS FOR COLORECTAL CANCER

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Objectives: Statins, inhibitors of HMG-CoA reductase (HMGCR)—the rate-limiting enzyme in cholesterol synthesis—are widely prescribed to lower blood cholesterol. Beyond their cardiovascular benefits, cholesterol is essential for cancer cell proliferation, supporting membrane synthesis, signaling pathways, and vesicular trafficking. This study aims to assess the potential of statins as a therapeutic strategy for colorectal cancer (CRC), the third most common cancer globally and the second leading cause of cancer-related mortality. Additionally, we investigate their impact on mitochondrial function, a crucial factor in cancer cell metabolism.

Materials and Methods: The study evaluated the effects of six statins—cerivastatin, atorvastatin, simvastatin, fluvastatin, pravastatin, and rosuvastatin—on colorectal cancer cell lines CaCo2 and HCT116. Key experiments included viability assays, apoptosis analysis via flow cytometry, mitochondrial activity assessment through high-resolution respirometry and Mitotracker Red imaging, and synergy calculations using Bliss' method. Additionally, kinome profiling was performed for simvastatin, atorvastatin, and cerivastatin, testing their influence on over 450 kinases, including wild-type and cancer-associated mutants.

Results: Statins demonstrated dose-dependent cytotoxicity in CRC cells at micromolar concentrations, with cerivastatin exhibiting the strongest anticancer effect. Synergistic activity was observed when atorvastatin, simvastatin, or fluvastatin were combined with 5-fluorouracil (5-FU), enhancing cell death. Statins induced both apoptotic and necrotic pathways. Mitochondrial analyses revealed a significant reduction in oxidative phosphorylation (OXPHOS), specifically affecting complexes I, II, and IV, while paradoxically increasing mitochondrial membrane potential. Furthermore, kinome profiling identified inhibitory effects on kinases such as CAMK1G and TSSK1B at micromolar concentrations, suggesting additional off-target mechanisms beyond HMGCR inhibition.

Conclusions: This study highlights the potential of statins as adjuvant agents in colorectal cancer treatment due to their cytotoxic effects and synergy with 5-FU. Their anticancer activity extends beyond cholesterol depletion, involving mitochondrial dysfunction and inhibition of specific kinases. These findings support further investigation into statin repurposing for CRC therapy, potentially improving treatment outcomes when combined with conventional chemotherapy.

PROTEIN-LIGAND BINDING AFFINITY PREDICTION USING DESCRIPTORS DERIVED FROM VORONOI TESSELLATION

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The estimation of protein and small drug-like ligand binding affinity is one of the important tasks in modern drug development. At the moment, numerous computational models aiming to predict the protein-ligand binding affinity have been developed. However, they possess various drawbacks, such as insufficient prediction accuracy, long computation time or dependence on data similarity to the training set. Therefore, designing a model able to precisely predict the binding affinity for diverse protein-ligand complexes remains an unsolved challenge [1].

Here we present the development of computational models for prediction of protein-ligand binding affinity based on Voronoi tessellation of the 3D structure of molecular complex [2]. Voronoi tessellation is a computational geometry approach which allows to compute molecular descriptors independently of subjectively chosen parameters, such as interatomic distance thresholds. Although Voronoi tessellation-based methods were successfully applied to analyse biomolecular interactions, this approach has been only rarely used for protein-ligand interactions. Attempting to exploit its advantages, we developed machine learning models for prediction of protein-ligand binding affinity using descriptors derived from Voronoi tessellation of the structures of protein-ligand complexes (interatomic contact areas, annotated according to atom properties, and molecular volume change upon complex formation), supplemented by the number of protein-ligand hydrogen bonds. Models were trained utilizing experimentally derived structures and binding affinity data from the high-quality subset of the PDBbind database [3] using different machine learning algorithms (Random Forest, Gradient Boosting Machine and Partial Least Squares).

We investigated the performance of our models using 4 independent datasets of protein-ligand complexes, and the correlation between experimental and predicted binding affinity values ranged from 0.41 to 0.75. Our models demonstrated better prediction results compared to the classical methods, although they were outperformed by some of the recent deep learning-based scoring functions. Furthermore, we demonstrated that the accuracy of prediction depends on the similarity of the target protein to the training set proteins even for such simplistic models.

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3D CELLULAR MODELS

EXPERIMENTAL CELL THERAPY AND ADVANCED CELLULAR MODELS TO REPLACE ANIMAL TESTING

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Cell therapy is a fascinating and highly interdisciplinary field, which integrates various research areas, such as cell biology, immunology, bio-analytics, imaging technologies, medical technology, process development and automation. Here our focus is the regenerative medicine including the development of new cell-based therapies (advanced therapy medicinal products, ATMP) using Mesenchymal Stromal Cells.

To replace animal models, we further aim to develop 3-dimensional cell culture models, to study experimental cell therapies but also for drug testing, e.g. in cancer research.

ADDING THIRD DIMENSION: CHANGES IN RESPONSE TO CYTOTOXIC AGENTS, RADIATION OR ENDOCRINE DISRUPTORS IN 3D VS 2D CELL CULTURE

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Unlike the traditional 2D cell culture, the recently emerged 3D systems are expected to mimic the native tissue context, featuring oxygen and nutrient gradients, limited diffusion of drugs, and intricate cell-cell and cell-matrix interactions. These differences influence cellular behavior, profile of biomarkers, and response to therapeutic agents. Hereby we provide examples based on our recent experience with spheroids generated from cancerous or non-cancerous cell lines.

When exploring efficacy of drug candidates in glioblastoma cell lines under normoxic and hypoxic conditions using both 2D and 3D models, we screened compounds targeting epigenetic regulators and cell cycle kinases [1]. Protein arginine methyltransferase 5 inhibitor on a metostat emerged as the lead, showing high potency under both normoxic and hypoxic conditions and inhibiting spheroid formation at sub-therapeutic concentrations compared to lomustine. The spheroid integrity was disrupted in a cell-line-specific manner: while U-87MG cells exhibited reduced spheroid size at higher onametostat concentrations, U-251MG spheroids showed size increases due to compromised cell-cell contacts. These findings highlighted the importance of considering 3D-specific dynamics in drug testing.

The dissection of the irradiation-induced proteomic changes in lung adenocarcinoma cell lines under 2D and 3D conditions by proteomic analysis revealed irradiation-induced upregulation of different resistance-related protein targets in spheroids versus adherent cells [2]. Functional assays confirmed that irradiation sensitized spheroids to Ephrin Type-A Receptor 2 inhibitorALW-II-41-27. Strikingly, this compound disrupted spheroid integrity at low nanomolar concentrations, demonstrating 3D-specific vulnerabilities of A549 cells.

Finally, investigation of the effects of the endocrine disruptor mono(2-ethyl-5-hydroxyhexyl)phthalate(MEHHP) in endometrial stromal cell line T-HESC indicated that short-term exposure altered plasma membrane fluidity, while prolonged exposure interfered with spheroid formation and disrupted zonula occludens1 protein-mediated cell-cell contacts [3]. These results emphasized that plasma membrane and membrane-bound organelles can serve asdirect targets of endocrine disruptors. Furthermore, dependent on the mode of T-HESC culturing, 72-h treatment with MEHHP caused distinct alterations in transcriptome –involving a set of pharmacologically relevant G-protein coupled receptors [4].

In conclusion, use of 3D models enhances our understanding of drug efficacy, resistance mechanisms, and potential targets, offering critical insights for preclinical research and toxicology studies.

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LOSING ADHESIONS TO PROMOTE PROSTATE CANCER

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The extracellular matrix (ECM) undergoes significant remodeling during prostate cancer (PCa) progression. Cell-ECM interactions, primarily mediated by integrins, play a critical role in regulating cell growth and polarity. We have investigated the functional roles of specific integrins in PCa revealing that the disruption of coordinated focal and hemidesmosomal adhesions contributes to tumorigenic properties of PCa cells. A meta-analysis of multiple PCa cohorts demonstrated that downregulation or genomic loss of ITGB4, ITGA1 and ITGA2 integrin genes correlated with tumorprogression. Specifically, the genomic deletion of both ITGA1 and ITGA2 activated epithelial-to-mesenchymal transition (EMT) in benign prostate epithelial cells, thereby enhancing their invasive potential in vitro and converting them in to tumorigenic cells in vivo. Mechanistically, EMT was induced by enhanced secretion and activation of autocrine TGFβ1 leading to nuclear targeting of YAP1. Our unbiased genome-wide co-expression analysis in large PCa cohort datasets identified the transcription factor TEAD1 as a key regulator of ITGA1 and ITGA2 expression. TEAD1 loss phenocopied the dual loss of α2- and α2-integrins both in vitro and in vivo. While b4-integrin depletion alone had modest effect, it synergized with protumorigenic genomic alterations, such as PTEN-loss, to enhance tumorigenicity. To further investigate the dynamics of these events during early PCa tumorigenesis, we are developing a co-culture model using primary prostate organoids.

IMPACT OF THE MICROENVIRONMENT ON CELL FATE DETERMINATION

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Cell fate is determined by a complex and dynamic interplay of biochemical, biophysical, and mechanical signals within the microenvironment. The extracellular matrix (ECM), substrate stiffness, topographical cues, and cell-cell interactions work in concert to regulate fundamental cellular processes, including differentiation, proliferation, migration, and responses to external stressors. Recent advancements in tissue engineering, regenerative medicine, and mechanobiology have provided new insights in to how the semicroenvironmental factors shape cellular behavior, influencing both normal tissue development and pathological conditions.

This talk will explore the intricate role of microenvironmental cues in directing cellfate, highlighting recent research on artificial tissue development, cancer cell drug resistance, and mesenchymal stem cell responses to environmental stress. By understanding the mechanisms by which cells sense and respond to their surroundings, we can refine biomaterial design, enhance scaffold fabrication techniques, and develop more effective regenerative therapies. These findings hold significant implications for improving clinical applications, from engineered grafts to targeted treatments for tissue repair and disease management.

PATIENT-DERIVED GLIOMA ORGANOIDS REAL TIME IDENTIFICATION WITH DIFFERENTIAL ION MOBILITY SPECTROMETRY

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Purpose: Extent of brain tumor resection continues to be one of the central decisions taken during standard of care in glioma patients. Here, we aimed to evaluate the most essential molecular factors, such as IDH (isocitrate dehydrogenase) mutation in gliomas classification with patient-derived glioma organoids (PGOs)using differential mobility spectrometry (DMS).

Methods: we prospectively recruited 12 glioma patients, 6 IDH-mutated and 6 IDH wild-type tumors, from which PGOs were generated *ex-vivo*. Altogether, 320 PGOs DMS spectra were analyzed with a classifier algorithm based on linear discriminant analysis (LDA).

Results: LDA modelclassification accuracy (CA) obtained between IDH-mutant and IDH wild-type PGOs was 90% (91% sensitivity and 89% specificity). Furthermore, 1p/19q codeletion classification within IDH mutant PGOs reached 98% CA (93% sensitivity and 99% specificity), while CDKN2A/B homozygous loss status had 86% CA (63% sensitivity 93% specificity).

Conclusion: DMS suitability to differentiate IDH-mutated PGOs was thus validated in *ex vivo*cultured samples, PGOs. Preliminary results regarding 1p/19q codeleted PGOs and CDKN2A/B loss PGOs identification endorse testing in a prospective intraoperative glioma patient cohort. Our results reveal a sample classification set-up that is compatible with real-time intraoperative surgery guidance.

OMICS AND SYSTEMS BIOLOGY

DECODING MOLECULAR PROPERTIES FROM SEEMINGLY CHAOTIC FLUORESCENCE INTENSITY TRACES USING FITSA

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Understanding how molecules move and behave inside live cells is crucial for modern biology, yet our tools for studying these processes are limited. One established technique, fluorescence correlation spectroscopy (FCS), allows us to characterize molecular movements in live cells. However, FCS has significant drawbacks: it requires intense laser light that can damage cells and needs long observation times under stable conditions to produce reliable results. Moreover, FCS is usually employed using problematic statistical assumptions that ignore the correlation between points used to fit the models.

We present a new method called fluorescence intensity trace statistical analysis (FITSA), which uses modern computational approaches and libraries that are also fundamental to current artificial intelligence applications. Instead of relying purely on pattern recognition, FITSA incorporates our understanding of biochemical and physical principles to analyze molecular behavior.

Our results show that FITSA can match the precision of conventional FCS while requiring significantly shorter measurement times, thereby reducing the total light exposure of the sample. This advancement is particularly valuable for studying sensitive biological samples where minimizing light exposure is crucial. FITSA thus represents a promising new method for researchers studying molecular processes in living cells.

ENDOCRINE DISRUPTIVE CHEMICALS CHANGE OVARIAN METABOLISM AND SUB-CELLULAR COMPOSITION LEADING TO REDUCED OVARIAN RESPONSE TO FSH STIMULATION

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The normal functioning of an ovary is the prerequisite for oocyte maturation and ovulation, but also for steroidogenesis, which affects most of the organs in the human body. The somatic cells of the ovarian follicle (FSCs) are supporting the oocyte metabolism and are responsible for steroid hormone production. The survival and proliferation of these cells are under the control of the pituitary gland, secreting the follicle stimulating hormone (FSH). Hence, female fertility is a result of a delicate interplay between the pituitary gland and the FSCs.

While women in the Western countries are postponing their motherhood, they are longer exposed to various environmental stimuli. We have shown that several substances in products of everyday use, but known for their properties as endocrine disruptive chemicals (EDCs), can be found in the humanovarian follicular fluid (FF). We show that higher levels of EDCs in FF correlate to a reduced ovarian response to FSH in women undergoing infertility treatment. This results in a lower number of oocytes retrieved after ovarian stimulation and a lower chance of pregnancy from in vitro fertilization procedure.

We chose di(2-ethylhexyl)phthalate (DEHP) as an example to demonstrate the molecular mechanisms of how the ovarian function is disturbed in case of high exposure. We observed that women with high levels of DEHP in their ovarian FF have alterations in the gene expression levels in the corresponding FSCs, intercellular communication by extracellular microRNAs and steroidogenesis. These changes can be partly attributed to a change in the proportions of somatic cell types in the ovary.

Our findings call for closer monitoring and regulation of known and novel substances on the market to protect female fertility and health.

EXPLORING LANDSCAPE OF PANCREATIC NEUROENDOCRINE TUMOUR MICROENVIRONMENT BY TRANSCRIPTOMICS

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Neuroendocrine tumors (NETs) are a heterogeneous group of malignancies arising in the neuroendocrine system. Pancreatic NETs (PanNETs) make up about 7% of all NETs and less than 2% of all pancreatic neoplasms. While the stromal cell contribution to the development of neuroendocrine tumor cells has been identified, currently the overall gene expression profiles of stromal cells, associated pathways and potential interplay mechanisms have not been yet described in PanNETs. In this study using a digital spatial profiling approach we characterized the gene expression profiles of tumor, α-SMA positive stroma, and adjacent normal tissues from eight retrospective PanNET cases. The spatial profiling revealed that stromal cell associated genes were mainly involved in pathways of mainly extracellular matrix modification, while in tumor cells the gene expression profiles were mainly associated with the pathways involved in cell proliferation. The comparison of gene expression profiles across all three PanNET grades revealed that heterogeneity is not only present within the tumor but also the α-SMA positive stroma. Furthermore, the comparison of tumor cells adjacent versus non-adjacent to α-SMA positive stromal cells revealed an overexpression of MMP9 in grade three tumor. Overall, this study provides in-depth characterization of gene expression profiles in both stroma and tumor cells of PanNETs and outlies potential crosstalk mechanisms.

DEEP LEARNING BASED HIGH CONTENT LIVE-CELL MICROSCOPY IMAGE ANALYSIS: FOCUS ON QUALITY BEYOND BLIND METRICS

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Machine learning, particularly deep learning methods like convolutional neural networks, has revolutionized high-content image analysis. Previously, analysis depended on carefully constructed image analysis algorithms. Nowadays, deep learning models rely on empirical data labeling for training, which can be done faster and without exceptional skill in computer vision. However, these models come with a cost: the explainability of their decisions is difficult to trace and fix. Often, the explainability aspect is ignored, and instead, some general quality metrics are calculated as-is. This can lead to a situation where a model optimal in terms of a default metric is not suitable for a specific biological question—metric blindness.

To address these issues, we have employed a biological hypothesis-guided model development approach. This approach is enabled by tight in-lab collaboration and emphasizes interdisciplinary understanding of both biological and computational domains. It is based on principles, methods, and quality control logic that focus on the end goal while treating machine learning as another tool in the biochemist's toolbox. We used the quantification of ligand binding dynamics to G protein-coupled receptors, particularly muscarinic acetylcholine and dopamine receptors in live cells, as a model system. However, the principles can be applied more generally. We have shown that imaging artefacts can significantly impact assay quality but can be efficiently detected by specific machine-learning models. Additionally, downstream analysis metrics, such as dose-response fit quality, serve as more reasonable metrics compared to image-level metrics like the F1 score. Finally, we demonstrate that dedicated narrow machine-learning models can be trained quickly for each experiment type and surpass a single pre-trained model relying on blind metrics, suggesting the usefulness of online and human-in-the-loop machine-learning approaches.

ANALYSIS AND INTEGRATION OF MULTI-OMICS DATA TO OVERCOME CHEMOTHERAPY RESISTANCE IN OVARIAN CANCER

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Computational methods are essential in modern cancer research, enabling the integration and analysis of vast multi-omics datasets to uncover mechanisms of disease progression and treatment resistance. In ovarian cancer, chemotherapy resistance remains a major challenge, requiring advanced analytical approaches to distill information from multi-modal data. Herein, I will present machine learning-based approaches to interpret patient-derived genomics, transcriptomics, epigenetics, histopathological and clinical data. Building on our recent efforts, such as Lahtinen et al., Cancer Cell 2023 and Häkkinen et al., Bioinformatics 2021, I demonstrate how computational strategies enhance our understanding of resistance mechanisms, paving the way for precision oncology.

SEMI-PERMEABLE MICROCAPSULES: EMERGING TECHNOLOGY FOR SINGLE-CELL -OMICS STUDIES

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Every tissue and organ in the human body comprises a diverse array of cells, each responding uniquely to external signals and stimuli. Understanding this cellular heterogeneity is closely tied to advancements in single-cell technologies. In this talk, I will introduce an emerging single-cell technology based on semi-permeable capsules (SPCs). SPCs are microscopic liquid droplets encased in a thin, porous membrane that remains stable under standard laboratory conditions, including thermocycling, centrifugation, solvent exposure, etc. Single cells isolated in SPCs can be processed through a series of independent biochemical reactions to profile both their genotype and phenotype. I will present how SPCs can facilitate high-throughput single-cell -omics studies, achieving sensitivity that surpasses traditional droplet microfluidics and microtiter plate-based approaches. Finally, I will present whole-transcriptome analyses of white blood cells from acute myeloid leukemia (AML) patients. Surprisingly, we observe that innate immune cells exhibit up-regulation of genes typically associated with the AML blast phenotype. This unexpected finding raises the possibility that either differentiated cells respond to the AML-induced microenvironment by activating gene programs similar to immature blasts, or that leukemic blasts themselves retain the capacity to differentiate into aberrant mature phenotypes.

CULTIVATION OF MICROBIAL CONSORTIA FOR FUTURE PROBIOTICS

Kaarel Adamberg

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Colon microbiota, composed of hundreds of different species, is closely associated with our health. Gastrointestinal tract is a dynamic environment in which microorganisms interact with the host via their metabolites. The most important of these are fermentation products such as organic acids and gases. Major substrates for colon microbiota comprise dietary fibres such as pectins and xylans but also mucins secreted by colon epithelial cells. The colon microbiota is like a microbial fingerprint of a person providing unique set of metabolites. We have shown the links between colonic pH, stool consistency and diet depending on the variation of the metabolic pools on the main routes of degradation of dietary fibres to acetate, butyrate, propionate, lactate and succinate.

To systematically elucidate cross-feeding patterns within the gut microbiota in vitro cultivation methods are combined with up-to-date analytics. A novel approach of change-stat culture to propagate functional microbial consortia was used. The cultured microbiotas maintained high diversity, showed dilution rate dependency and good reproducibility. Based on the metabolic model calculations the cross-feeding via acetate, lactate, succinate and 1,2-propanediol was estimated to 10-40 %.

Chemostat culture has high potential for several applications including phage propagation to overcome potential threats of faecal transplantation. Our cultivation approach may constitute the first step of developing novel therapeutic tools with high reproducibility and low risk of infection from the donor material to target gut-related disease.

QUANTITATIVE SYSTEMS BIOLOGY ANALYSIS OF LIPID-PRODUCING YEAST *RHODOTORULA TORULOIDES* CULTIVATION

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Using microbes as biocatalysts to convert abundant feed stocks into fuels, chemicals and food ingredients is one of the ways to reduce the industrial dependence on fossil-based resources. Yeasts are well-known for their fermentation capabilities, but less known for their natural ability to synthesize fats and oils. Red yeast *Rhodotorula toruloides* can naturally accumulate neutral lipids up to 70% of its dry weight, consume wide variety of carbon sources and tolerate toxic compounds often generated during the feedstock pre-treatment processes. Xylose being one of the chemical building blocks in the abundant and locally available waste lignocellulosic feedstock is one of the carbon sources *R. toruloides* can consume to produce lipids. With synthetic biology and rational metabolic engineering, yeast lipid metabolism is able to produce specialty lipids and oleochemicals. Although fatty acid biosynthesis is a well-known biochemical pathway, the metabolic regulation of lipid synthesis in oleaginous yeasts is not well understood.

We have developed a systems biology platform for quantitative studies of yeast lipid metabolism capable of building data-driven links among the central carbon, redox and energy metabolic pathways. We used a stirred tank submerged batch cultivation coupled to quantitative proteomics to reconstruct *R. toruloides* metabolism using an in-silico enzyme-constrained genome-scale metabolic model and flux analysis. Metabolic modelling could accurately predict growth phenotypes and provide first suggestions on metabolic pathways responsible for lipid synthesis. We further established a CRISPR/Cas9 genome editing tool for engineering the lipid synthesis pathways and advancing the understanding of lipid metabolism through functional genomics studies. The analysis of gene knockout mutants helped to understand which metabolic pathways were responsible for lipid synthesis on common carbon substrates, such as glucose, xylose and acetic acid.

Our work provides insights into future biorefinery of *R. toruloides* and advances the fundamental understanding of yeast lipid metabolism. It is also valuable for rational metabolic engineering strategies to improve the performance of lipid-producing yeasts.

EDUCATION AND SCIENCE POLICY

CONDITIONS FOSTERING AND IMPEDING ACADEMIC FREEDOM IN LITHUANIAN ACADEMIA

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Today more than ever academic freedom, the fundamental value enshrined in the Constitutions and laws of different democratic countries is threatened by geo-political changes and redefined science and higher education governance regimes. Drawing on recent studies carried out for the European Parliament (2024) and the European University Association (2025) as well as the findings of the Academic Profession in Knowledge Societies multi country survey study (Leišytė, Marquina and Jones, forthcoming), this session focuses on the question How is academic freedom fostered and what challenges does it face in the Lithuanian academia? Here we would like to discuss the political imperatives, research funding as well as governance arrangements, as well as media role in fostering institutional autonomy of universities on the one hand, and individual academic freedom, on the other hand in Lithuania in the context on similar developments internationally. We draw on research around academic governance and institutional autonomy as the discretion universities have to decide what is important (Christensen 2011; Maassen, Gornitzka, Fumasoli, 2017). The various degrees of autonomy universities have at faculty, department and individual levels are manifested in the control academics (and academic bodies) exercise over the definition of curricula & research, setting standards of quality, recruitment of academic staff and students.

The session will consist of the presentation of the APIKS survey findings focusing on academic perceptions on their agency and the imperatives of management and external funding agencies in Lithuania and internationally. Further, the panel discussion with different stakeholders of higher education policy and academia in Lithuania will reflect on the conditions fostering and impeding academic freedom in Lithuania.

WHY ESTABLISH A JUNIOR SECTION?

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Junior sections (JS) of national societies bring together junior researchers to organise events and provide support from students to students, often centred around networking and career development. FEBS JS unites the national junior representatives with a goal to network, learn from each other and encourage mobility. This talk will showcase the relevance of junior sections and their activities as well as share experiences from establishing the Finnish Biobio JS.

POSTER PRESENTATIONS

GENE EDITING

GE-1

EFFECTIVE SCREENING OF ACTIVE TNPB VARIANTS FOR TAM-DEPENDENT DNA CLEAVAGE

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RNA-guided nucleases Cas9 and Cas12a are essential tools in genome editing field for their ability to efficiently introduce a double stranded DNA breaks in eukaryotic cell genomic DNA[1]. One of the main drawbacks for the successful application of these proteins in therapy is their large size (> 1300 aa) that provides significant constraints on their encoding genes' delivery to the cells, therefore there is a need for smaller nucleases. To tackle this problem, a new class of RNA-guided nucleases has been characterized[2]. Miniature TnpB protein, found in transposon of *Deinococcus radiodurans*, was proven to be an ancestor protein of CRISPR-Cas nucleases and a programmable RNA-guided endonuclease itself capable of efficient cleavage of genomic DNA[3]. ISDra2 TnpB applicability is diminished by its relatively stringent TAM (analogous to PAM sequence for Cas proteins) sequence requirement (5'-TTGAT-3') for the target DNA sequence which reduces the spectrum of potential genomic targets. This limitation can be tackled by using rational protein design that was enabled by determination of ISDra2 TnpB structure[4] which revealed the molecular details of TAM sequence recognition. To investigate ISDra2 TnpB engineering potential for recognizing alternative TAM sequences we adopted high-throughput mutant library screening approach to evaluate TAM recognition by ISDra2 TnpB variants. We discovered the possible recognition of alternative TAM recognition by ISDra2 TnpB variants that helped to establish principles for future engineering efforts. Overall, this study reveals the potential of high-throughput screening system to investigate TnpB variants' potential to cleave DNA and discover key molecular determinants of TAM recognition.

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UNVEILING THE DIVERSITY OF TRANSPOSON-ENCODED PROGRAMMABLE TNPB NUCLEASES

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CRISPR-Cas nucleases have become essential genome-editing tools, transitioning from clinical trials to approved applications for treating genetic disorders. The technology relies on complexes of Cas proteins and guide RNA molecules (gRNAs), which function as RNA-programmed DNA nucleases. The primary proteins used for genome editing, Cas9, and Cas12a, exhibit high activity but have notable limitations, including the requirement for a PAM sequence, non-specific activity, and the large size of their encoding genes, complicating their delivery into the cells. As a result, research efforts have been focused on identifying new nucleases with improved properties. Our previous studies showed that the ISDra2 transposon-encoded TnpB, an evolutionary relative of Cas12a, functions as an RNA-guided nuclease capable of human genome editing in a TAM (Transposon-Associated Motif)-dependent manner. Recent studies show that only a small fraction of the naturally occurring TnpB proteins can be used for genome editing. Here we present a novel set of diverse TnpB proteins with promising potential for genome editing. We produced TnpB orthologs in human cell culture and used the TnpB-containing lysates for in vitro TAM determination assay. Our results revealed a wide variety of TAM sequences for TnpB proteins from the IS200/IS605 family transposable elements. These results contribute to the ongoing efforts to expand the genome editing toolbox with novel RNA-guided TnpB editors that may offer advantages in size, specificity, and targeting flexibility compared to conventional CRISPR systems.

FUNCTIONAL CHARACTERISATION OF CRISPR/CAS9-MEDIATED MIR-30A DELETION IN LLC1 CELLS

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MicroRNAs (miRNAs) are small (21–25 nucleotides), non-coding RNAs that regulate gene expression at the post-transcriptional level. They play critical roles in various biological processes, including cancer progression and metastasis. miRNAs have been implicated in key metastatic processes such as epithelial-mesenchymal transition (EMT), migration, invasion, and colonization of distant organs. Altered miRNA expression profiles are frequently observed in tumor cells and are associated with tumor development, progression, metastasis, and response to treatment, suggesting their potential as diagnostic and predictive biomarkers[1].

Previous studies in our laboratory have identified differences in miRNA expression between mouse Lewis lung carcinoma (LLC1) cells cultured in 2D and extracellular matrix (ECM)-based 3D environments. These findings led to the identification of a subset of 77 miRNAs potentially related to LLC1 cell metastasis, including miR-30a[2]. To further investigate the functional role of candidate miRNAs, loss-of-function studies are important, with CRISPR/Cas9 genome editing serving as a powerful tool for this purpose.

The aim of this study was to investigate the functional role of miR-30a in LLC1 cells by using CRISPR/Cas9-mediated gene deletion, followed by a characterization of the resulting cell lines. The miR-30a encoding DNA sequence was excised using the CRISPR/Cas9 system with two distinct guide RNAs, generating sublines with monoallelic and biallelic deletions. Quantitative PCR confirmed a significant reduction in miR-30a expression in these sublines. Functional analyses revealed that miR-30a deletion did not affect cell morphology, proliferation, or cell cycle progression in monolayer culture. However, wound healing assay demonstrated that miR-30a deletion significantly enhanced cell migration. Further gene expression analysis showed that biallelic deletion of miR-30a led to the upregulation of its predicted target gene, *Prrx1*, a key EMT regulator.

This study demonstrates the efficacy of a dual-gRNA CRISPR/Cas9 approach for miRNA deletion in cancer research. The findings suggest that miR-30a may regulate metastatic properties in LLC1 cells, although further investigations are necessary to fully elucidate its role in lung cancer progression.

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CLONING AND PURIFICATION OF A TYPE III CRISPR-CAS-ASSOCIATED PUTATIVE DNA NUCLEASE

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Bacteria constantly encounter attacks from mobile genetic elements, such as plasmids and bacteriophages. To counter these threats, they have evolved diverse defense mechanisms, including the type III CRISPR-Cas immune system. This system not only degrades foreign nucleic acids but also generates cyclic oligoadenylates (cOA) as secondary signaling molecules. These molecules activate ancillary proteins, enhancing infection clearance and promoting bacterial survival. Despite recent discoveries, many cOA-regulated proteins remain unidentified, and their functions are not yet fully understood.

In this study, we cloned and purified a novel putative DNA nuclease associated with the type III CRISPR-Cas system. This protein contains a CRISPR-associated Rossmann Fold (CARF) sensory domain fused to a predicted nuclease-like domain, suggesting a role in nucleic acid processing. Our findings provide the first insights into the biochemical properties and potential function of this CARF-associated nuclease, contributing to a broader understanding of CRISPR-Cas-mediated immunity.

STUDY OF TYPE IV-A2 CRISPR-CAS SYSTEMS

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CRISPR-Cas systems assure adaptive immunity against invading mobile genetic elements in bacteria and archaea. Among them, type IV-A CRISPR-Cas systems stand out due to their distinct mechanism. Type IV-A systems lack the typical DNA/RNA nuclease activity, instead, these multisubunit systems employ a helicase DinG, which unwinds the DNA rather than cleaving it, thus causing transcriptional interference [1][2]. While subtypes IV-A1 and IV-A3 have been thoroughly characterized through structural and biochemical studies, subtype IV-A2 remains largely unexplored. This is mainly because type IV-A2 systems diverge from other subtypes with the absence of a Cas8 homologous protein, which in type IV-A1 and A3 systems, together with Cas5, is responsible for protospacer adjacent motif (PAM) recognition [1][3][4]. However, in our study using a bioinformatic guilt-by-association approach, it was previously discovered that CRISPR-Cas type IV-A2 systems co-occur with a small, conserved gene near the cas operon, which is transcribed in the opposite direction. Investigating the function of the protein encoded by this gene will not only deepen the understanding of type IV-A2 mechanisms but also lead to their potential applications in gene editing in the future. Here, we present the preparatory studies laying the groundwork for the structural and functional characterization of one of type IV-A2 systems.

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FUNDAMENTAL AND APPLIED BIOCHEMISTRY

FA-1

INFLUENCE OF THE PGLX PROTEIN MUTATIONS ON THE BREX PROTECTION

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The ongoing co-evolution and co-adaptation of bacteria and bacteriophages has led to the development of antiviral defense systems that are encoded in regions of the bacterial genome called defense islands [1]. One of the bacterial antiviral defense systems is BREX (Bacteriophage Exclusion). It is present in about 10 % of prokaryotic genomes. This system inhibits bacteriophage replication by an unknown mechanism of action [2].

Type 1 BREX system consists of a cassette of 6 protein-coding genes: *brxA, brxB, brxC, pglZ, brxL, pglX*. The *pglX* gene encodes the m6A DNA methyltransferase, which methylates specific sequences in the host genome to distinguish itself from foreign DNA [2]. Bacteriophages can evade BREX protection by encoding protein inhibitors. The Ocr protein encoded by the T7 bacteriophage binds specifically to the PglX protein in a way that mimics the double-stranded helix of DNA and blocks BREX protection [3]. In this study, we analysed the influence of PglX mutations on BREX activity and its inhibition.

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ENERGY METABOLISM IN CACO-2 CELLS VS. CRC: A COMPARATIVE ANALYSIS

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Colorectal cancer (CRC) is characterized by significant metabolic alterations, including changes in glycolytic enzyme activity and lactate metabolism. Understanding these metabolic shifts is essential for developing kinetic models of tumor metabolism. A hallmark of cancer metabolism is the Warburg effect, where tumor cells rely heavily on glycolysis for energy production even in the presence of oxygen. This shift provides cancer cells with a rapid energy supply, supporting their proliferation, resistance to apoptosis (cell death), and adaptation to the tumor microenvironment.

In vitro models typically use high glucose concentrations (25 mM), which do not accurately reflect physiological conditions. Since the physiological glucose levels in the human body are lower than 25mM, reducing glucose in culture media helps to create a reliable model of tumor and normal tissue metabolism.

For this reason, to improve the physiological relevance of our study, we cultured Caco-2 cells in DMEM under two glucose conditions: 25 mM and 5 mM. Additionally, future experiments will further refine this system by lowering oxygen levels to better mimicking the hypoxic environment of tumors.

Our study focuses on two aspects of cancer metabolism: enzyme kinetics (Vmax) and lactate production. To investigate these, we used Caco-2 cells, a well-established human colorectal adenocarcinoma cell line, as an in vitro model, alongside clinical CRC samples, to compare metabolic behaviors and provide insights for kinetic modeling.

Enzymatic analysis of glycolytic enzymes revealed that Caco-2 cells cultured in DMEM with 5 mM glucose showed statistically significant differences compared to both intact clinical samples and Caco-2 cells cultured in DMEM with 25 mM glucose. This indicates that glucose availability plays a crucial role in regulating enzyme activity, leading to metabolic adaptations in vitro.

Lactate production further supports the enzyme kinetics findings: cells cultured with 5 mM glucose produced significantly less lactate than those with 25 mM glucose, especially in glucose-free Krebs-Ringer buffer. However, when 10 mM glucose was added, lactate production became comparable between both conditions, emphasizing how changes in glucose availability drive metabolic adaptations.

Ultimately, this study contributes to developing a kinetic model of CRC metabolism by integrating data from both in vitro and clinical samples. This model bridges the gap between experimental and clinical findings, enhancing our understanding of metabolic reprogramming in CRC and aiding in the identification of potential therapeutic targets. Furthermore, it supports the development of more physiologically relevant cell models for future research.

HEALTH CARE BIOSENSOR FOR l-ARGININE MONITORING IN DAILY INTAKES

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Introduction. L-arginine (L-arg) belongs to the most metabolically versatile amino acids and it plays a vital role in metabolism since, apart from being a component of proteins and a precursor of a wide range of other essential compounds in the body, it is the only physiological donor of nitrogen for NOS-catalyzed reactions. Its uptake and metabolism are intricately linked to oxidative stress, apoptosis, and tissue-specific responses, making it a critical focus for understanding and mitigating cellular damage in various diseases. L-arg plays a dual role in cellular physiology, acting as both a vital nutrient and a potential source of damage under certain conditions. Additionally, L-arginine supplementation shows promise in reducing obesity-related fat gain and enhancing reproductive outcomes in animal models. Thus, further research is needed to understand the full implications of L-arg intake in humans. Considering the above, fast and reliable L-arg detection methods are required. In this study, we propose the electrochemical method based on a biosensor consisting of a bienzyme system.

Results. To develop an amperometric biosensor for fast and accurate detection of L-arg, the multilayer membrane containing an immobilized two-enzyme system consisting of arginase and urease was developed. The membrane served as a selective recognition element of biosensors while acting in modeling and real samples. For this biosensor, the main analytical characteristics were studied: linear range 0.2–2 mM, Ki=1.5 mM of L-arg, response time 1.5 minutes, reproducibility throughout the day was high (rsd=2.7 %), after 4 days of operation and storage of the biosensor at room temperature in 20 mM phosphate buffer pH 7.8, the residual activity was 61%.

Conclusions. A novel amperometric biosensor of L-arg was developed, and the possibility of measuring L-arg in real samples was demonstrated. Considering the advantages of electrochemical biosensors, such as their suitability for working with turbid biological samples without additional preparation, the possibility of miniaturization, and the relative cheapness of materials, such biosensors open up new opportunities in healthcare applications.

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SYNTHESIS AND ESTERASE-CATALYSED HYDROLYSIS OF 5'-*O*-AMINO ACID DERIVATIVES OF URIDINE

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Amino acid and nucleoside esters are important class of chemical compounds with significant application in medical chemistry, biochemistry and prodrug development. The conjugation of nucleoside and amino acid serves to improve prodrug permeability and solubility [1,2]. Furthermore, the substitution of the nucleoside 5'-hydroxyl group enhances the prodrug's resistance to deamination thereby improving its stability [3]. This study explores synthesis and enzymatic hydrolysis of 5'-*O*- derivatives of uridine, where the amino acid or dipeptide is attached via ester bond to the 5'-position of ribose.

The synthesis of amino acid-nucleoside conjugates was performed in a three-step process, including amino acid blocking, conjugate synthesis via formation of an ester bond, and conjugate deprotection. The initial step involved the protection of the amino group of selected amino acids using di-tert-butyl dicarbonate. The Boc-protected amino acids were treated with DCC to generate activated amino acid, which simultaneously reacted with 2',3'-*O*-isopropylideneuridine. The synthesized nucleosides were deprotected using trifluoroacetic acid and water mixture. A total of ten 5'-*O*-amino acid and five 5'-*O*-dipeptide derivatives of uridine were successfully synthesized.

The enzymatic hydrolysis of synthesized esters to nucleoside and corresponding amino acid or dipeptide was tested with 47 esterases. Esterases PLCDK52, PLCD83, PLCD14D, SVG1, SVG3 and 24T1 exhibited the highest enzymatic activity, demonstrating the ability to utilize all tested nucleosides as substrates. Actually, 5'-*O*-amino acid derivatives of uridine undergo hydrolysis more efficiently than their peptide analogues. In addition, L-amino acid derivatives of nucleosides were degraded more efficiently than their D-amino acid counterparts.

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SYNTHESIS OF NOROVIRUS-LIKE PARTICLES IN DIFFERENT YEAST EXPRESSION SYSTEMS

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Virus-like particles (VLPs) are self-assembling multimeric nanostructures that resemble the morphology of native viruses. They mimic the form and size of viruses but lack their genomic material, which prevents them from replicating and causing infections. VLPs can be utilized as delivery systems for various antigens, which can either be presented on their surface or encapsulated within their structure. They can potentially be used in allergen-specific immunotherapy, where they are being studied as carriers for allergenic molecules. VLPs offer a promising platform to enhance the immune system's uptake, recognition, and processing of antigens. Due to their small size, VLPs can diffuse through the 200 nm pores of lymphatic vessels and efficiently travel from the injection site to the draining lymph nodes. Furthermore, VLPs are easily taken up and actively transported by antigen-presenting cells.

Yeast has proven to be an effective platform for producing VLPs, due to its ability to efficiently synthesize heterologous proteins. Additionally, yeast cultivation process is cost-effective, scalable, and simple. The most commonly used yeast species for VLP production are Saccharomyces cerevisiae and Pichia pastoris. However, other yeast species, including Kluyveromyces lactis and Kluyveromyces marxianus have also been successfully used for VLP expression and purification. All of these yeast species are classified as Generally Recognized As Safe (GRAS) and have the Qualified Presumption of Safety (QPS) status, making them suitable for therapeutic product production.

In the present study, Norovirus-like particles (NoV VLPs) were studied. NoV is a single-stranded RNA virus with a genome of approximately 7,500 nucleotides, consisting of three open reading frames (ORFs). ORF2 encodes the major capsid protein VP1 (~58 kDa), which assembles into VLPs measuring either 38-40 nm or 23 nm in diameter. Previous analyses of NoV VLPs indicated that their structural properties are potentially suitable for presenting specific antigens in a nanoparticle structure. In this research, NoV VLPs were produced in four different yeast expression systems: S. cerevisiae, K. lactis, K. marxianus, and P. pastoris. The VLPs were partially purified by ultracentrifugation in a CsCl gradient and visualized using Transmission Electron Microscopy (TEM). The research is funded by the Research Council of Lithuania (LMTLT), agreement No S-MIP-24-41.

DEVELOPMENT OF A MICROFLUIDICS-BASED ULTRA-HIGH THROUGHPUT PLATFORM FOR THE ISOLATION OF SINGLE DNA MOLECULES

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Multiple displacement amplification (MDA) of single DNA molecules isolated in water-in-oil droplets produces condensed DNA microparticles (DNAps) containing between 10^4 and 10^5 copies of a clonally amplified gene. These microparticles form due to interactions between Mg^{2+} ions from the reaction buffer, the amplified DNA, and pyrophosphate, a byproduct of the reaction [1,2]. DNAps hold potential for use in developing a microfluidics-based method for *in vitro* directed evolution. The method would involve generating a gene library, synthesizing DNAps from single DNA molecules, expressing them *in vitro* and using fluorescence-activated droplet sorting to identify enzyme variants with improved catalytic activity.

Our research team has already established a method for DNAp synthesis and *in vitro* expression using regular microfluidic chips. However, the throughput of this approach–approximately 10^6 droplets per hour–is relatively low for large-scale directed evolution applications. Also, the MDA reaction may begin outside the droplets and the Phi29 polymerase used in the reaction could lose its initial activity. To prevent these issues, a method capable of generating droplets at much higher throughput is required.

In this work, we aimed to validate the design of a microfluidic chip and optimize flow parameters to achieve an encapsulation rate of 10^6 single DNA molecules per minute. We used a step emulsification chip, whose geometry allows for throughput exceeding 10^4 droplets per second [3], minimizing the time the reaction mixture remains outside the droplets. To balance the efficient isolation of single DNA molecules in droplets with reagent cost considerations, we selected 4 pL droplets. Unlike in regular microfluidic chips, in step emulsification chips, droplet size does not depend on the flow rate of the oil phase. To achieve the desired 4 pL droplet volume, we experimented with a silicone oil that interacts with the chip, causing channel narrowing due to swelling. By using a low-viscosity silicone oil and a step emulsification chip at an encapsulation rate of 10^6 single DNA molecules per minute, we generated a monodisperse emulsion with water-in-oil droplets approximately 4 pL in volume. The ultra-high-throughput droplet generation achieved in this work should enhance directed evolution experiments that require screening large numbers of enzyme variants *in vitro*.

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EVALUATING THE INFLUENCE OF LIGHT SOURCE WAVELENGTH ON BACTERIORUBERIN SYNTHESIS IN *HALOBACTERIUM SALINARUM* USING A NOVEL 3D-PRINTED CULTIVATION SYSTEM

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Bacterioruberin (BRE) is a C50 carotenoid produced by the halophilic archaeon *Halobacterium salinarum*. It is known for its exceptional antioxidant properties and is therefore widely used in the cosmetics industry [1]. Moreover, *H. salinarum* growth is closely linked to light conditions, owing to its photon-dependent proton pumps [2] and the protective role of BRE in its cell membrane against UV radiation [1]. Thus it is important to understand how specific light conditions influence both the growth of *H. salinarum* and the synthesis of BRE. In this study, we investigated the impact of seven distinct light sources on *H. salinarum* growth and BRE biosynthesis.

To facilitate continuous illumination during cultivation, we developed a novel device and methodology. Using Onshape computer aided design software, we designed custom 3D-printed Polyethylene Terephthalate Glycol (PETG) lids for Erlenmeyer flasks that integrate a 5 mm LED. The chosen light sources for analysis were as follows – red (626 nm), yellow (590 nm), green (525 nm), blue (470 nm), UV (385 nm), warm white (3000 K), cold white (10000 K). The design allows for uninterrupted growth in a shaker incubator, ensuring cultivation simplicity while providing specific wavelengths and constant intensities of light directly into the flask. The system is both cost-effective and easily adaptable for various photobiological studies.

To assess the effects of light conditions on *H. salinarum* growth, we measured optical density at 600 nm (OD_{600}) every 12 hours and prepared growth curves. Additionally, after cultivation, we extracted bacterioruberin using solid–liquid extraction using γ-Valerolactone [3] from the cell cultures and analyzed extracts' absorption at 494 nm to quantify carotenoid content. Lastly, to examine potential differences in synthesized carotenoids, we performed thin-layer chromatography (TLC) using a 1:1 acetone:dichloromethane solvent system [4].

Contrary to initial expectations, our results indicate that neither bacterioruberin synthesis nor cell growth was significantly affected by differences in light wavelength. Cultures grown under each tested light source displayed comparable OD_{600} growth profiles and bacterioruberin yields to control cultures grown in the dark, with no differences in TLC carotenoid migration patterns.

These observations suggest that factors other than light wavelength may play a greater role in regulating bacterioruberin production in *H. salinarum*. The novel 3D-printed lids and integrated LED setup represent a valuable tool for future investigation of photobiological processes in halophilic archaea and other microorganisms, however further optimisation of light intensities is necessary.

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SYNTHESIS OF THE *ARTEMISIA VULGARIS* ALLERGEN ART V 3 IN DIFFERENT YEAST SPECIES: PROTEIN PURIFICATION, AND ANTIGENICITY TESTING

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Allergies are a common health issue worldwide. Based on the statistical research data, allergy prevalence varies between countries from 20% to 40% of the population (Boehmer *et al*., 2018). According to the World Health Organization, by 2050, the incidence of allergic diseases is projected to reach 50% of the world's population (Dbouk *et al*., 2022). Allergens are molecules, which can trigger allergic reactions. Different types of allergies include inhalant, food, contact, drug, and insect. Allergy can cause numerous symptoms like watery eyes, itchy throat, etc. It could also develop into chronic conditions like eczema and allergic asthma or even cause anaphylactic shock (van Ree *et al*., 2022). Allergy diagnostics and immunotherapy are still based on whole-allergen extracts from natural sources. However, the use of these extracts is limited by several factors. Natural extracts are difficult to standardize because of a variable number of different allergens, different biological activities of those allergens and potential contaminants. These issues can result in inaccurate results and ineffective treatment. Single recombinant allergen molecules could help to overcome these problems (Valenta *et al*., 2018).

Yeasts are commonly used for recombinant protein synthesis. They have the advantages of both prokaryotic and eukaryotic organisms. Yeast cultivation is simple, cost-effective, and allows the production of recombinant proteins in high yields. Yeast can perform eukaryotic post-translational modifications, including disulfide bond formation and glycosylation (Schütz *et al*., 2023).

The main object of this study was the synthesis of recombinant allergen Art v 3 from *Artemisia vulgaris* (common mugwort). *A. vulgaris* belongs to the *Asteraceae* plant family and is found in Europe, North America, and parts of Asia. This plant has six different allergens (Art v 1-6). Art v 3 belongs to the family of non-specific lipid transfer proteins. About 70% of people allergic to common mugwort are sensitized specifically to Art v 3 (Hauser *et al*., 2022). During this work, recombinant protein Art v 3, fused with maltose binding protein (MBP), was synthesized using *Saccharomyces cerevisiae*, *Kluyveromyces lactis*, and *Pichia pastoris* yeasts. Recombinant proteins were purified and primary antigenicity experiments were conducted. The research is funded by the Research Council of Lithuania (LMTLT), agreement No S-ST-24-73.

CHARACTERIZATION OF HYBRID CLASS 1 AND CLASS 2 CRISPR-CAS SYSTEMS

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CRISPR-Cas systems are diverse microbial RNA-guided adaptive immune systems that protect bacteria from viruses and other mobile genetic elements by using guide RNAs to recognize and target them. These systems have also served as the foundation for several molecular technologies, most notably programmable genome editing. CRISPR-Cas systems are classified into two classes. In class 1 CRISPR-Cas, the effector complex consists of several different Cas proteins to recognize foreign DNA, while a separate component – Cas3 – is responsible for cutting the target DNA. Although Class 1 systems have been used to induce targeted deletions in genomic DNA, the number and size of their constituent components limit their wider application in the genome editing field (Dolan et al., 2019). In class 2 CRISPR-Cas systems, the effector complex comprises a single protein responsible for both target recognition and cleavage. Because of this feature, the class 2 RNA-programmed Cas9 and Cas12 effector proteins have been widely adapted to cut genomic DNA, which can be exploited to introduce precise changes in the genome (Wang and Doudna, 2023).

A recent study has identified previously unknown associations with core CRISPR effector modules (Altae-Tran et al., 2023). One of the associations included hybrid Cas12m-Cas3 systems, where Cas3 nuclease-helicase, characteristic of class 1 CRISPR-Cas systems, is located in the same genomic locus as class 2 Cas12m proteins. Interestingly, the compact Cas12m proteins of this type cannot cut DNA but can strongly bind it in a PAM (short motif adjacent to the DNA target recognized by the Cas effector complex) dependent manner (Bigelyte et al., 2024). The Cas12-Cas3 system is a putative class 1-class 2 hybrid system in which a Cas12m may have associated with a Cas3 helicase-nuclease that might enact an interference mechanism beyond DNA binding.

This project aims to biochemically characterize Cas12m-Cas3 hybrid systems to gain a better understanding of their molecular mechanisms and function. The successful implementation of this project could lead to novel tools with additional functionalities for biotechnological applications.

EXPANDING THE ARSENAL: FIRST PEEK AT A NOVEL TYPE III CRISPR-CAS EFFECTOR

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Clustered Regularly Interspaced Short Palindromic Repeats (CRISPR) loci and CRISPR-associated proteins (Cas) enable bacteria and archaea to defend against bacteriophages by specifically targeting and degrading viral nucleic acids. Among CRISPR-Cas systems, type III stands out by employing a unique signaling pathway. When the type III CRISPR-Cas interference complex identifies a foreign RNA transcript, the Cas10 subunit starts to synthesize cyclic oligoadenylates. These signaling molecules bind to the sensory domains of ancillary effector proteins, triggering the activation of their effector domains. Once activated, these effectors can degrade RNA or DNA, inhibit protein synthesis, or compromise cell membrane integrity, thereby preventing viral replication or killing infected cells [1,2].

Recent studies analyzing previously uncharacterized genes within CRISPR-Cas operons have uncovered a wider variety of effector proteins [3], with predicted biochemical activities not previously linked to CRISPR-Cas systems. Despite that, the exact roles of these newly identified effectors in type III CRISPR-Cas immunity remain poorly understood. Here, we present the initial studies on novel putative type III CRISPR-Cas effectors demonstrating their activity in the heterologous *E. coli* host.

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CHARACTERIZATION OF SIGMA-1 RECEPTOR CONTAINING EXTRACELLULAR VESICLES FOLLOWING SIMULTANEOUS EXPRESSION WITH TETRASPANINS

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Background. The sigma-1 receptor (S1R) is an endoplasmic reticulum protein involved in cellular stress responses and autophagy. S1R has been detected in blood plasma and has been shown to indirectly influence extracellular vesicle (EV) release. Recent finding suggests that modulating S1R activity with its ligands may serve as a novel approach to altering EV content and production. However, direct evidence confirming the presence of S1R on EVs is still lacking.

Methods. The MultiBacMam system was used for the simultaneous co-expression of S1R and tetraspanins: S1R-mCherry with EGFP-CD9 and S1R-EYFP with either CD63-mCherry or CD81-miRFP670 in human ovarian adenocarcinoma SK-OV-3 cells. The EVs were isolated from conditioned cell culture media by tangential flow filtration and concentrated using 8% polyethylene glycol (PEG6000). Emission spectra measurements were used to confirm the presence of specific fluorescent proteins in isolated samples while SDS-PAGE and Western blot was used to quantify the expression levels. A multi-well total internal reflection fluorescence (TIRF) microscopy system was used for the analysis of isolated EVs at a single particle level.

Results. S1R-mCherry labelled EVs were detected in extracellular space during live cell imaging and confirmed in isolated EV samples. Compared to S1R-EYFP, S1R-mCherry was more prone to intramolecular protein cleavage following sample denaturation by heating. Detailed characterization of S1R-EVs using TIRF microscopy showed that 17 ± 10% of Sig1R-enriched particles were associated with CD81, while 40 ± 7% were together with CD9. The strongest spot coupling was observed between Sig1R and CD63-labeled particles, with 60 ± 14% of Sig1R-EYFP-labeled spots colocalizing with CD63-mCherry. Analysis of SK-OV-3 cells revealed no significant impact of S1R overexpression on the endogenous levels of tetraspanins CD63 and CD9, suggesting that S1R overexpression does not increase the endogenous production of these tetraspanins to enhance EV generation and release.

Conclusions. S1R was detected on tetraspanin-labeled EVs, suggesting its potential as a novel EV marker. This finding warrants further investigation to elucidate S1R role in EV biogenesis, composition, and potential diagnostic applications.

STUDIES OF BACTERIAL MICROCOMPARTMENT CORE ENZYME INTERACTION NETWORK

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Bacterial microcompartments (BMCs) are polyhedral organelle-like structures in bacteria, composed of a semi-permeable protein shell and an enzymatic core. Depending on the composition of the enzymatic core, microcompartments can be involved in a variety of metabolic processes, facilitating carbon fixation in carboxysomes, as well as the execution of various catabolic processes in metabolosomes, including the degradation of compounds such as ethanolamine, propanediol, fucose, rhamnose, and others.

Although the majority of the identified BMCs belong to the class of metabolosomes, little information has been gathered so far regarding the interactions within the enzymatic core of this group and any potential regularities. Previous studies have identified that encapsulation peptides—short, often amphipathic sequences within the core enzymes—are involved in the formation of the core-shell interaction. However, the principles governing the interactions within the core itself have been minimally explored.

In our study, we conducted a systematic analysis of enzymatic interactions within metabolosomes, including representatives from seven metabolosome classes. We tested interactions in a total of 14 full-length enzymes with 33 potential encapsulation peptides, identifying associations with eight encapsulation peptides from three bacterial microcompartment classes. Our results illustrate the crucial role of encapsulation peptides in these interactions.

α-LIPOIC ACID AS POTENTIAL COPPER REGULATOR IN CASE OF ALZHEIMER'S DISEASE

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Copper metabolism is dysregulated in Alzheimer's disease (AD), which may trigger the development of AD pathology. AD is accompanied by substantially elevated levels of copper in extracellular space and simultaneous copper deficiency in brain tissue. These changes result in decoppering of cellular copper proteins, which is detrimental to neurons. In the current study, we follow the widely accepted hypothesis that the normalization of copper metabolism leads to the prevention or slowing of the disease and search for new copper-regulating ligands, such as natural ligand α-lipoic acid (LA). We have previously shown that LA could redistribute copper between extra- and intracellular environment in cell culture without being toxic[1]. However, the precise mechanism of action and role of LA in copper metabolism remained elusive. In this study, we compared the cellular effects of LA with those of different synthetic copper-binding ligands: diethyldithiocarbamate (DETC), clioquinol (CQ), D-penicillamine (D-PA) and elesclomol (ES).

We used SH-SY5Y neuroblastoma cell culture (WT and CTR1C-/-) and 3T3-L1 mouse adipocytes cell culture (WT and ATP7A-/-) and conducted ICP-MS and vitality experiments. To study the effect of LA on AD phenotype, the following *Drosophila melanogaster* AD lines were used –UAS-Abeta.1-42.VK00033 ($A\beta^{42}$); UAS-Abeta.11-42.VK00033/TM3, Sb[1] ($A\beta^{11}$) and UAS-APP.Aβ42.D694N.VTR (Iowa) for negative geotaxis.

Experiments with cell culture showed that LA can redistribute copper between intra- and extracellular environments in a safe manner. We demonstrated that LA accumulation in SH-SY5Y WT cells increases slowly but is enhanced by copper. Adding the isotope ^{65}Cu revealed LA's role in shifting extra- and intracellular copper equilibrium. This shift occurred rapidly, altering isotope ratios without disrupting copper transport[2]. We did similar experiments with other cell lines, where CTR1C and ATP7A were knocked down to understand more about the mechanism of LA action. Copper supplementation decreased climbing score of $A\beta^{11}$ and $A\beta^{42}$ flies, whereas LA prevented this decline, indicating that LA can prevents copper toxicity. In summary, we hypothesize that LA can attenuate copper toxicity without affecting cellular functioning. To understand more about the effects of LA on dysregulated copper metabolism, more experiments are planned with transgenic AD flies where different copper transport proteins are silenced.

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MECHANISM OF MINIATURE TYPE III CRISPR-CAS ASSOCIATED RING NUCLEASE

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Type III CRISPR-Cas systems protect prokaryotes from viral infection by RNA-guided degradation of phage nucleic acids and activation of effector proteins via a signaling cascade [1, 2]. Upon recognition of foreign RNA during phage DNA transcription, the Csm/Cmr interference complex synthesizes cyclic oligoadenylate (cOA) molecules, which allosterically activate CARF/SAVED domain containing accessory proteins. While these effectors enhance host immunity, their prolonged activation can be damaging, potentially leading to dormancy or cell death. To prevent toxicity, cOA levels are tightly regulated by target RNA cleavage and termination of cOA synthesis, and degradation by specialized ring nucleases [3] or by the effector itself [4-6].

In this study, we characterize a novel type III CRISPR-Cas-associated ring nuclease [7] and demonstrate its activity both *in vitro* and *in vivo*. We resolve the crystal structure of ring nuclease in complex with cA4 and provide mechanistic insights into its function.

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SYNTHESIS OF VB_ECOS_NBD2 BACTERIOPHAGE-ORIGINATED POLYTUBES IN DIFFERENT YEAST EXPRESSION HOSTS

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Virus-like particles (VLPs) or virus-based nanoparticles (VNPs) are protein structures morphologically similar to viruses. Due to their structural versatility and safety, these nanostructures have extensive applications in biomedicine, including vaccine development, and immunotherapy[1,2].

gp39 is a tail tube protein of the bacteriophage vB_EcoS_NBD2. Previous studies have shown that the tubes formed by these proteins expressed in *S. cerevisiae* are immunogenic, resistant to extreme environmental conditions such as pH and temperature changes, and stable in the presence of denaturing agents[3]. These structures could present a new approach in immunotherapy, particularly in developing chimeric proteins by conjugating allergens to enhance immune response modulation. Nevertheless, the production of the humanized gp39 protein remains a challenge. For this purpose, yeast-based recombinant protein expression systems are beneficial. Their main advantage is that they support eukaryotic-specific post-translational modifications, making these proteins suitable for biomedical applications[4]. However, different yeast strains exhibit distinct characteristics, such as varying glycosylation patterns, which could be important for further protein use.

The aim of this study was to analyse the synthesis of bacteriophage vB_EcoS_NBD2 gp39 nanotube protein in different yeast expression systems, specifically, in *Kluyveromyces lactis* and *Kluyveromyces marxianus*. Initially, yeast cells were transformed with vectors carrying the cloned gp39 protein gene, followed by protein synthesis experiments. These results provide important findings on the most suitable yeast expression systems for tail tube protein production for biomedical applications.

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TYROSINASE IMMOBILIZATION ON ELECTROPOLYMERIZED FILMS FOR ENHANCED DOPAMINE BIOSENSING

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Dopamine (DA) is a crucial catecholamine neurotransmitter in the brain, playing a vital role in regulating motivation, cognition, and motor behaviour. Deregulation of DA levels is implicated in several neurological disorders, including Parkinson's disease, Huntington's disease, attention deficit hyperactivity disorder, and schizophrenia.[1] Consequently, the accurate and sensitive detection of DA is crucial for both clinical and research purposes.[2], [3] While DA is an electrochemically active molecule, developing a highly selective and sensitive method for the quantification of DA remains a significant challenge, particularly in complex biological matrices.[4]

This work presents developing and characterising novel biosensors for DA detection, utilising mushroom tyrosinase (TyrOx) as the biorecognition element. TyrOx, a monophenol monooxygenase, was immobilised onto electropolymerised films of poly-dopamine, poly-noradrenaline, and poly-adrenaline, which served as oxygen-sensitive layers on nanostructured gold electrodes. The resulting enzymatic electrochemical biosensors demonstrated high selectivity for DA and good stability, offering a promising approach for DA quantification.

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A POTENTIAL UTILIZATION OF A THERMOBIFIDA FUSCA CUTINASE AND A METAGENOMIC ESTERASE TB10_7T FOR CYTOSOLIC PROTEIN SECRETION

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Escherichia coli is a widely used protein expression platform due to its fast growth rate, ability to utilize cheap nutrient media and high yields of expressed proteins. Despite of its numerous advantageous characteristics, *E. coli* also suffers from a few drawbacks, among them, an inefficient natural protein secretion [1]. An ability of a microbial host to secrete expressed proteins is desired, because it facilitates protein purification and downstream processing, furthermore, cytosolic protein secretion can serve as a basis for a creation of a method to screen for enzymes, that are active on substrates, which have an impaired access to the cytosol of microbial cells.

Protein secretion in bacteria is usually mediated by an N-terminal or a C-terminal signal peptide, which is recognized by a certain transporter protein complex, called a secretion system [2]. There are multiple different types of secretion systems reported in literature [2]. Additionally, non-classically secreted proteins, which are secreted in spite of lacking any recognizable signal peptides are known [3-5]. Depending on exact secretion mechanism, particular events of non-classic secretion may happen independently of any secretion machinery [3], allowing proteins to be effectively secreted in a heterologous host, such as *E. coli*. Fusion to non-classically secreted proteins, could constitute an attractive strategy for cytosolic protein secretion in *E. coli*.

Thermobifida fusca cutinase is a non-classically secreted enzyme, that has been reported previously [4], and is thought to possess phospholipase activity, which may be responsible for its secretion. Esterase Tb10_7T is an enzyme, that has been discovered by a metagenomic analysis in earlier works of our department [6]. According to bioinformatic analysis, Tb10_7T does not contain any recognizable signal peptide.

In this work, secretion mechanisms of *T. fusca* cutinase and Tb10_7T are investigated by assaying phospholipase activity of *T. fusca* cutinase and determining an influence of an unstructured N-terminal region of Tb10_7T on its secretion. Additionally, *E. coli* cells, expressing either of the enzymes, are imaged by scanning electron microscopy. Furthermore, subcellular localization and activity of fusion proteins, comprised of various cytosolic enzymes and either a *T. fusca* cutinase or a Tb10_7T are determined in this work.

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SYNTHESIS AND EVALUATION OF MUTANT VARIANTS OF THE *ARTEMISIA VULGARIS* ALLERGEN ART V 3 TO IDENTIFY IGE-BINDING EPITOPES

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Over 20% of the world‘s population suffers from allergic diseases, such as allergic rhinitis, allergic asthma, and conjunctivitis, with prevalence increasing annually (Pfaar *et al.*, 2023). Allergies occur when the immune system overreacts to substances that are harmless to most individuals, such as pollen, dust mites, certain foods, and pharmaceuticals. In response, the immune system produces antigen-specific immunoglobulin E (sIgE), leading to symptoms like sneezing, coughing, skin rashes, and runny nose (Wang *et al.*, 2023). Although allergen-specific immunotherapy (ASIT) is the only allergic disease-modifying treatment, it can induce systemic inflammation and other side effects. To overcome severe side effects, such as life-threatening anaphylaxis, which is caused by IgE-mediated mast cell and basophil degranulation, new strategies have been developed to reduce IgE reactivity with allergy vaccines (Campana *et al*., 2010). Identifying IgE binding sites on allergens is the first step in designing new forms of immunotherapy, such as hypoallergen vaccine candidates (Mueller *et al*., 2021).

Airborne pollen is a frequent trigger of respiratory allergies, especially in a changing global climate. Pollen allergies affect many people worldwide (Oh, 2022). *Artemisia vulgaris*, known as a common mugwort, is a highly allergenic plant that releases large quantities of wind-borne pollen. *Artemisia vulgaris* pollen is a major cause of allergic reactions in Europe (Wofner *et al*., 2005). One of *Artemisia vulgaris's* six allergen components is the non-specific lipid transfer protein Art v 3. Its IgE epitopes have not yet been identified. The reactivity of this proteins with sIgE from *Artemisia vulgaris* allergic patients varies from 22% to 70% (Gadermaier *et al*., 2014).

The aim of this research was to generate mutants of the common mugwort allergen component Art v 3 and to conduct a primary antigenicity assay. Mutations in the Art v 3 gene were generated by using the site-directed mutagenesis method. Mutant variants were synthesized in *E. coli*, purified, and tested for sera IgE reactivity. Accurately identifying epitopes recognized by antibodies in patients' sera provides a deeper insight into immunological diseases and could guide the development of new therapies.

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YEAST SURFACE DISPLAY OF EXTREMOENZYME ESTERASE FROM *GEOBACILLUS STEAROTHERMOPHILUS*

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Yeast surface display system is a widely used protein engineering technology that enables the anchoring of recombinant proteins to the yeast cell wall. Our technique uses the a-agglutinin system, where the Aga1 and Aga2 subunits form disulfide bridges. By fusing the target protein with the Aga2 subunit, it can be effectively exposed on the yeast surface [1]. This approach has broad applications in biotechnology, including enzyme immobilization, biocatalysis, and industrial processes. It simplifies substrate accessibility and allows continuous enzymatic reactions. Moreover, this system reduces the risk of enzyme contamination in the final product, as the enzymes remain immobilized on yeast cells and are not released into the reaction mixture.

This study aimed to express and display the extremoenzyme esterase from *Geobacillus stearothermophilus* [2] on the surface of *Saccharomyces cerevisiae* using five distinct yeast expression vectors. Previously developed with various linker peptides between Aga2 and the target protein [3], these vectors were designed to enhance protein synthesis and enzymatic activity. Previous studies have demonstrated that lipase from mesophilic bacteria was successfully displayed on the yeast surface and had enzymatic activity [4]. Since lipases and esterases belong to the same enzyme class, these findings suggest that our yeast surface display system could be also an effective platform for esterase displaying. Furthermore, displaying extremoenzymes like esterase on the yeast surface could enable their function under harsh industrial conditions, including high temperatures that usually inactivate conventional enzymes. The constructed plasmids will be tested further to evaluate their enzyme display and activity efficiency. This study contributes to expanding the application of yeast display in biocatalysis and industrial enzyme production.

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LINKERS AS PART OF NANOPARTICLES IMMOBILISING AMIDOHYDROLASE YqfB: INVESTIGATION OF ENZYMATIC ACTIVITY AND STRUCTURAL CHANGES

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Controlled self-assembly systems are used in various applications, such as developing biosensors and bioimaging devices, manufacturing nanoelectronic materials and vaccines, cellular delivery of therapeutic agents, and tissue engineering. Particular attention is being paid to expanding the application of self-assembling nanosystems as special platforms for the attachment and display of functional amino acids, peptides, or enzymes [1]. The key component in this process is the choice of linkers, which bind different types of molecules or fragments into conjugates and should be considered when designing nanomaterials for targeted delivery or other applications [2].

We aimed to determine the most suitable linker for the construction of an immobilization system based on tail sheath protein gp041 of the bacteriophage vB_KleM-RaK2, which can self-assemble into tubular structures of variable length *in vivo* and *in vitro* [3]. Cryo-EM allowed us to obtain a detailed atomic structure of this protein and the nanotubes it forms. Based on the structure analysis, the C-terminus of the truncated gp041 protein was chosen for display of the *Escherichia coli* amidohydrolase YqfB [4]. The linkers of different lengths and amino acid composition were inserted between the structural (gp041) and functional (YqfB) part of the construct, making the junction flexible ((GGGGS)$_2$, (GGGGS)$_3$, (GGGGS)$_4$), semi-rigid (EAAAK+GGGGS)$_2$ or rigid (EAAAK)$_3$. Constructed chimeric proteins were successfully synthesized in *E. coli* cells. All of them possessed an enzymatic activity which was confirmed by hydrolysis of *N*$_4$-acetyl-2'-deoxycytidine to 2'-deoxycytidine. The specific activity of hybrid proteins and temperature stability were determined and compared to free YqfB. Different types of linkers had only a minor effect on the biological activity of the chimeric amidohydrolase. This study has enabled us to determine which type of linker gives the highest degree of thermostability and the highest level of activity to the engineered nanotubes.

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BIOCHEMICAL STUDY OF THE INTERFERENCE STAGE IN TYPE I-F CRISPR-CAS EFFECTOR COMPLEX

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Prokaryotes have a wide range of defense mechanisms against invading nucleic acids. CRISPR-Cas is by far the most extensively studied and successfully applied anti-phage system. The vast diversity of CRISPR-Cas systems is divided into two classes and six types with different modes of action. The focus of this study is the type I-F CRISPR-Cas defense system found in pathogenic bacteria *A. actinomycetemcomitans* [1]. This system encodes four different proteins (Cas5, Cas6, Cas7, Cas8), which together with crRNA form the ribonucleoprotein effector complex called Cascade (Csy), and *in trans* acting Cas2/3 nuclease-helicase[2]. Cascade binds to the invading DNA site that is complementary to the crRNA, forming the R-loop structure. The R-loop triggers the Cas2/3 to degrade the foreign DNA eliminating the infection[3].

We have recently solved the cryo-EM structure of Cas2/3 bound to the Cascade-DNA complex and determined the interaction surfaces of Cas2/3 with Cascade. Here, we analyse the importance of these surfaces for the DNA interference in the type I-F system.

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EPOXIDATION OR *N*-OXIDATION: REGIO- AND STEREOSELECTIVE OXIDATION USING PmlABCDEF MONOOXYGENASE

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A wide variety of industrial products can be obtained through oxidation reactions. These reactions are appealing not only because of the potential applications of their products but also because they offer opportunities for further modifications of oxidized compounds. In industry, these reactions are typically conducted using traditional chemical reagents such as *meta*-chloroperoxybenzoic acid (*m*CPBA) or hydrogen peroxide (H_2O_2), which are capable of oxidizing a wide range of functional groups. However, these methods encounter challenges when attempting to oxidize complex substrates with multiple oxidation sites. Chemical oxidation of such compounds is often characterized by low regio- and stereoselectivity. A promising alternative for the oxidation of these complex substrates is bioconversion using various oxygenases. The non-heme diiron monooxygenase PmlABCDEF can perform several types of oxygenation reactions, including *N*-oxidation and C=C double bond epoxidation. *O*-alkenyl substituted pyridines possess two potential oxidation sites: the *N*-heteroatom and the C=C double bond, making them interesting candidates as substrates for this enzyme. Using *Pseudomonas putida* KT2440 bacteria producing PmlABCDEF monooxygenase, *O*-alkenyl substituted pyridines were oxidized, resulting in the formation of epoxides, *N*-oxides, and molecules containing both epoxide and *N*-oxide moieties. Notably, this whole-cell biocatalysis method allows for the introduction of an epoxide group into *O*-alkenyl substituted pyridines without the accompanying *N*-oxidation. In contrast, chemical oxidation with *m*CPBA typically results in *N*-oxidation first, followed by oxidation of the double bond. Furthermore, a chiral analysis by a high-performance liquid chromatography with UV detection (HPLC-UV) using Chiracel® OD-H column revealed that PmlABCDEF monooxygenase also exhibits significant stereoselectivity. For instance, the bioconversion of 3-prop-2-enoxypyridine to its corresponding epoxide resulted in the formation of 3-(*S*)-((oxiran-2-yl)methoxy)pyridine, which showed a high enantiomeric excess of 84.5%.

PIONEERING LA-LUS VLP AS POTENTIAL NANODELIVERY SYSTEM

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Virus-like particles (VLPs) are promising nanoscaffolds as nanodelivery systems. In addition to being able to be produced efficiently in a number of expression systems, they also provide a wide range of functionalization options through genetic engineering. In order to carry loose macromolecules, such as proteins, DNA, or RNA, it requires disassembly and assembly in mild controlled conditions to encapsulate cargo. We characterized the Saccharomyces cerevisiae LA-lus VLPs expressed and purified from *Saccharomyces cerevisiae* cells. Using the dynamic light scattering analysis, we tested VLPs under different buffer solutions and pH. The particles exhibited size stability in different buffer solutions over a month at temperatures of 4°C and 37°C. Optimal pH was about or near-neutral. We also tested various denaturing chemical reagents, which may destabilize VLP. Results indicate the majority of chemicals used as denaturants may increase aggregation. Further research is needed to comprehend the optimal conditions for disassembly.

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COMPARATIVE ANALYSIS OF SERUM ACYLCARNITINE PROFILES DURING THE MIXED MEAL TEST IN HEALTHY SUBJECTS, PREDIABETES, AND DIABETES MELLITUS PATIENTS

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Insulin is crucial for regulating carbohydrate and lipid metabolism. In insulin resistance, alterations in serum acylcarnitine (AcylCarn) profiles between fasted and fed states may occur early, reflecting metabolic dysregulation. However, few have examined AcylCarn changes in diagnosing fatty acid metabolism disturbances in disorders like prediabetes and diabetes mellitus (DM). Given the limitations of current diagnostic criteria for prediabetes and DM, this study aimed to compare serum AcylCarn profiles during a mixed meal test (MMT) in healthy individuals, prediabetes patients, and those with type 2 DM.

The study involved 45 participants: 13 healthy controls, 17 with prediabetes, and 15 with type 2 DM. Ages ranged from 28 to 76 years (median 48). The MMT meal contained yogurt and a muesli bar, with plasma samples collected preprandially and at 30, 60, and 120 minutes postprandially to assess serum glucose, c-peptide, and AcylCarn profiles (short-chain AcylCarn (SCAC) C2–C4, medium-chain AcylCarn (MCAC) C5–C10, and long-chain AcylCarn (LCAC) C12–C18). Statistical analysis was performed using the Kruskal–Wallis test.

The control group had significantly lower baseline glucose (4.7mmol/L, 95%CI[4.5;5.0]) compared to prediabetes (5.4mmol/L, 95%CI[5.0;5.7],p=0.044) and DM groups (6.2mmol/L, 95%CI[5.3;7.2],p=0.002). The control group also showed lower glucose elevations across all time points. AcylCarn concentrations changed significantly during the MMT. At 30 minutes, SCAC concentrations decreased more in the control group than in the prediabetes group (p=0.008), but not in the DM group. Similarly, MCAC decreased more in the control group compared to both prediabetes (p=0.0004) and DM groups (p=0.017). LCAC also decreased more in the control group than the prediabetes group at 30 minutes, but not in the DM group. At 60 minutes, SCAC decreased by 33.6% from baseline in the control group (IQR 28.6–37.2), significantly lower than in the prediabetes (5.1%, IQR -1.6–20.3,p=0.004) and DM groups (15.9%, IQR 0–23.1,p=0.045). MCAC decreased by 42.1% (IQR 38.5–54.7) in the control group, more than both prediabetes (27.8%, IQR 4.1–34.9,p=0.002) and DM groups (13.9%, IQR 5.4–41.9,p=0.008). LCAC decreased by 39.5% (IQR 54.4–78.3) in the control group, more than the prediabetes group (19.6%, IQR 6.4–27.1,p=0.011), but not the DM group. At 120 minutes, only SCAC decreased significantly more in the control group than in prediabetes (p=0.043).

These findings suggest that changes in serum AcylCarn concentrations during the MMT could be a useful tool for diagnosing prediabetes and possibly DM. Further studies are needed to establish specific diagnostic cut-off values.

CONSTRUCTION OF BACTERIOPHAGE GENOMIC DNA FRAGMENT LIBRARIES

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Bacteria have developed a wide range of antiviral defenses to protect themselves from infection by their own viruses (bacteriophages) (Wang, et al., 2024). One of the defense strategies used by bacteria against bacteriophages is abortive infection (Abi). Abi is a group of different systems that work by the same mechanism, where phage infection initiates cell death or metabolic slowdown to prevent the completion of phage replication cycle and infection of neighboring cells (Lopatina, et al., 2020).

Many mechanisms of defense systems underlying abortive infection remain poorly understood and identifying genes that activate abortive infection remains a major challenge (Aframian, et al., 2023). So far, in the study of bacterial defense systems, one approach to finding factors that activate abortive infection has been the isolation and sequencing of bacteriophage mutants that escape the effects of defense systems. However, isolation of such phage mutants is successful only for a smaller part of the systems, and other assays are slower and of low-throughput (Stokar-Avihail, et al., 2023).

Our work aims to address the shortcomings of phage mutant assays and to propose a new, faster method to simultaneously screen more bacterial defense systems for their activating factors. This method involves construction of bacteriophage genomic DNA fragments libraries. To construct these phage libraries, first we amplified phage genomic DNA using Oxford Nanopore PCR Barcoding Kit, which allowed us to obtain 3000 kb and longer DNA fragments. These DNA fragments were then cloned into high copy cloning vector. The number of colonies obtained was within the target number of colonies ($>3 \times 10^4$), which is sufficient for complete phage genome coverage. The quality of constructed libraries was then assessed using the Oxford Nanopore sequencing method. The sequenced reads of the phage library were then aligned to the reference phage genome. The reference phage genome was almost completely and evenly overlaid, which showed that the phage libraries were constructed successfully. The libraries will be further screened in cells, coding Abi systems of interest. The constructed phage library is transformed together with the Abi system, and library genes lost due to activated Abi proteins potentially encoding activators would be identified by sequencing.

INVESTIGATING A tRNA-TARGETING HEPN PROTEIN INVOLVED IN BACTERIAL ANTIVIRAL DEFENSE

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Our research is focused on one of the recently discovered bacterial antiviral defense systems from *E. coli*, which comprises a single protein featuring a HEPN (Higher Eukaryotes and Prokaryotes Nucleotide-binding) domain (1). HEPN domains are known for their metal-independent ribonuclease activity, yielding 5′-hydroxyl and 2′,3′-cyclic phosphate ends. The goal of our study is to uncover the precise mechanism by which this protein confers immunity against various phages.

HEPN ribonucleases are known to target a variety of RNAs (2). To identify the specific target of this particular HEPN protein, we added it to *E. coli* cell lysates and conducted small-RNA sequencing of the cleavage products. The results revealed that the primary targets are several tRNAs, with cleavage mainly occurring in the anticodon loop. This tRNA cleavage activity aligns with the abortive infection mechanism observed *in vivo* for this antiviral defense system (1).

It has also been noticed that HEPN domains require dimerization to form a functional active site (2). SEC-MALS measurements indicated that the antiviral protein assembles into much larger homooligomers (decamers or even larger). Currently we perform structural analysis of the protein using cryoelectron microscopy and analyze how it is activated upon phage infection.

Our findings provide some insights into the structure and function of this HEPN-based antiviral defense system, paving the way for a deeper understanding of bacterial immunity against phages.

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UNCOVERING NEW PROKARYOTIC ANTIVIRAL DEFENSE SYSTEMS: PD-T4-1, PD-T7-1, AZACA AND MOKOSH I

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The ongoing evolutionary battle between bacteria and bacteriophages has led to the emergence of diverse prokaryotic antiviral defense systems. Historically, research focused on well-known defense mechanisms like restriction-modification (RM), abortive infection (Abi) systems and the widely-recognized CRISPR-Cas system. However, recent advancements in exploring the vast bacterial pangenome, the collective genetic pool of a species, are revealing a hidden arsenal of previously unknown defense systems clustered in "defense islands" [1, 2]. In this study, we explore the molecular mechanisms and functional optimization of four novel defense systems: PD-T4-1, PD-T7-1, Azaca and Mokosh I. Our research starts by testing whether the defence systems are able to protect against bacteriophages. We have tested the defence systems against bacteriophages from the BASEL collection and have shown that they are active and able to defend against some of the bacteriophages in the collection. We were also able to isolate several bacteriophage mutants (escapers). In future studies, the genomic DNA information isolated from these bacteriophage mutants will provide a more detailed understanding of the defence systems we are studying. Subsequently, we purified wild-type and mutant proteins from the Azaca, PD-T4-1, and PD-T7-1 systems and conducted nuclease activity assays using various DNA substrates. These experiments revealed the specificities and efficiencies of the nucleases, highlighting key functional domains and residues. Finally, we performed optimization studies to determine the optimal conditions for the nuclease activity of the wild-type proteins, enhancing our understanding of their biochemical properties and potential applications.
This work not only advances our knowledge of prokaryotic antiviral defense mechanisms but also lays the groundwork for the development of novel biotechnological tools and therapeutic strategies.

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NOVEL PROKARYOTIC ANTIVIRAL DEFENSE SYSTEMS – OLOKUN AND PD-T7-5

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The long ongoing fight between bacteria and bacteriophages has led to bacteria evolving and/or acquiring diverse defense mechanisms. Many of these have become irreplaceable tools in molecular biology and biochemistry. CRISPR-Cas systems for genome editing and restriction-modification systems (RM) for manipulating nucleic acids are all derived from prokaryotic antiviral defense systems [1]. However, there is always a need for new tools to be discovered. Fortunately, recent bioinformatics approaches have identified "defense islands" (DIs), genomic regions enriched in defense-related genes, leading to the identification of numerous novel and uncharacterized defense systems [2, 3].

This study aims to characterize two novel prokaryotic antiviral defense systems, PD-T7-5 and Olokun, both discovered in the *E. coli* UMB0934 strain. To better understand their underlying mechanisms, site-specific mutagenesis targeting the predicted active centers of each system was performed. Following mutagenesis, Western blotting was used to confirm the expression of the mutant defense systems, while phage plaque assays assessed the effects of these mutations on their ability to protect bacteria from bacteriophage infection *in vivo*. Additionally, the purified mutant proteins were subjected to biochemical assays to evaluate their nucleolytic activity *in vitro*. For PD-T7-5, crystallization screening was conducted to determine optimal crystallization conditions, with the long-term goal of solving its structure.

This study provides critical insights into the mechanisms of these two antiviral defense systems, expanding our understanding of bacterial immunity against phage infection.

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A NEGATIVELY CHARGED CLUSTER IN THE DISORDERED ACIDIC DOMAIN OF GPIHBP1 PROVIDES SELECTIVITY IN THE INTERACTION WITH LIPOPROTEIN LIPASE

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GPIHBP1 is a membrane protein of endothelial cells that transports lipoprotein lipase (LPL), the key enzyme in plasma triglyceride metabolism, from the interstitial space to its site of action on the capillary lumen. An intrinsically disordered highly negatively charged N-terminal domain of GPIHBP1 contributes to the interaction with LPL. In this work, we investigated whether the plethora of heparin-binding proteins with positively charged regions found in human plasma affect this interaction. We also wanted to know whether the role of the N-terminal domain is purely non-specific and supportive for the interaction between LPL and full-length GPIHBP1, or whether it participates in the specific recognition mechanism. Using surface plasmon resonance, affinity chromatography, and FRET, we were unable to identify any plasma component, besides LPL, that bound the N-terminus with detectable affinity or affected its interaction with LPL. By examining different synthetic peptides, we show that the high affinity of the LPL/N-terminal domain interaction is ensured by at least ten negatively charged residues, among which at least six must sequentially arranged. We conclude that the association of LPL with the N-terminal domain of GPIHBP1 is highly specific and human plasma does not contain components that significantly affect this complex.

RATIONAL DESIGN OF CYTIDINE DEAMINASES: UNVEILING OF AMINO ACIDS DETERMINING THE SUBSTRATE SCOPE

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Non-natural nucleosides are used in molecular therapies, vaccines, and diagnostics, for the development of high-throughput enzyme screening systems, and to expand the genetic alphabet. In order to expand the offer of synthetic nucleosides, various enzymes active towards modified nucleosides have been identified and applied.

The aim of this research was to broaden a substrate promiscuity of the metagenomic tetrameric cytidine deaminase CDA_F14 by investigating amino acids responsible for the interactions with the ribose ring. Typical cytidine deaminases (CDAs; EC 3.5.4.5.) catalyze deamination of cytidine or 2'-deoxycytidine to uridine or 2'-deoxyuridine, respectively. However, some prokaryotic tetrameric CDAs showed ability to convert N_4-acyl, N_4-/O_4-/S_4-alkyl, and N_4-/O_4-/S_4-arylpyrimidine nucleosides into derivatives of uridine except for the substrates with substitutes on the ribose ring [1]. The cytidine or uridine compounds substituted at 2'-, 3'- or 5'-position could be potential pharmaceuticals against cancer, viral or fungal diseases.

Based on the structural and molecular docking analysis, five amino acids (Ala46, Tyr48, Asn42, Asn52 and Gly54) potentially influencing the enzyme promiscuity towards substituted cytidine analogs on the ribose ring were identified. All constructed mutants showed changes in the values of kinetic constants and substrate specificity compared to the wild-type CDA_F14. Meanwhile, substrate scope analysis of these mutants led to the discovery of new activities towards cytidine analogues substituted on 5'-position. These results indicate possibilities for further broadening of CDAs' promiscuity towards structurally diverse substrates including ones with substitutes on the ribose ring.

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XYLOSE TRANSPORT INTO MODIFIED *OGATAEA POLYMORPHA* YEAST DURING ALCOHOLIC FERMENTATION

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Growing concerns about the depletion of fossil fuels and their impact on the environment are driving the search for alternative energy sources. Bioethanol, produced from renewable materials, is a promising alternative. When burned, it releases less nitrogen oxides and particulate matter into the environment. In particular, second-generation bioethanol, produced from lignocellulosic biomass derived from agricultural and forestry waste, makes it a more sustainable option as it uses raw materials that are not food-based [1].

Xylose, which is the second most abundant monosaccharide after glucose and the most abundant pentose sugar, is a key component of the hemicelluloses [2]. The utilization of xylose can increase the bioethanol yield from feedstocks, and maximizing xylose uptake by yeast cells is crucial to improving its use in lignocellulosic bioethanol production.

Ogataea polymorpha, a thermotolerant methylotrophic yeast, can ferment xylose at elevated temperatures. However, a challenge for efficient lignocellulosic ethanol production is the transport of xylose into yeast cells in the presence of both glucose and xylose. In xylose-utilizing wild-type yeast cultures, xylose consumption does not begin until glucose has been depleted, resulting in longer fermentation times and incomplete conversion of the sugars from the lignocellulose hydrolysates [3]. This problem arises because the transporters that are responsible for the uptake of xylose have a lower affinity compared to glucose. Furthermore, some potential xylose transporters are removed from yeast plasma membranes at low glucose levels, so making modifications to increase their stability are essential [4]. For this reason, the transporters found in *Saccharomyces cerevisiae* Gal2 and Hxt7 or *O. polymorpha* Hxt1 are considered to be potential xylose carriers and mutagenesis could improve the uptake of xylose and its utilization in alcoholic fermentation. This research project aims to investigate the importance of the *O. polymorpha* Hxt1 transporter and heterologous modifications of the *S. cerevisiae* Gal2 or Hxt7 transporters in *O. polymorpha* yeast, in particular for high-temperature alcoholic fermentation.

The rate of glucose and xylose uptake into the cells can be determined by recording the activity of energy metabolism - respiration, glycolysis, and ATP synthesis. In our experiments, the respiration and glycolysis of *O. polymorpha* cells were evaluated under different environmental conditions, such as reduced or increased sugar concentrations, in a broad interval of temperatures, buffers, and/or in the growth media. Results of the experiments showed that a high concentration of one of the sugars (glucose or xylose) inhibited the entry of the other into *O. polymorpha* cells.

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MICROBIAL BREAKDOWN OF A-RIBAZOLE PHOSPHATE: TRACING THE FATE OF VITAMIN B12 LOWER LIGANDS

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Corrinoids, including vitamin B12, are among the most complex organometallic cofactors found across all domains of life, facilitating essential biochemical processes such as methyl group transfer, carbon skeleton rearrangement, and reductive dehalogenation. Fully assembled corrinoids (cobamides) consist of an upper Coβ ligand, a central cobalt-containing corrin ring, and a lower Coα base, which is part of the nucleotide loop attached to the corrin ring. For example, vitamin B12 (cyanocobalamin) carries an artificial cyano group as its upper Coβ ligand and 5,6-dimethylbenzimidazole (DMB) as the lower base. In nature, the nucleoside base of cobamides varies depending on the microorganism that synthesizes them. While the biosynthesis and function of different lower ligands are well understood, their fate after they are no longer needed remains largely unknown.

In this study, we sought to identify catabolic pathways for α-ribazole phosphate (αRP), the primary lower ligand of vitamin B12. To do so, we screened various surface soil and water samples for αRP degradation activity. Five samples were selected for further analysis, from which we successfully isolated several microorganisms from the *Ochrobactrum* and *Agromyces* genera capable of removing DMB from αRP. The active fraction was purified from the *Ochrobactrum* lysate, and proteomic analysis identified the responsible enzyme as an ADP-ribosylglycohydrolase family protein. Ongoing research is focused on determining the phylogenetic distribution of these enzymes and exploring the substrate range of the identified enzyme.

In summary, this study explores the degradation of α-ribazole, the primary lower ligand of vitamin B12, by identifying environmental microorganisms capable of breaking it down and characterizing an ADP-ribosylglycohydrolase enzyme responsible for its catabolism.

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REGULATION OF LARGE TERMINASE SUBUNIT NUCLEASE ACTIVITY BY THE PORTAL PROTEIN IN NOVEL JUMBO PHAGE KLEB27-3

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The study of bacteriophages, viruses that infect bacteria, has greatly advanced molecular biology and biotechnology. Among these, jumbo phages, with genomes over 200 kilobases, stand out for their genomic complexity and partial independence from host machinery. Jumbo phages have a broad host range, infecting diverse bacterial species, including multidrug-resistant pathogens [1]. They play key roles in microbial ecosystems by regulating pathogenic populations, making them valuable for environmental and clinical applications. Given the growing threat of antibiotic resistance, jumbo phages hold promise as therapeutic agents.

Within the Podoviridae family, the jumbo phage KLEB 27-3, a double-stranded DNA (dsDNA) virus, provides an intriguing model for studying viral assembly and genome packaging [2]. A critical step in this process is the packaging of viral genomes into preformed capsids, facilitated by the terminase complex (TerC) and regulated by the portal protein. The large terminase subunit performs ATP-dependent genome translocation and cleavage, yet how the portal protein regulates the large subunit‘s nuclease function remains unclear.

This study investigates the portal protein of KLEB 27-3 aiming to identify amino acid residues responsible for regulating TerL nuclease activity. Using AlphaFold3 structural modeling, mutagenesis, and DNA curtains it aims to uncover the molecular mechanisms governing this regulation, offering insights into jumbo phage biology and genome packaging dynamics.

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INVESTIGATION OF THE RELATIONSHIP BETWEEN SHORT PAGOS AND THE RNA CHAPERONE HFQ THROUGH NAD+ DETECTION

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Since the recent unraveling of the biology of different prokaryotes and the ever-growing prospects of antibacterial treatment and genome editing, antiviral defense mechanisms in bacteria have drawn immense attention. Due to their complexity and sheer quantity, these systems require in-depth research on their distinct processes [1].

Short prokaryotic Argonaute linked with Toll-Interleukin Receptor (TIR) effector (SPARTA) proteins have been shown to use RNA guides (gRNA) to detect DNA targets (tDNA), which results in the depletion of nicotine adenine dinucleotide (NAD+). Although the Hfq protein has been implicated in many RNA-related processes thus far, including RNA matchmaking, which facilitates base pairing between sRNAs and mRNAs, Hfq interacts with rRNAs to influence rRNA processing, ribosome biogenesis, and translation fidelity, as well as with tRNAs to influence its maturation [2]. Despite this, the relationship between Hfq and the immune response in conjunction with pAgos has not yet been demonstrated. This study aims to investigate how the ubiquitous bacterial RNA chaperone Hfq interacts with short prokaryote Argonautes (pAgos). By analyzing the interactions between the Hfq protein and various homologous SPARTA, as well as the influence of the RNA chaperone on the NADase activity within the defense system, we hope to gain a better understanding of SPARTA functionality and its reliance on other molecules.

Investigating antiviral defense mechanisms and their relationships with other molecules provides a deeper understanding of the connections between bacteria and phages, while also opening new avenues for research in biotechnology and genetic engineering.

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EFFECTS OF FIREWEED (*CHAMERION ANGUSTIFOLIUM* L.) LEAF EXTRACT ON MITOCHONDRIAL FUNCTION IN GASTRIC CANCER CELLS

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Introduction. Fireweed (*Chamerion angustifolium* L.) is a medicinal plant that is effective in treating gastrointestinal inflammation, irritation, gastric and duodenal ulcers, gastritis and colitis, acid reflux [1]. Fireweed is included in a European Medicines Agency (EMA) monograph stating that it can be used to relieve lower urinary tract symptoms associated with benign prostatic hyperplasia [2]. Fireweed contains abundant polyphenolic compounds (tannins, flavonoids, phenolic acids, etc.) and has anti-cancer, anti-inflammatory and anti-bacterial properties [3, 4]. All living cells, whether healthy or cancer, require energy that is synthesized in the mitochondria. Currently there is no scientific research on fireweed leaf extract effect on cancer cell mitochondrial function.

Aim. The aim of the study was to determine the effect of fireweed *(Chamerion angustifolium L.)* leaf extracts, derived from plants grown under different conditions, on mitochondrial function in gastric cancer (AGS) cells.

Methods. The fireweed leaves were collected in Jonava district, Šafarka village (55°00′22″ N; 24°12′22″ E) in 2023 July. The aqueous extracts from unfermented leaves were prepared from plants grown under natural, organic and biodynamic farming conditions. Gastric cancer cells (AGS) were treated with the determined IC_{50} doses for 48 hours. The IC_{50} values were calculated as follows: 0.457 mg/ml for natural, 0.508 mg/ml for organic farming, and 0.590 mg/ml for biodynamic farming conditions. The impact of the extracts on mitochondrial function was evaluated using high-resolution respirometry Oxygraph-2k at 37 °C with glutamate/malate and succinate as substrates [5].

Results. The mitochondrial functions of the AGS gastric cancer cell line were most significantly affected by the fireweed leaf extract obtained from ecologically grown plants. The respiratory rate in leak state (L) with glutamate/malate increased by 168% and in leak state (L+CAT) with succinate by 318% ($p<0.05$), suggesting potential damage to the inner mitochondrial membrane. The respiration rate in the uncoupled state (E, induced by DNP) decreased by 30% ($p<0.05$), indicating impairment of the mitochondrial respiratory chain. Additionally, the cytochrome c effect increased 1.3-fold ($p<0.05$), pointing to damage to the outer mitochondrial membrane. Respiratory control ratio decreased by 62% with glutamate/malate and by 72% with succinate ($p<0.05$) in cells treated with fireweed leaf extract.

Conclusions. The aqueous extract from unfermented leaves of fireweed (*Chamerion angustifolium* L.) noticeably supresses mitochondrial function in gastric cancer cells (AGS). The extract derived from ecologically grown fireweed had the most significant effect. Treatment with the fireweed extract increased the permeability of both, the inner and outer

mitochondrial membranes, thus inhibiting the ability to synthesize ATP, underscoring the potential as a therapeutic agent against gastric cancer.

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THE ROLE OF TUMOR–ASSOCIATED MACROPHAGES IN SECONDARY TUMOR REJECTION IN A CONCOMITANT IMMUNITY MODEL

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Background. Immunotherapy enhances the immune system ability to eliminate tumors, primarily through T lymphocytes, which recognize tumor cells through antigen presentation and cytotoxic responses. However, macrophages also play a key role in the tumor microenvironment, either promoting tumor growth or enhancing anti-tumor immunity. Concomitant immunity inhibits secondary tumor growth without eliminating the primary tumor. Large Peritoneal Macrophages (LPMs) and Small Peritoneal Macrophages (SPMs) exhibit distinct functions: LPMs are predominantly immunosuppressive (M2-like phenotype), while SPMs express MHC-II and respond dynamically to inflammation (Bou Ghosn et al., 2010). This study aimed to assess macrophage subpopulation differentiation during secondary tumor rejection in a DBA/2 mouse - SL2 tumor model.

Methods. A concomitant SL2 tumor immunity model was used in DBA/2 mice, divided into three groups: (1) concomitant immunity, (2) tumor-only, and (3) control. Flow cytometry was used to assess immune cell populations in peritoneal samples, with cells stained using CD19–PerCP–Cy5.5 (B cells), CD11b-APC (myeloid cells), CD11c-PE (dendritic cells), and F4/80-FITC (macrophages). One-way ANOVA followed by post hoc Tukey's test was used for statistical comparisons ($p < 0.05$ considered significant).

Results. Small peritoneal macrophages (SPMs) levels were significantly higher in the concomitant immunity group (19.2%) compared to the tumor-only group (5.6%) ($p = 0.001$), suggesting their active role in tumor rejection. $CD11c^+$ dendritic cells exhibited significant difference ($p < 0.001$) with elevated levels in the tumor-only group, indicating their potential regulatory role in tumor progression. $CD19^+$ B cells were significantly lower in tumor-bearing groups, suggesting a shift in immune regulation ($p = 0.043$).

Conclusions.

- SPM levels were significantly higher in the concomitant immunity group, suggesting their role in secondary tumor rejection.
- $CD11c^+$ dendritic cells varied significantly between groups, with higher levels in the tumor-only group, suggesting a regulatory function in tumor immunity.
- $CD19^+$ B cells were reduced in tumor-bearing groups, indicating alterations in immune regulation, possibly reflecting changes in humoral immunity or immune suppression in the tumor microenvironment.

Discussion. These findings support the hypothesis that SPMs contribute to tumor rejection, while LPMs maintain a homeostatic role. The distinct functions of macrophage subsets emphasize the complexity of the tumor immune microenvironment. Future studies should investigate SPM-driven inflammation, cytokine signaling, and macrophage-T cell interactions to optimize immunotherapeutic strategies.

SITE-SPECIFIC TYROSINE BIOCONJUGATED GLUCOSE OXIDASE FOR BIOELECTRONIC APPLICATIONS

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Single-enzyme-based bioelectronics hold promise for diverse applications, ranging from highly sensitive biosensors to logic elements [1]. While enzymes are typically considered insulators, they can exhibit semiconducting behaviour under specific conditions, enabling charge carrier transport. Redox-active amino acids, such as tyrosine (Tyr) and tryptophan (Trp), play a critical role in facilitating this charge carriers transfer within biological systems[2,3]. Furthermore, bioconjugating enzymes with redox mediators, creating "wired enzymes", significantly enhances their potential for bioelectronic applications.

This work investigates the potential of self-assembly monolayer of bioconjugated glucose oxidase (GOx-PTZ) for bioelectronic applications. We employed an electrochemical method to selectively bioconjugate phenothiazine and phenoxazine redox-active groups to tyrosine residues on the GOx surface. Successful modification of these tyrosine residues was confirmed through mass spectrometry analysis. The resulting bioconjugated GOx exhibited improved electrochemical properties, including increased anodic currents in the absence of oxygen and a broader operational potential window compared to the native enzyme.

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DNMT3A METHYLTRANSFERASE CLINICALLY RELEVANT VARIANT INTERACTION WITH DNA

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In eukaryotes, DNA methylation is important for a large part of cellular processes. These include cell type and lineage determination, transcription, chromatin structure and genome stability. Disruption of DNA methylation and its control mechanisms can lead to atypical DNA methylation, misregulation of cell cycle or DNA repair genes, and chromosomal instability, leading to various diseases. This crucial epigenetic mark is installed by specific enzymes – DNA methyltransferases (MTases) DNMT3A, DNMT3B and DNMT1 by attaching a methyl group to a cytosine, mostly in CpG sequences. DNMT3A and DNMT3B MTases carry out de novo methylation that shapes cell fate . Tatton-Brown-Rahman syndrome (TBRS) is a congenital autosomal overgrowth syndrome, which is caused by heterozygous mutations in the DNMT3A MTase gene. The syndrome is characterized by increased height and/or head circumference, obesity, intellectual disabilities and seizures. Subtle dysmorphic features are also common. The syndrome was first described in 2014, with data from 13 sequenced genomes and found to have heterozygous DNMT3A variants. The majority of these variants were located in one of the three known functional domains of the MTase: the PWWP domain, which binds the methylated H3K36 histone, the ADD domain, which binds the non-methylated H3K4 histone, and in the domain of the MTase that is responsible for the catalytic DNA methylation activity.

In 2024, another unique TBRS-causing mutation in DNMT3A MTase was described - Ser775Tyr. It was identified in a patient who was diagnosed with cardiovascular disorders at VU Hospital Santaros Clinics [3]. Ser775Tyr mutation is located in the catalytic domain of the DNMT3A MTase, but relatively distant from the active site and DNA binding loops. The seemingly indirect effect of the mutation to the catalysis mechanism, yet obvious phenotypic penetrance prompted us to investigate deeper into the molecular pathogenesis pathway of this curious case. Bioinformatical analysis suggested that the mutation may change the DNA binding loop flexibility through the network of amino acid interactions. To test that experimentally, recombinant human DNMT3A wt and Ser775Tyr variants were purified from yeast and their DNA binding affinity determined by electrophoretic mobility shift assay (EMSA). As DNMT3A is a large multidomain protein, special EMSA conditions had to be optimized. The results show that Ser775Tyr variant has significantly lower DNA binding affinity as compared to the wt DNMT3A.

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ENVIRONMENTAL AND PLANT BIOCHEMISTRY

EP-1

EDNA-BASED DETECTION OF *SARCOCYSTIS* SPP. IN LITHUANIAN LIVESTOCK FARMS

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Sarcocystis are intracellular protozoan food-borne parasites that can threaten both animal and human health. However, due to the lack of precise molecular methods sarcocystosis is being neglected worldwide. To date, there is a lack of information on *Sarcocystis* spp. occurrence in livestock farms. Whereas cysts can transmit protozoa through contaminated water or food, this study aims to develop suitable methods for detecting *Sarcocystis* from the environmental DNA (eDNA) samples.

In the summer of 2024, environmental water, hay, and soil samples were collected from the animal grazing areas and a nearby water body from twelve livestock farms in Lithuania. A previously developed water filtration method was used to collect and concentrate *Sarcocystis* spp. sporocysts. Species-specific primers targeting the COX1 gene were selected to detect nine *Sarcocystis* species' DNA using a nested PCR.

During this study, samples were analysed for the presence of *S. arieticanis*, *S. bertrami, S. bovifelis*, *S. capracanis*, *S. cruzi*, *S. hominis*, *S. miescheriana, S. tenella* and *S. suihominis* that form sarcocysts in meat-producing animals. However, only six species were detected during the study, the most common species identified being cattle-infecting *S. cruzi*, which is widely found worldwide. Horse-infecting *S. bertrami* was observed with a moderate frequency, while the overall detection rates of *S. arieticanis* and *S. capracanis* were lower. Only one sample was found to contain the DNA of *S. bovifelis*, which infects cattle. In each case estimated intraspecific and interspecific genetic differences did not overlap. Moreover, it is important to emphasize that one of the species detected was the zoonotic *S. hominis*. Based on single nucleotide polymorphism (SNP), 5 haplotypes of *S. hominis* were determined differing by one to three single nucleotides. According to the results, the highest overall detection rate of *Sarcocystis* spp. DNA was detected in water and hay samples. Furthermore, *Sarcocystis* DNA was detected twice as rarely in soil samples. Three or four different species were most often found in an individual farm.

The results obtained indicate a significant potential for animals to become infected with *Sarcocystis* parasites on farms. Meanwhile, the detection of zoonotic species raises questions about sanitary conditions and the potential risk to human health. Therefore, it is important to continue environmental studies on livestock habitats to understand possible routes of parasite spread.

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THE INFLUENCE OF NUCLEAR DNA HYPOMETHYLATION ON SOME MORPHOLOGICAL PARAMETERS OF WHEAT (*TRITICUM AESTIVUM* L.)

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Wheat is one of the most important food crops, especially in Europe and North America with great economic importance. Wheat is a powerful genetic model for studying cereal domestication. Epigenetic mechanisms play a critical role in regulating gene expression affecting plant growth, development, responses to stress and diseases thought small RNAs, histone modifications, DNA methylation etc. Enzymatic nuclear DNA methylation is an epigenetic mechanism involving the transfer of a methyl group onto the C5 position of the cytosine to form 5-methylcytosine. DNA methylation play an important role in plant growth and development and it varies in different environments. 5azacytidine (5-azaC) is a nucleoside-based DNA methyltransferase inhibitor that induces nuclear DNA hypermethilation. Heritable phenotypic changes induced by 5-azaC in progeny were observed in many plants, for example rice, flax, tobacco, triticale and canola. It was demonstrated to increase the phenotypic variation in the progeny. The aim of this research is to study the role of nuclear DNA hypomethylation, induced by 5azaC, on some morphological parameters of wheat seedlings.

The object of this study was etiolated seedlings of wheat (*Triticum aestivum* L., cv. Skagen, Galerist and Creator). The wheat seedlings are convenient models for studying plant physiology and molecular biology because their growth and development are synchronous throughout ontogeny. After 24 hours of germination on moist filter paper at 26ºC etiolated wheat seedlings of equal length were transferred to polyethylene pots containing a solution of 5azaC (50 ng/ml) (Sigma-Aldrich) and continue growing for up to 6 days at a temperature of 26ºC. The maximum length of root, length of first leaf and coleoptile, fresh (FW) weights at the whole plant level was evaluate. The nuclear DNA methylation level was calculated used the LUMA method, which based on combined DNA cleavage by methylation-sensitive restriction enzymes and polymerase extension assay by pyrosequencing (PyroMark Q24).

It was shown that increase of maximum of length of root, length of first leaf and coleoptile, fresh (FW) weights were Creator 13, 37, 48, 13 Δ% and Skagen 2, 36, 17, 17 Δ%, but at varieties Galerist -20, 8, -38, -7 Δ% this effect was not found. Therefore, the hypomethylation effect of total DNA methylation was different in sensitive and not sensitive to environmental wheat varieties. Our work in process.

COMBINED EFFECTS OF SEED TREATMENT WITH COLD PLASMA AND SEEDLING WATERING OR SPRAYING WITH PLASMA ACTIVATED WATER (PAW) ON LETTUCE (LACTUCA SATIVA) GROWTH AND BIOCHEMICAL PROCESSES

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The combined effects of seed treatments with different types of cold plasma and seedling watering or leaf spraying with plasma activated water (PAW) on plant growth and leaf biochemical composition were studied on seedlings of red lettuce cultivar 'Cervanek' cultivated in field plots for 56 days. Seeds were treated with low-pressure air plasma for 3 min or vacuum for 5 min (further denoted as CP3 and V5 groups) or atmospheric dielectric barrier discharge (DBD plasma) for 5 min (further – DBD5 group). The gliding arc discharge plasma was used for production of plasma-activated water (PAW). The physico-chemical composition of PAW was analysed and depended on the type of used water, treatment duration and the plasma discharge parameters. Strong positive impact of seed treatments on lettuce growth was observed in V5 group – in comparison to control plants, seedling weight, height, width of the above-ground part, and leaf number were increased by 32,17,9 and 17□, respectively. In contrast, DBD5 effects were negative – these parameters were reduced by 10-35□. In control plants, PAW watering reduced seedling width only. However, in V5 and CP3 groups effects of PAW watering were negative (weight, height, width, leaf number were lowered by 40,12,13 and 20□ in V5 and by 0,12,15,15□ in CP3 group). PAW watering had positive impact on growth in DBD5 group only. Leaf spraying with PAW reduced all growth parameters of V5 seedlings (50, 8, 9, 13□), had no effect in CP3 group and increased DBD5 seedling height and width (by 21 and 18□). Seed treatment with V5 reduced leaf antioxidative activity (AA, tested by DPPH scavenging) and total amount of phenolic compounds (TPC) by 29 and 18□, respectively. PAW watering did not change TPC but decreased AA (43□) in V5 group. PAW spraying reduced AA in control plants by 27□, but 21-24□ increased AA in seedlings from all treated groups. PAW spraying effectively increased TPC in treated groups (by 26,30 and 43□ in V5, CP3 and DBD5 group, respectively). Thus, the obtained results indicate that effects of seed treatments and PAW watering or spraying on lettuce growth and biochemical composition are not additive. The biochemical changes induced in seedlings by spraying lettuce leaves with PAW were stronger compared to PAW watering, and the response was different in groups of seedlings growing from treated groups compared to the control.

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STEVIOL GLYCOSIDES (SGS) CONCENTRATION CHANGES INDUCED BY COLD PLASMA (CP) IN STEVIA REBAUDIANA BERTONI PLANTS: SOIL VS. AEROPONIC CULTIVATION

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Steviol glycosides (SGs) are the main sweetening compounds (diterpene glycosides), known for their high-intensity sweetness and zero-calorie profile. Beyond their natural sweetness, these glycosides (SGs), mainly stevioside (Stev) and rebaudioside A (RebA), extracted from Stevia rebaudiana Bertoni leaves offer several health benefits (exhibit antioxidant, anti-inflammatory, and antimicrobial activities). Commercially, SGs are incorporated into sugar-free beverages, confectionary, and dietary supplements. Pre-sowing treatment with cold plasma (CP) was shown to stimulate SGs biosynthesis/accumulation up to several folds. Stevia plants are known for their low-germination and vitality rates; therefore, it is commercially important to cultivate the highest yield as possible. Plants cultivation without soil (aeroponics), where plant roots are sprayed with nutrient medium in controlled environment (temperature, humidity, day/night regime) could be a solution for achieving such goal. This study aimed to evaluate the universality of two types of CP-induced biochemical changes in stevia by comparing SGs concentration in stevia plants, cultivated in soil and aeroponics. Seeds of stevia cultivar SHUG HIGH A3 (HYBRID) were treated for 5 min. with two different CP equipment types – capacitively coupled (CC - referred CC5 group) and dielectric barrier discharge (DBD – referred DBD5 group respectively). Additionally, V5 group, seeds treated with partial vacuum (100 Pa) for 5 min., was included as additional control. As per CC equipment design – a pressure is needed for the plasma discharge. After 6 days of treatment, seeds were sown in soil and in aeroponic system. Different cultivation conditions resulted in higher by 2-folds SGs concentration in aeroponics. Cumulative (RebA+Stev) SGs concentration in control group was 71,84 mg/g dry weight in soil, in aeroponics - 151,82 mg/g dry weight. Whereas effect of CP-treatments resulted dependently on cultivation conditions. CC and DBD did not affect SGs concentration in soil-cultivated plants, however CC plasma increased RebA concentration in aeroponically cultivated plants. In addition, this plasma treatment increased RebA/Stev ratio up to 1,7. This ratio is used to evaluate the taste properties (mainly, licorice-like aftertaste existence) of steviosides. The higher this ratio is – the better taste it has. In conclusion, seed treatment with CC plasma induced SGs concentration increase due to RebA biosynthesis stimulation in aeroponically cultivated plants, whereas DBD plasma did not affect SGs concentration.

DIVERSITY OF MICROBIAL COMMUNITIES IN A MUNICIPAL WASTE LANDFILL

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Despite global efforts to reduce waste production, improve treatment methods such as incineration, advance new technologies, and enhance recycling efficiency, waste management remains insufficient to address the problem. In the EU, over 23% of municipal waste is still landfilled, while in Latvia, this figure reaches 50%. As municipal waste consists of diverse materials, landfills create a complex environment rich in microbial resources but also contain various inhibitors, promoting the development of diverse microbial communities. Investigating landfill microbial communities can improve our understanding of waste degradation processes and support the development of novel biological approaches for more effective waste management.

This study aimed to assess the diversity of microbial communities in a managed municipal waste landfill on the outskirts of Riga. Soil fraction samples were collected from an old landfill site active from the early 1990s to 2000. A trench (~4 m deep, 18 m long) was excavated, and 28 soil samples were collected at four different depths.

DNA was extracted using the FastDNA Spin Kit for Soil, and metagenomic shotgun sequencing was performed on a DNBSEQ-G400 sequencer (PE150 flow cells), generating ~20 million 150 bp paired-end reads. Raw reads were quality-filtered (Trimmomatic v.0.39, Q30 threshold). Taxonomic classification was conducted using Kraken2 (NCBI RefSeq release 98). Functional characterization utilized DIAMOND with the UniRef90 and PlasticDB databases. Data analysis was performed in RStudio using packages including Maaslin2, vegan, FactoMineR, and ComplexHeatmap.

Landfill microbial communities were dominated by bacteria (~23%). A total of 58 phyla and ~1,700 genera were identified (minimum relative abundance >0.01%). The predominant phyla were *Pseudomonadota*, *Actinomycetota*, and *Firmicutes*, with *Streptomyces*, *Pseudomonas*, and *Thiobacillus* as the most abundant genera. *Pseudomonas* and *Streptomyces* harbor plastic-degrading species, and functional analysis indicated plastic biodegradation as a potential process, with ~200 enzymes, including hydrolases, PETases, and laccases, identified.

Archaea (~2% of reads) were primarily *Methanobacteriota*, with *Methanotrix*, *Methanoculleus*, and *Methanoregula* as the dominant methanogenic genera. Other identified archaea included halophiles and nitrogen turnover-related taxa. Fungi, mainly *Ascomycota* and *Basidiomycota*, formed a minor fraction. Taxonomic composition remained consistent across depths, but bacterial and archaeal abundances correlated with increasing electrical conductivity (EC). Higher EC levels negatively affected *Streptomyces* and *Bradyrhizobium* but positively correlated with sulfur-metabolizing bacteria.

In conclusion, landfills harbor diverse microbial communities dominated by soil-associated taxa. Soil salinity, measured by EC, appears to be a key factor shaping bacterial and archaeal populations.

THE ENZIMATIC NUCLEAR DNA METHYLATION OF EURASIAN PERCH FROM FRAGMENTED AND NONFRAGMENTED POPULATION BY DAM IN DAUGAVAS RIVER

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Fish resources are one of the most valuable biological resources in Latvia. Anthropogenic activity is diverse, but it can, in fact, be reduced to a few main effects, like the development of barriers in fish migration paths, transformation of habitats and a reduction in water quality. The Eurasian perch *Perca fluviatilis* (L.) is a freshwater, is a quite common fish species in Europe. Eurasian perch was used for investigations the relative importance of genetic variation and plasticity on phenotypic variation. The modified DNA methylation triggers phenotypic plasticity and promotes rapid adaptation and transgenerational inheritance. The aim of this work was to investigate the level of enzymatic methylation of nuclear DNA in fragmented and non-fragmented perch populations.

Tissue samples were collected from a total of 80 perch individuals from five different Daugava River sites in Latvia: fish from the Daugava River collected in the Rîga Hydroelectric Power Plant (HPP) (Tome), in Ķegums HPP (Ķegums), and near the estuary in Riga city (Voleri), and Staņķu Lake. The ages of fishes were determined by Sample tissues were stored at –20 °C. DNA by the salt extraction method. DNA was quantified and qualified spectrophotometrically and electrophoretically. The nuclear DNA methylation level was calculated used the LUMA method, which based on combined DNA cleavage by methylation-sensitive restriction enzymes and polymerase extension assay by pyrosequencing (PyroMark Q24). It was shown, that perch population from the Daugava River in Riga (Voleri_D) formed a separate group from the other Daugava River perch populations.

HSGA20OX1, HSGA3OX1 AND HSGA2OX1 INVOLVED IN ENDOGENOUS GIBBERELLIN REGULATION WITHIN *HERACLEUM SOSNOWSKYI* OVARIES IN RESPONSE TO EXOGENOUS GA_3

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Gibberellins (GAs) are crucial plant hormones that regulate growth and developmental processes in various plant species. Their biosynthesis involves a series of enzymatic reactions across multiple organelles, forming bioactive GA_4 and GA_1. Late GA metabolism is tightly regulated by GA-oxidase (GAox) enzymes, specifically GA20ox, GA2ox, and GA3ox. In this study, we performed a genome-wide identification of GAox enzymes, their gene expression, and endogenous GA analysis in a highly invasive plant *Heracleum sosnowskyi*, in response to exogenous GA_3 treatment.

A total of 27 GAox proteins were identified in the *H. sosnowskyi* genome, including nine GA20ox, thirteen GA2ox, and five GA3ox enzymes containing 2OG-Fe(II) oxygenase (PF03171) and non-haem dioxygenase N-terminal (PF14226) domains specific to 2-oxoglutarate-dependent dioxygenase superfamily. Genes encoding these GAox enzymes are distributed across eleven chromosomes, and the expressed proteins are localized in the cytoplasm. Phylogenetic analysis revealed clustering of *H. sosnowskyi* GAox proteins into three families: GA20ox, C19-GA2ox, and GA3ox, with no members assigned to the C20-GA2ox family. Gene structure and amino acid motif analysis confirmed conserved evolution across species, with distinct patterns of exon-intron organization and motif composition among three GAox families. For the first time, the *HsGA20ox1*, *HsGA3ox1* and *HsGA2ox1* genes, which exhibited the highest expression in developing ovaries, were cloned. Expression analysis of selected *GAox* genes in response to exogenous GA_3 treatment demonstrated differential expression patterns in developing *H. sosnowskyi* ovaries. *HsGA3ox1* expression was significantly upregulated 10-days after treatment, while *HsGA20ox1* expression decreased. Endogenous GA levels varied with GA_3 treatment; GA_1 levels significantly increased in the lateral part of the umbel but decreased in the central part 10-days after GA_3 application. GA precursors (GA_{44}, GA_{19}, GA_{20}) peaked at earlier developmental stages, and GA catabolites (GA_8, GA_{29}) showed distinct accumulation patterns. Phenotypic analysis indicated that GA_3 treatment significantly reduced ovary size and weight in both parts of the umbel. Ten-days after treatment, ovary length, width, and weight decreased by 21%, 30%, and 59% in the central, and by 20%, 31%, and 41% in the lateral part, respectively.

Our comprehensive genome-wide identification and functional analysis of GAox genes in *H. sosnowskyi* provides new insights into GA metabolism and its response to exogenous GA_3. The differential gene expression and endogenous GA fluctuations in response to GA_3 highlight their role in ovary development and seed formation. These findings contribute to understanding GA regulation in invasive plant species and may inform future strategies for controlling the spread of *H. sosnowskyi*.

Antibiotic-Resistant Bacteria in Ecological Farmland: Distribution and Persistence

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Antibiotic-resistant bacteria (ARB) are an increasing concern in agricultural environments, even in the absence of antibiotic use. These bacteria can spread through soil, water, and animal feed, facilitating resistance gene transfer to pathogenic bacteria and posing risks to human and animal health. Understanding ARB persistence in ecological farming systems is crucial for assessing natural resistance levels and potential risks. This study examines bacteria distribution as well as ARB distribution and resistance patterns in Lithuanian farmland ecosystems, focusing on soil, feed, and freshwater.

The bacteria were identified using shotgun metagenomic sequencing, combined with cultural and molecular methods on media supplemented with ampicillin, streptomycin, tetracycline, and chloramphenicol. The samples were collected from three dairy farms practicing ecological farming.

The metagenomic analysis revealed that in soil, the most abundant genera were *Pseudomonas, Nocardioides, Rhizobium*, and *Streptomyces*. Grass samples were dominated by *Pseudomonas, Sphingomonas, Rhizobium,* and *Massilia*. Water samples exxibited lower bacterial abundance, with *Acidobacteria, Nocardioides*, and *Pseudomonas* being the most prevalent. A total of 47 bacterial strains were isolated and subjected for further resistance analysis. To assess ARB distribution, we applied statistical methods including relative frequency distribution, Chi-square test, and correlation analysis. Resistance levels were determined by minimum inhibitory concentration (MIC) tests followed CLSI and EUCAST guidelines. Cultivatable ARB occurrence was highest in soil, followed by feed, and freshwater. A significant associations between bacterial genera and environmental sources was identified (Chi-square = 42.28). MIC results revealed the highest resistance against ampicillin, followed by chloramphenicol, streptomycin, and tetracycline. Nearly 90% of isolates exhibited multidrug resistance.

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EFFECTS OF SEED TREATMENT WITH COLD PLASMA ON LETTUCE (*LACTUCA SATIVA)* GROWTH IN AEROPONIC SYSTEM AND BOCHEMICAL PROCESSES

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Aeroponic plant cultivation is a novel technology explored for potential of use for indoor vertical farming. It is expected to resolve several plant physiological constraints occurring during hydroponic cultivation, including greater oxygen availability within the root bed and enhanced water use efficiency. The aim of this study was to evaluate effects of seed treatments with cold plasma (CP) on growth, physiological processes and biochemical parameters in two lettuce cultivars - green variety 'Perl Gem' (PG) and red variety 'Chervanek' (Ch) cultivated in aeroponic system („Baltic Freya“) for 45 days. Seeds were treated with low-pressure air plasma for 3 min (further denoted as CP3 group) or atmospheric dielectric barrier discharge (DBD plasma) for 3 and 5 min (further – DBD3 and DBD5 groups). The effects on seed treatments on parameters of seedling growth, photosynthetic efficiency, amounts of photosynthetic pigments, anthocyanins, total phenolic compounds (TPC) and antioxidant activity (AA) in seedling leaves were estimated. The parameters of biomass gain were higher in PG seedlings compared to Ch (seedling weight, root weight and leaf weight was 28,35 and 27% larger, respectively) although the number of leaves in PG seedlings was 1.7 times lower than in CH, and the length of the seedlings did not differ. The indices of photosynthetic efficiency were cultivar dependent – photosynthetic performance index PI in PG leaves was higher by 49%, and Fv/Fm ratio – by 2.3%, in comparison to Ch. In control Ch seedlings TPC, amount of anthocyanines and AA was by considerably higher (by 53,60 and 69%, respectively) but the amount of chlorophylls – slightly lower, compared to PG. In both cultivars, seed treatments did not induce significant changes in morphometric parameters of seedlings. However, treatments induced increase in photosynthetic performance in both cultivars – CP3 and DBD5 increased PI in PG leaves by 28 and 36%, and DBD3 enlarged PI by 57% in Ch leaves as well as considerable changes in biochemical parameters. In PG seedlings from CP3 treated seeds, TPC was 53% and AA – 2.4 fold higher compared to control, but DBD5 treatment did not change TPC and resulted in reduced AA (-34%). DBD5 treatment reduced both TPC and AA in Ch leaves (by 14-15%). Thus, the obtained results indicate that although seed treatments with CP did not have impact on biomass gain of lettuce grown in aeroponic system, appreciable changes in plant photosynthetic performance, amounts of pigments, protective phenolic compounds and antioxidant activity were observed.

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COMPARISON OF ESSENTIAL OIL AMOUNT IN DIFFERENT PARTS OF VARIOUS SPECIES AND CULTIVARS OF MINTS DURING THE FIRST AND SECOND FLOWERING PERIODS

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Mints are pharmacologically valuable essential oils bearing plants of genus *Mentha*, which are widely distributed and cultivated not only in Lithuania, but also throughout the world. The amount of essential oil in mints may depend on the taxon (species, hybrid, and variety) and part of the plant. If mints are cut during flowering, they regrow and bloom a second time in the same vegetation season. Therefore, the amount of essential oil during the first and second flowering may also differ. The aim of this study was to compare the amounts of essential oil in the leaves, inflorescences and stems of *Mentha longifolia*, *Mentha spicata*, *Mentha villosa*, *Mentha suaveolens*, *Mentha* × *piperita* 'Zgadka' and *M.* × *piperita* 'Prilukskaja' during the first and second flowering. All mints cultivated in the collection of medicinal and aromatic plants of the Nature Research Centre Field Experimental Station were harvested during the first and second flowering period and dried. Essential oils were extracted from leaves, inflorescences and stems of all mints separately by hydrodistillation and expressed as % (v/w); hydrodistillation time – one and a half hours. The highest amounts of essential oils were established during the first flowering in the inflorescences: *M. suaveolens* – 5.28 ± 0.09%, *M. spicata* – 2.50 ± 0.06%, *M. villosa* – 2.04 ± 0.03%, *M. longifolia* – 1.01 ± 0.09%, *M.* × *piperita* 'Zgadka' – 3.45 ± 0.04%, and *M.* × *piperita* 'Prilukskaja' – 2.52%. In the first flowering, the essential oil amounts in the leaves of all mints were found to be lower (in some mints even 1.5–2 times) than in the inflorescences, and in the stems their amounts often did not reach 0.2%. During the second flowering, the essential oil amounts both in the inflorescences and leaves of *M.* × *piperita* 'Prilukskaja', *M. villosa* and *M. suaveolens* were significantly lower than during the first flowering; however, in the leaves of *M. spicata* and *M.* × *piperita* 'Zgadka' and in leaves and inflorescences of *M. longifolia,* more essential oil was found during the second flowering. It can be concluded that the first flowering is more important for the extraction of essential oils, but it is also useful to take into account the taxon.

CONDITIONAL EFFICIENCY OF PROPIDIUM MONOAZIDE IN REAL-TIME PCR-BASED VIABILITY TESTING OF *SARCOCYSTIS* SPP. OOCYSTS

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Sarcocystis spp. are protozoan parasites with a two-host life cycle. Sarcocysts develop in the muscles of intermediate hosts, while sporocysts form in the intestines of definitive hosts and are excreted into the environment. Infection occurs when intermediate hosts ingest contaminated food or water. Infected animals may suffer from rapid weight loss, anemia, fever, and, in severe cases, mortality. Detecting viable *Sarcocystis* sporocysts in environmental samples is challenging due to their low concentration and inability to be cultured. Quantitative PCR (qPCR) is highly sensitive for DNA detection but cannot differentiate between viable and non-viable cells. This study aimed to evaluate propidium monoazide (PMA) for assessing *Sarcocystis* sporocyst viability.

Environmental samples were collected from two ponds in the Vilnius district. Water was filtered through membranes of varying pore sizes, rinsed with sterile water, and divided into two groups: PMA-treated and untreated (controls). After photoactivation, genomic DNA (gDNA) was extracted, and a qPCR assay targeting the *COX1* gene was performed using species-specific primers.

Previous studies yielded unexpected results, where PMA-treated *Sarcocystis* DNA amplified earlier (Ct23) than untreated DNA (Ct29). Further tests with frozen and heated water samples suggested that high concentrations of microbial DNA interfere with PMA binding to *Sarcocystis* DNA. Control samples confirmed this, as their amplification curves rose earlier than those of PMA-treated samples. However, after freezing or heating, the curves of DNA with and without PMA aligned, indicating that temperature affects PMA efficiency. These findings suggest *Sarcocystis* sporocysts are highly resistant to temperature fluctuations, as their gDNA concentration remained stable after 24 hours at -20°C or 1 hour at 98°C, regardless of PMA treatment. Additionally, microbial DNA may interfere the interaction of DNA polymerase and primers with *Sarcocystis* DNA. Further research revealed that amplicon length influences PMA effectiveness. Short amplicons are not fully covered by the dye, while longer ones are more likely to bind effectively, improving differentiation between viable and non-viable cells.

It was concluded that PMA dye is unsuitable for quantitative viability studies of Sarcocystis spp. in natural samples. Alternative methods, such as flow cytometry, are more appropriate for assessing their viability.

STEROID HORMONES IN GREY SEAL FUR: DIFFERENCES BETWEEN REHABILITATED AND HEALTHY PUPS

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Grey seals are top predators and important indicators of marine ecosystem health. Exposure to pollutants in the Baltic Sea may disrupt their endocrine system. Steroid hormones measurement in fur offers a minimally invasive tool for monitoring health, though this method remains unexplored in grey seal pups.

This study examines cortisol (COR), cortisone (CORNE), estradiol (E2), testosterone (T2), dehydroepiandrosterone (DHEA), progesterone (P4), and 17-hydroxyprogesterone (17-OHP4) levels in rehabilitated (Lithuanian Sea Museum) and wild (Estonia) grey seal pups during early development. Fur samples from 24 seals (14 males, 10 females) were analyzed using LC-MS/MS. Generalized Linear Mixed Models (GLMMs) assessed the effects of sex, body mass, fur type (lanugo vs. regular), and life history (rehabilitated vs. wild).

Rehabilitated pups had 1.6 times higher COR levels and reduced DHEA, T2, E2, and 17-OHP4 levels compared to wild pups. Sex significantly influenced COR, P4, and 17-OHP4: males exhibited 2.1 times higher COR levels, while females had 7.4 times higher P4 and 2.8 times higher 17-OHP4 levels than males. Body mass was positively associated with T2 and 17-OHP4 levels. Fur type significantly affected CORNE and DHEA: pups with permanent dark fur had higher CORNE levels and 60% lower DHEA levels compared to lanugo.

This study demonstrates the value of fur as a tool for assessing steroid hormones in grey seal pups. Monitoring these hormones provides insights into the health of seals in polluted environments, emphasizing the need for continued endocrine monitoring in conservation efforts.

CHRYSEOBACTERIUM NEMATOPHAGUM AS A NOVEL MATRIX DIGESTING PATHOGEN OF NEMATODES *CAENORHABDITIS ELEGANS*

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Chryseobacterium genus comprise gram-negative environmental bacteria form phylum Bacteroidota found in various terrestrial and aquatic domains. Lately several species of *Chryseobacterium* have been identified as fish or human pathogens. Our main research subject *Chryseobacterium nematophagum* displays a drastic virulence behaviour towards nematodes that normally feed on bacteria. After being ingested, *C. nematophagum* attaches to anterior pharynx of its host, destroys the pharyngeal lining and spreads throughout the entire body cavity, causing disintegration of the nematode. Worm-killing ability was observed towards several parasitic nematodes making *C. nematophagum* as a good potential agent against emerging drug-resistant species. Thus far the molecular mechanisms of *C. nematophagum* virulence have not been explored and by examining this model we can explain how *Chryseobacterium* develops its pathogenic behaviour.

For bacteria, secretion of proteins into the surroundings contribute to the pathogenicity, uptake of the resources or biofilm formation. To achieve this, bacteria developed highly specialised multi-protein nanomachines called the secretion systems (TSS) capable secreting proteins or DNA across bacterial cell membranes. TSS gained traction as a potential target for therapies after connection between TSS, and virulence has been observed in many human pathogens. Genome analysis of *C. nematophagum* revealed the presence of three distinct TSS: T4SS, T6SS, and T9SS. Using mutant strains, we have shown that T9SS is responsible for secretion of the vast majority of the proteins found in the cell culture supernatant. Moreover, worm-killing assays revealed that T9SS secretion pathway is essential for pathogenicity.

Thus far, only two strains of *C. nematophagum*, JUb275 and JUb129 were shown to exhibit worm-killing capacity, while other *Chryseobacterium* species were reported to be benign. We opted to reconstruct the phylogenetic tree by comparing the entire core genomes (gene set shared by all strains) of multiple strains or species. 102 genomes of *Chryseobacterium* spp. were used to construct the new tree and two additional strains - *C. potabilaquae* and *C. fistulae*, were identified as closely related to *C. nematophagum*. Both species were found positive for worm-killing phenotype in contrast to other phylogenetically more distant strains tested. We conclude that these four strains comprise worm-killer clade that could all be named as species *C. nematophagum*. Comparative genetics of all worm-killer strains further allowed us to identify potential virulence factors responsible for worm killing.

DNA METHYLATION AS EPIGENETIC MECHANISMS OF PLANT ADAPTATION TO ABIOTIC STRESSES

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Abiotic stresses negatively impact plant growth and development, with severe effects on crop yield leading to huge economic losses. Plant response to abiotic stresses is complex and involves multiple mechanisms, activated and controlled by massive changes in gene expression and nuclear organization. Environmentally induced epigenetic variation can result in heritable phenotypic plasticity, which may play a major role in adaptation to changes in the environment. Epigenetic mechanisms regulate high-order DNA structure and gene expression without changes to the underlying DNA sequence. Epigenetic mechanisms play a critical role in regulating gene expression affecting plant development, responses to stress and diseases through small RNAs, histone modifications, DNA methylation etc.

The most thoroughly studied type of epigenetic phenomena in plants is DNA methylation [1,2]. Amajor part of methylated cytosine residues (m5C) in plants, like in animals, occurs in the symmetric CG sites. Unlike animals, plants also display significant methylation in the symmetric CHG sites and asymmetric CHH sites (here H is any nucleotide except G). All three methylation contexts are present in repeat and transposable element (TE) sequences, while the protein-coding gene sequences are mostly methylated at CG sites. In recent years, it was shown that pattern of DNA methylation in model plants is sensitive to various environmental stressors. Changes in DNA methylation in some genomic regions are inherited through mitotic cell divisions and can be heritable over many generations. There is growing evidence that epigenetic mechanisms contribute to stress responses and memory in plants. Therefore, it is tempting to speculate that many of the observed transgenerational responses to stress conditions could be attributable to epigenetic mechanisms. It has been shown that plants have an elaborate reprogramming system for epigenetic marks during sexual reproduction. It was shown, that repeated stress conditions lead to the acquisition of a stable epigenetic memory on the level of DNA methylation.

The studies of epigenetic variability in the natural plant populations show that plants are quite similar genetically, but very different epigenetically. The pairwise comparisons showed that epigenetic differentiation occurred independently of genetic differentiation. The question that remained unanswered is whether the high epigenetic variability allowed the invasive plans to succeed in colonizing the new habitats, or different new habitats induced the epigenetic differences.

PLANT-BASED SUBSTANCES FOR VIRUS INACTIVATION

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The emergence of novel and drug-resistant viruses is a cause for concern and a stimulus for researching alternative antiviral agents. Plant-based extracts, such as essential oils (EOs), are a promising prospect due to their eco- and human-friendly nature as well as increasing evidence for their broad spectrum of bioactive properties. EOs are hydrophobic liquids primarily comprised of volatile terpenes and phenylpropanoids. The antiviral efficacy of some EOs and their components has been proven against certain viruses, but in most cases, detailed information regarding their mechanisms of action is still lacking.

For this work, seven EOs (those of lemon myrtle, lemongrass, lemon verbena, coriander, lavender, mandarin, and tea tree) and some of their primary components were selected. The compositions of EOs were determined by GC/MS. The antiviral properties of selected substances were investigated by utilizing *Saccharomyces cerevisiae* yeast L-A virus as a model system and the effect of EOs in their liquid and vapor phases was evaluated by measuring the incorporation of radio-labeled nucleotides into newly synthesized viral RNA. Citral and citral-rich EOs displayed the greatest overall antiviral efficacy, whereas limonene and mandarin EO exerted a strong effect only in the vapor phase. To gain insight into their antiviral mechanisms, a T7 phage RNA polymerase-based *in vitro* transcription system was employed. By exposing separate transcription reaction components to the vapor of EOs and their constituents, the action of tested substances on the viral RNA polymerase was investigated. In addition, TEM imaging was used to analyze the effect of EOs on *S. cerevisiae* L-A virus morphology.

The results of this study aid in elucidating the antiviral potential and mechanisms of action of plant-based substances, opening new horizons for the sustainable application of EOs in the control of viral infections. This study was funded by the Research Council of Lithuania (project no. S-MIP-24-55).

STIMULATION OF STEVIOL GLYCOSIDES BIOSYNTHESIS IN STEVIA GROWN IN AEROPONICS BY A COMBINATION OF COLD-PLASMA SEED TREATMENT AND PLASMA-ACTIVATED WATER LEAF SPRAYING

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Stevia rebaudiana Bertoni seed treatment with cold plasma (CP) was shown to stimulate the biosynthesis of its secondary metabolites steviol glycosides (SGs), which are widely used in the food industry as health-beneficial natural sweeteners. This effect is observed when stevia is grown in soil. In plants cultivated in aeroponics, SG concentrations are higher than in soil-cultivated plants, but the stimulating CP effect is diminished. This study aimed to investigate the effect induced by the combined treatment of CP on seeds, plant cultivation in aeroponics, and leaf spraying with plasma-activated water (PAW). We have demonstrated that a short (5 min) seed treatment with two types of CP (dielectric barrier discharge (DBD) and capacitively coupled (CC) CP) didn't stimulate the most abundant SGs rebaudioside A (RebA) and stevioside (Stev) SGs biosynthesis, DBD treatment even decreased RebA and Stev by 20%. However, DBD didn't change RebA/Stev ratio (the indicator of taste quality of SGs) compared to the control, which was 1.0, but CC increased it up to 1.7. PAW was produced from tap water using a gliding arc discharge plasma for 5 min. PAW treatment (leaf spraying for 12 days) increased RebA concentration in control (plants from CP-untreated seeds) and DBD groups by 71% and 67%, respectively. Consequently, RebA/Stev ratio increased from 1.0 up to 3.5 and 2.4 in the control and DBD groups, respectively. PAW treatment did not affect SGs of the CP group. From this study, it can be concluded that PAW treatment of stevia growing in aeroponics may be used to ameliorate the taste quality of natural sweeteners, except in combination with CC treatment.

This work was supported by the Research Council of Lithuania (S-MIP-23-8).

3D CELLULAR MODELS

IDENTIFICATION OF POTENTIAL GENE TARGETS IN PRIMARY AND SECONDARY GLIOBLASTOMA CELL LINES USING MULTI-CELLULAR SPHEROID CELL CULTURE MODEL

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Glioblastoma originates from astrocytic glial cells and is classified as a grade IV malignant glioma by the World Health Organization (WHO). This highly aggressive tumor is associated with a poor prognosis, with a median survival of approximately 15 months following diagnosis. 90 % of glioblastomas develop *de novo*, while 10 % are from low-grade gliomas via different pathways. Secondary glioblastoma predominantly affects younger patients, exhibits a higher incidence in females compared to males, and is associated with a more favorable prognosis in females. However, the differences between these two gliomas are not well characterized, although researchers are actively investigating potential diagnostic and prognostic biomarkers. By integrating 3D cell culture models of two cell lines corresponding to two types of GBM with gene expression analysis techniques, it is possible to gain insights into intricate cellular regulatory networks, some of which may play a crucial role in tumor development and finding new potential targets for therapy.

This study aims to compare the expression changes of genes associated with metabolic pathways, cell interactions, cancer pathway targets, and immune response between primary and secondary glioblastoma representing cell lines U87 and A172 (respectively) using a 3D multi-cellular spheroid model system.

The U87 and A172 cells were cultivated in 2D and 3D model systems. mRNA was extracted on days 2 and 6 of their growth, and qPCR analysis was performed to assess gene expression. The changes in gene expression between cells on days 2 and 6 were compared, and data was analyzed using GraphPad v.9.

Earlier, the genome-wide analysis revealed the gene expression profiles of glioblastoma U87 2D and 3D cultivated cell lines. KEGG functional enrichment analysis sorted out the main functional groups: immune response, cell adhesion, metabolic, and cancer pathway targets, which have been validated by qPCR. Gene expression analysis of U87 and A172 cell lines revealed distinct patterns between day 2 and day 6. On day 2, before the development of hypoxia and nutrient gradients within cell spheroids, significant differential expression was observed in metabolic and cancer pathway target groups. Conversely, day 6 exhibited divergent gene expression profiles between U87 and A172 cell lines across all gene groups, highlighting the temporal significance of these genes and pathways. Significant differences of *HMGCS1*, *CDH4*, *CACNG8*, *FGF7*, *IL1RAP*, and *IL1β* expression at day 6 imply potential functional roles in the biological processes under study and, consequently, they are considered therapeutic targets.

EXPLORING 2D AND 3D ENDOMETRIAL CANCER MODELS FOR PRECLINICAL DRUG TESTING

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Endometrial cancer is the 6th most common cancer in women, with Lithuania and Poland leading in Europe (Bray et al., 2024). The increased risk of endometrial cancer is associated with increased age, certain ethnicities, higher BMI, endogenous or exogenous estrogen exposure, early menarche, late menopause, lower parity, metabolic syndrome, family history and genetic predisposition (Makker et al., 2021). Surgery is typically the primary treatment for endometrial cancer, and if there is a risk of recurrence, chemotherapy is administered afterward. However, commonly used drugs like paclitaxel, carboplatin, and cisplatin have significant drawbacks, including a lack of specificity. Targeted therapies are being actively developed and explored as an alternative to conventional chemotherapy (Janssens and Remmerie, 2018). These compounds are also widely applied in personalized medicine. Several preclinical models are currently available for evaluating drug efficacy and predicting patient outcomes in endometrial cancer. Traditionally, 2D monolayer cell cultures have been the standard in cancer research, but they fail to accurately replicate the complex molecular mechanisms of tumors in vivo. The development of 3D cell culture models has introduced a more physiologically relevant alternative, better reflecting key tumor characteristics such as cellular heterogeneity, interactions with the extracellular matrix (ECM), hypoxic microenvironments, and ECM-dependent signaling pathways (Salinas-Vera et al., 2022). Moreover, 3D cultures demonstrate more realistic growth kinetics, drug responses, gene expression patterns, and epigenetic profiles, making them a powerful tool for studying cancer biology.

In this study, we developed 2D and 3D cultures from novel endometrial cancer cell lines that were originally derived in our laboratory from tumor tissues of Lithuanian endometrial cancer patients. The drug effects were evaluated based on alterations in spheroid or aggregate size and metabolic activity following treatment. A range of clinically approved targeted therapies, including PI3K/AKT/mTOR and receptor tyrosine kinase inhibitors, as well as regulators of cell cycle, proliferation, and survival, were examined. Our findings identified the most effective compounds as the nuclear transport inhibitor selinexor, PI3K/AKT/mTOR inhibitors such as sapanisertib, samotolisib, apitolisib, and MK-2206, along with the proliferation regulator selumetinib (in 3D models) as well as cell cycle regulator BI 2536 (in 2D models).

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MICROFLUIDIC ORGAN-ON-CHIP PLATFORM FOR PERSONALIZED CHEMOTHERAPY TESTING IN PANCREATIC DUCTAL ADENOCARCINOMA

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Background. Pancreatic ductal adenocarcinoma (PDAC) is a highly aggressive cancer with poor survival rates and rising global incidence. Advances in tumor staging, classification, and functional mutation discovery have enabled more precise treatments, potentially improving outcomes. To enhance personalized therapies, in vitro models that enable functional drug testing and accurately mimic the disease at the organ level are needed. This study tested a microfluidic system simulating blood vessel–PDAC tubule interactions using primary human pancreatic organoids (hPO) and human umbilical vein endothelial cells (HUVEC). It assessed drug distribution through a simulated desmoplastic environment, the efficacy of gemcitabine and SN38 (irinotecan's active metabolite), and fluctuations in tumor-specific markers (CA-19.9, TIMP-1, osteopontin, MIC-1, ICAM-1, sAXL) and cellular well-being markers (LDH, fractalkine) across organ-on-a-chip (OOC) channels under treatment.

Aim. To assess the feasibility of PDAC OOC in drug sensitivity assessment, using two most commonly used chemotherapy drugs gemcitabine and SN38.

Methods. Primary PDAC organoids and HUVECs were co-cultured in a layered microfluidic chip made of cyclo-olefin copolymer and a porous PET membrane. HUVECs were seeded in the lower channel and cultured for one week before PDAC organoids were added to the upper channel. The media flow rate was maintained at 4 μL/min. To assess drug effects, 10 μM gemcitabine and 10 nM SN-38 were introduced into the endothelial channel for 72 hours. Outflows from all channels were collected for cell viability assessments and biomarker analysis via multiplex and ELISA assays daily. Additionally, membrane sections were fixed, stained, and analyzed for apoptosis marker $Casp3^+$ using fluorescence microscopy.

Results. Gemcitabine successfully absorbed from the vascular to the PDAC channel, aligning with $Casp3^+$ apoptosis data, inducing over a twofold increase in number of apoptotic PDAC cells (Control = 20±8; Gemcitabine = 57±16; $p<0.05$ (%)), and a fivefold increase in $Casp3^+$ HUVECs (Control = 4±0.6; Gemcitabine = 22±13; $p<0.05$ (%)). SN38 was undetected in the PDAC channel and showed no significant apoptotic effect on both cell types. Secretome analysis revealed decreased CA19-9 levels and increased LDH and fractalkine after gemcitabine treatment.

Conclusion. This study demonstrates that a microfluidic organ-on-chip platform effectively replicates the pancreatic ductal adenocarcinoma microenvironment, enabling personalized chemotherapy testing by revealing gemcitabine's superior efficacy over SN-38 in inducing tumor cell apoptosis, while also highlighting the potential of such systems for precise drug evaluation and improved patient treatment outcomes.

IMPACT OF SKOV3 CELL LINE VARIABILITY ON 3D SPHEROID FORMATION AND TRANSCRIPTOMIC PROFILES

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The adoption of 3D cell culture models has significantly improved the relevance of *in vitro* cancer research by better replicating the tumour microenvironment. However, variations within cell lines remain a challenge, affecting experimental reproducibility. In this study, we investigated the ability of two authenticated SKOV3 ovarian cancer cell strains, S1 and S2, to form 3D spheroids, analyzing their growth dynamics, structural characteristics, and transcriptomic profiles. Experiments were conducted between passages 20–30, with cells seeded at densities ranging from 500 to 7000 cells per well. Spheroid formation was monitored over 20 days and imaged every three days. The SKOV3 S1 strain consistently formed compact, spherical spheroids as early as day 3, independent of seeding density. In contrast, S2 spheroids only became visible between days 6 and 9, depending on the number of cells seeded, with optimal spheroid formation observed at higher densities (5000–7000 cells per well). Quantitative analysis confirmed that S2 spheroids were significantly larger in diameter and volume than those formed by S1. However, S1 spheroids exhibited higher sphericity and compactness, suggesting tighter cellular aggregation, while S2 spheroids, despite their larger size, displayed less compact 3D structures. Transcriptomic analysis further highlighted differences between the strains in 3D culture conditions. Gene expression profiling revealed that S1 spheroids were enriched for pathways related to epithelial-mesenchymal transition (EMT), extracellular matrix remodeling, and cell adhesion. In contrast, S2 spheroids showed upregulation of MYC-regulated pathways related to proliferation and metabolism. Both strains exhibited upregulation of genes associated with hypoxia, angiogenesis, and tumour progression when compared to 2D cultures. Overall, this study demonstrates significant strain-dependent variability in SKOV3 3D models, affecting spheroid formation and gene expression. Given the increasing reliance on 3D models for studying tumour biology and drug resistance, these results highlight the necessity of optimizing culture conditions and considering cell line heterogeneity in experimental design.

BIOCHEMISTRY OF INFECTIOUS DISEASE

ID-1

PLASMID GENOMIC VARIATION IN CLINICAL *ACINETOBACTER BAUMANNII* ISOLATES

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Gram-negative opportunistic pathogen *Acinetobacter baumannii* is notorious for its ability to cause difficult to treat nosocomial infections. This bacterium has the ability to acquire numerous resistance and virulence determinants through mobile genetic elements such as plasmids. The aim of this research was to identify the diversity of plasmids of *A. baumannii* isolates obtained from clinical settings.

In total 228 *A. baumannii* isolates were obtained from National Cancer Institute and Santaros Clinics in the years 2013-2019 and 2022-2023, respectively. Plasmids were identified using PCR-based replicon typing and pulsed-field gel-electrophoresis (PFGE). Representative isolates (n=22) were selected for whole genome sequencing (WGS). WGS was performed using MinION (Oxford Nanopore Technologies, UK) sequencing device. Isolate plasmids were then annotated using Prokka (v. 1.14.5) and their pangenomes were generated by Panaroo (v. 1.5.1). Sequenced plasmids were typed by a scheme proposed by Lam *et al*. and visualized using Snapgene viewer (v. 7.0.2).

PBRT and PFGE analysis revealed a wide variety of plasmids contained carried by *A. baumannii* isolates. PBRT analysis identified 13 different plasmid types. PFGE profiles identified plasmid-like fragments ranging from 10 kb to almost 300 kb. Isolates (n=22) with most diverse plasmids according to their replicon types and sizes were selected for sequencing. Analysis of sequencing data revealed 43 plasmids that belonged to 14 different types, thereby extending the profile obtained by PBRT analysis. Plasmid sizes were found to vary from 2 kb to 180 kb. Some plasmids contained more than one replicon. Moreover, sequencing data demonstrated that plasmids varied significantly in their genomic composition. Within some plasmid types (GR6 (rP-T1)), gene composition varied less than within other plasmid types (GR13 (r3-T7)). Additionally, the most common type, GR2 (r3-T1) plasmids in *A. baumannii* isolates from National Cancer institute displayed varying genomic composition, while their counterparts found in isolates from Santaros clinics had a lesser variety. Most of the gene functions were unknown, however, some plasmids contained genes responsible for plasmid maintenance or putative virulence factors. A few plasmids also contained resistance genes, such as *sul2*, *aph(3')-VIa*, *bla*OXA-23 and *bla*OXA-72, conferring resistance to sulfonamides, aminoglycosides and carbapenems, respectively.

These findings demonstrate high plasmid variety in *A. baumannii* isolates, collected from two hospitals over the period of 10 years. Isolates carried numerous plasmids, that exhibited pangenomic variation, containing an assortment of genes. This proves that plasmids can accumulate a wide variety of different genetic determinants, that can represent potential increasing chance of survival and infection.

IMPACT OF EXTRACELLULAR INFLAMMASOME COMPONENTS – ASC SPECKS – ON MACROPHAGE ACTIVATION

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Innate immune system activation occurs through various signaling pathways, including inflammasome activation, which drives inflammation and pyroptosis. Inflammasomes can be activated by numerous factors, including microbes, facilitating the maturation of pro-inflammatory cytokines IL-18 and IL-1β. Inflammasomes are multiprotein complexes, all containing the common adapter protein - apoptosis-associated speck-like protein containing a CARD (ASC), which is crucial for their assembly and activation. During inflammasome activation, ASCs oligomerize into around 1μm size ASC specks. These specks can accumulate in inflamed tissues and continue to mature cytokines or be phagocytized by surrounding cells to activate inflammasomes further. Prolonged inflammasome activation is linked to chronic diseases like Alzheimer's, atherosclerosis, and autoimmune disorders. ASCs are thus potential therapeutic targets for inflammation control. This research aimed to investigate how ASC specks affect macrophage activation by viral antigens.

We prepared ASC speck fractions using human THP1-ASC-GFP cell line. We settled on an optimal technique to gather ASC specks using various cell lysis methods and centrifugation steps. THP-1 macrophage-like cells were treated with ASC specks and viral antigens – human KI polyomavirus virus-like particles (KIPyV VLPs). We assessed cell viability and inflammatory response via cytokine secretion to analyze the effect of ASC specks and KIPyV VLPs on macrophages. Cell viability was evaluated by lactate dehydrogenase (LDH) assay. Enzyme-linked immunosorbent assay (ELISA) was applied to measure the release of cytokines, including IL-8, IL-1β, and CCL2.

Our findings showed that ASC specks, alone or combined with KIPyV VLPs, significantly increased LDH release, indicating pyroptosis. We also observed that higher ASC speck concentrations correlated with increased LDH levels in the cell medium. KIPyV VLPs alone caused a mild, insignificant LDH increase. We also found that KIPyV VLPS induced a higher IL-8 and CCL2 secretion in macrophages than ASC specks. However, macrophages treated with ASC specks released more IL-1β than viral antigen-activated cells. Interestingly, ASC specks significantly reduced the KIPyV VLPs-induced effect on macrophages regarding CCL2 secretion. A mixture of ASC specks and KIPyV VLPs induced different secretion of CCL2 and IL-1β than KIPyV VLPs alone. However, a mixture of ASC specks and viral antigens had a bigger impact on IL-8 secretion than ASC specks alone.

Our results suggest that ASC specks promote inflammasome activation and pyroptosis in macrophages. KIPyV VLPs stimulate pro-inflammatory cytokine release, while ASC specks modulate this response. In conclusion, ASC specks affect the KIPyV VLP-induced inflammatory response in macrophages.

EVALUATION OF TDV1 DERIVED VLPS INTERNALIZATION INTO A549 CELLS

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Virus-like particles (VLPs) are self-assembling protein nanoparticles (NPs), mimicking the structure and shape of viral capsids without retaining the viral genome. VLPs are promising substitutes for conventional NPs. The amino acid sequence of VLPs can easily be modified genetically or chemically offering a set of advantageous characteristics including precise targeting, biocompatibility, immunogenicity, solubility, and structural stability of encapsulated cargo as it protects against degradation. The current study investigates the VLPs from Torulaspora delbrueckii TdV1 virus as protein cargo carriers in mammalian cell cultures to overcome the limitations of conventional nanocarriers e.g. potential cytotoxicity and inefficient endosomal escape. The unique characteristics of TdV1-derived VLPs include encapsulating any peptide sequence attached to the C-terminal of capsid protein as well as a replaceable hypervariable region, ideal for the insertion of the desired sequence. For the experimental validation, TdV1-derived VLPs were expressed in both bacterial and yeast expression systems. To evaluate the applicability, expressed VLPs were labelled with fluorescent protein tags mCherry or msGFP2. After purification, VLPs were introduced into A549 cell lines. Uptake and subcellular location of the TdV1-derived VLPs were analysed by the confocal fluorescence microscope while the Western blot was used to access the entry dynamics. The viability of transduced cells was checked by resazurin and MTT assay. The therapeutic potential of TdV1-derived VLPs was accessed by herpes simplex virus thymidine kinase (HSV-TV) on prodrug-treated A549 cells. The study highlights the potential of TdV1-derived VLPs as nanocarriers, while further research is required to elucidate their entry mechanisms to optimize these virus-based nanocarriers for targeted therapeutic applications.

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INTERACTION OF RIBOSOMAL PROTEIN s1 WITH VIRAL PROTEIN

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Viruses, including bacteriophages (or phages), exploit the resources of infected cell to produce their progeny virions. Depending on their genome size, viruses encode different sets of essential genes required for their reproduction, but their protein synthesis is entirely dependent on the host's translational machinery. To direct host translation apparatus toward the synthesis of viral proteins, viruses use both intrinsic (*cis*-acting mRNA regions) and extrinsic mechanisms, such as protein modifications [1]. Ribosomal proteins and translation factors are the most common targets for modification by viral proteins.

Here, we describe in vitro and in vivo studies of the detected interaction between the phage VpaE1gp09 small protein and *Escherichia coli* ribosomal protein (RP) S1. The RP S1 is a multifunctional protein composed of six homologous RNA-binding domains, four of which are essential for cell viability [2]. Phages tend to modify this protein by viral proteins binding or by covalently attaching different chemical groups [3, 4,]. However, there are not many such examples, and the functional outcome of these modifications remains unclear. Thus, the aim of this work was to reveal the nature of this newly detected interaction using in vitro and in vivo systems in order to predict its potential impact on the translational machinery of the infected cell.

The results obtained by pull-down and native electrophoresis assays using purified recombinant proteins, as well as those obtained using a bacterial two-hybrid system, revealed a relatively weak interaction between RP S1 and VpaE1 protein suggesting that this interaction can be indirect. Given the RNA-binding function of RP S1, the interaction may be mediated by a particular RNA whose intrinsic properties allow it to interact with both proteins.

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DRUG DISCOVERY AND THERAPY APPROACHES

DT-1

SCREENING FOR APOPTOSIS IN PATIENT-DERIVED HUMAN ENDOMETRIAL CANCER CELLS

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Endometrial cancer ranks as the sixth most common cancer among women, with Lithuania and Poland reporting the highest incidence rates in Europe (Bray et al., 2024)). While established risk factors include older age, obesity, hyperglycemia, diabetes, and certain genetic predispositions, the reasons behind the particularly high prevalence in Lithuania and Poland remain unclear. Surgery is the primary treatment for endometrial cancer, often followed by chemotherapy in cases where a risk of recurrence is present. However, widely used chemotherapeutic agents such as paclitaxel, carboplatin, and cisplatin come with limitations – lack of specificity and associated side effects. Thus, targeted therapy drugs are being actively explored as promising alternatives to conventional chemotherapy (Remmerie et al., 2018). These novel approaches are also playing a crucial role in the advancement of personalized medicine (Saeed et al., 2023).

In this study, we aimed to assess the effects of various clinically approved compounds — including standard chemotherapeutic drugs, PI3K/AKT/mTOR inhibitors, receptor tyrosine kinase inhibitors, and cell cycle regulators — on endometrial cell lines derived from tumor tissue of Lithuanian endometrial cancer patients. To identify the most effective agents, we evaluated cell viability after the treatment. Subsequently, we determined the activation of apoptosis in the most sensitive endometrial cancer cell line CRL-230407. Our findings revealed that nine of the tested compounds triggered apoptosis in CRL-230407 cells, with PI3K/AKT/mTOR inhibitors, including MK-2206, apitolisib, and samotolisib, demonstrating the strongest pro-apoptotic activity.

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DESIGN AND IN VITRO CHARACTERIZATION OF PERIODONTITIS VACCINE PROTOTYPES

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The human microbiome plays a crucial role in immunity, with alterations linked to autoimmune diseases. Chronic diseases, rising globally across all socioeconomic groups, share risk factors such as poor diet, inactivity, tobacco use, alcohol abuse, and stress. Periodontal diseases (PDD), affecting up to 90% of the global population, rank among the most prevalent human diseases. Gingivitis, the mildest form, results from bacterial biofilm accumulation but is reversible. In contrast, periodontitis (PD), affecting ~11% of people worldwide, is a chronic inflammatory disease-causing progressive tissue destruction and potential tooth loss. Genetic predisposition, environmental factors, and tobacco use contribute to its onset. PD is also associated with systemic conditions, including cardiovascular disease, diabetes, rheumatoid arthritis, neurodegenerative diseases, cancer, and adverse pregnancy outcomes.

Porphyromonas gingivalis (Pg), a key PD pathogen, produces virulence factors, notably gingipains, which are citrullinated by Pg's peptidylarginine deiminase (PPAD). PPAD is a promising target for drug and vaccine development. Our study aimed to develop a vaccine targeting Pg PPAD using virus-like particles (VLPs) derived from plant virus coat proteins (CPs). VLPs, a proven vaccine platform, were assembled using CPs from potato virus M (PVM), potato virus Y (PVY), potato virus X (PVX), and eggplant mosaic virus (EMV).

Genetically fused constructs were designed for efficient vaccine production. Direct fusion constructs formed soluble aggregates, but the mosaic expression system successfully produced CP-PPAD VLPs. Immunogenicity studies in BALB/c mice tested three constructs: mEMV-PPAD, mPVM-PPAD (PPAD fused at CP C-terminus), plain PPAD, and a chemically coupled variant, cPVM-PPAD. The highest antigen titers were observed against plain PPAD, followed by cPVM-PPAD (16.6% conjugation efficiency). Due to low antigen incorporation (0.2%), mPVM-PPAD titers appeared only by day 28, whereas mEMV-PPAD (49% incorporation) induced specific antibodies with high avidity, emphasizing the importance of antigen incorporation and orientation.

To enhance antigen presentation, we explored SpyCatcher/SpyTag conjugation, which preserved the VLP structure. Co-expression with PVY resulted in stable VLPs. The mosaic PPAD constructs of PVY, PVX, and the SpyCatcher/SpyTag variant were tested via western blot using antibodies from the mEMV-PPAD immunization experiment to evaluate vaccine variants in vitro before conducting mouse immunization tests. Western blot analysis demonstrated antibody specificity to CP-PPAD. Side-by-side immunization tests will help identify the most promising vaccine candidate for evaluation in a PD animal model. These findings highlight the potential of plant virus-derived VLPs as a flexible and effective vaccine platform for targeting PD-associated pathogens.

VACCINE PROTOTYPE DEVELOPMENT AND IMMUNOGENECITY TEST FOR AUTOIMMUNE DISEASES CAUSED BY CHRONIC INFLAMMATION

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Autoimmune diseases, affecting 5–10% of the population, arise from chronic inflammation due to innate and adaptive immune system dysfunction. Current therapies are often ineffective, expensive, and associated with side effects or intolerance. The IL-17/IL-23 axis is a major pathway in chronic inflammation, with IL-23 serving as a key therapeutic target. A promising approach involves anti-cytokine vaccines that induce endogenous antibody (Ab) production, minimizing immune rejection by avoiding xenogeneic epitopes. We explored an innovative strategy using virus-like particle (VLP)-based vaccines to induce endogenous Ab production against IL23A, leveraging plant virus coat proteins (CPs) for self-assembly.

Three plant virus-derived CPs—potato virus M (PVM), potato virus Y (PVY), and eggplant mosaic virus (EMV)—served as VLP platforms. The mIL23A antigen was genetically fused to CPs, but aggregation issues necessitated a mosaic VLP approach, co-expressing plain CPs with antigen-fused CPs. This enabled VLP formation for mPVM and mEMV but not for mPVY. Densitometry analysis revealed mIL23A-CP incorporation of ~0.56% for mPVM and ~22% for mEMV, indicating EMV CP's role as a fusion partner for soluble protein expression.

Alternative conjugation strategies were explored using fusion tag systems—SZ1/SZ2, SZ17/SZ18, and Ecoil/Kcoil. These platforms facilitate antigen presentation while preserving VLP structure. Co-expression of mIL23A (fused with tags—SZ2, SZ17, and Kcoil) with EMV CPs fused to SZ1, SZ18, and Ecoil tags yielded stable VLPs, with conjugation ratios of 0.3%, 1.56%, and 3.4%, respectively. However, co-expression with PVM CP-Ecoil disrupted VLP formation, highlighting limitations in filamentous VLP-based conjugation strategies.

Immunogenicity studies were conducted in BALB/c mice, immunized subcutaneously with 30 μg of vaccine on days 0, 14, and 28. ELISA detected anti-mIL23A Abs after the second boost for most prototypes, indicating the impact of antigen density and spatial arrangement on immunogenicity. All vaccines induced anti-carrier Abs after the first immunization, demonstrating the effectiveness of pathogen-associated molecular patterns in immune stimulation.

To evaluate immune polarization, IgG1 and IgG2a titers were measured on day 42. An IgG1/IgG2a ratio <0.5 (e.g., 0.29 for mEMV-mIL23p19) indicated a Th1-dominant response, which may mitigate IgE-mediated allergic reactions. The observed isotype switching to IgG2a is likely driven by TLR ligands such as ssRNAs naturally encapsulated in VLPs during *E. coli* expression. These results support the potential of VLP-based IL-23-targeted vaccines as a novel immunotherapeutic strategy for chronic inflammatory diseases.

THE EFFECT OF PROBIOTIC BACTERIA ON DECIDUALIZATION OF HUMAN REPRODUCTIVE SYSTEM STROMAL CELLS

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Various factors can cause female infertility, most of which fall in the category of female reproductive system disorders, however, approximately 10% of women face another diagnosis – infertility of unknown origin. A growing number of studies discovered a possible connection between infertility and female reproductive system microbiota. Interestingly, in most of the studies conducted, a recurring trend is associated with the dominance of *Lactobacillus* genus in the uterus and successful conception.

The human endometrium is a dynamic tissue that goes through more than 400 cycles of renewal, differentiation, and shedding throughout a woman's reproductive years. The changes occurring in the endometrium, such as decidualization, are regulated by hormones in the body and stromal cells that make up the endometrium (EnSCs). Decidualization is a process when EnSCs change their morphology and gene expression patterns to create a suitable environment for embryo implantation in the uterine lining. Ineffective decidualization is associated with various reproductive issues: infertility, miscarriage, and uterine dysfunction.

The main goal of this study was to investigate the microbial profile of endometrial tissue and to evaluate the effect of probiotic bacteria *Lacticaseibacillus casei* on EnSCs decidualization. For this reason, we isolated EnSCs from females that were able to conceive and not been able to conceive and induced EnSCs decidualization *in vitro*. During decidualization, EnSCs were cultured together with 5X, 20X, 50X and 100X *L. casei* supernatant dilutions for 72 hours. After this period, we assessed EnSCs cell death using Annexin V and propidium iodide binding and flow cytometry. Then, by employing RTqPCR, we determined the expression of decidualization related genes. Lastly, we performed western blot analysis to examine changes in levels of reproduction associated proteins in EnSCs after the treatment with *L. casei* supernatant.

After 16S rRNA sequencing, we determined that genus *Lactobacillus* was predominant in both patient groups, comprising about 40% of all identified genera. Moreover, we showed that 5X *L. casei* supernatant dilution significantly reduced EnSCs viability. RT-qPCR results revealed that EnSC decidualization was successfully induced, as visible by the upregulation of *IGFBP1* and *PRL* expression levels in cells after 72 h of decidualization. The highest gene expression levels of decidualization related genes were reached in cells treated with 20X dilution of *L. casei* supernatant, showing the possible significance of *L. casei* metabolites for gene expression regulation during decidualization.

INTRACELLULAR DELIVERY OF L-BC-1 VIRUS-LIKE PARTICLES

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Traditional methods used for the treatment of cancer and other disease often cause undesired side effects and work only temporarily, demonstrating the high importance of alternative treatment methods. The development of targeted nanocarrier systems have emerged as a promising technique for improved treatment of such diseases. Targeted nanodelivery allows to control the delivery of therapeutic drug to targeted cells more efficiently, therefore causing minimum damage to healthy cells. Various types of nanoparticles are used for the development of such systems, including non-infectious virus-like particles (VLPs). Different methods like gene fusion, chemical conjugation, passive diffusion, and re-assembly are used to incorporate the desired cargos and targeting ligands into the interior or exterior of VLPs to increase the likelihood of efficient cargo delivery to specific cells. VLPs of different origins are studied to search for effective nanodelivery systems that could improve existing treatment methods.

In this work, potential application of recombinant ScV-L-BC-1 virus major capsid protein Gag-formed VLPs for nanodelivery was evaluated. Gag-formed particles were synthesized in two expression systems: bacteria *E. coli* and yeast *S. cerevisiae*. Using gene fusion method Gag-originated particles were encapsulated with red fluorescence protein mCherry, and entry of such particles into lung carcinoma epithelial tissue A549 line cells was evaluated by fluorescence imaging. Results show that Gag-mCherry particles successfully enter A549 line cells after 4 hours of incubation without a need of transduction enhancers or additional functionalisation of the particles. The relative viability studies of A549 line cells treated with Gag-mCherry particles revelied that only bacteria-derived particles have a cytotoxic effect on cells, whereas yeast-derived particles did not significantly reduce the relative stability of A549 line cells.

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ENHANCING CAR-NK CELL THERAPY: INVESTIGATING THE IMPACT OF EPIGENETIC MODIFIERS ON ANTI-TUMOR EFFICACY

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Chimeric Antigen Receptor Natural Killer (CAR-NK) cells have emerged as a promising immunotherapy for cancer due to their potent cytotoxicity, safety profile, and ability to target tumor cells with reduced risk of graft-versus-host disease. However, limitations such as exhaustion, limited persistence, and suboptimal efficacy necessitate further optimization. Epigenetic modifications play a crucial role in regulating NK cell function, persistence, and metabolic fitness. Epigenetic modifications are known to regulate immune cell activity and may provide a strategy to enhance CAR-NK function. Understanding the epigenetic landscape of CAR-NK cells and its modulation could provide novel strategies to optimize their therapeutic potential, ultimately leading to more effective and durable cancer immunotherapies.

In this study, we investigated the impact of epigenetic modifiers on CAR-NK cells, assessing their effects on gene expression and cytotoxic potential. We treated CAR-NK cells with selected epigenetic agents like Zebularine as DNMTi or Sodium butyrate as HDACi. RNA sequencing (RNA-seq) analysis revealed significant alterations in DNA methyltransferases (DNMTs), histone deacetylases (HDACs), and other methylases, indicating epigenetic remodeling. Additionally, we observed changes in the expression of key CD markers, perforin, and granzyme—molecules crucial for NK cell-mediated cytotoxicity. These findings suggest that epigenetic modulation can potentially enhance CAR-NK cell function, providing a novel strategy to improve the persistence and anti-tumor activity of CAR-NK therapies.

Our results pave the way for future therapeutic strategies that leverage epigenetic reprogramming to optimize CAR-NK therapy, ultimately improving treatment outcomes for cancer patients. Further investigations are needed to identify the most effective epigenetic agents and to validate their functional impact in preclinical and clinical settings.

PICHIA PASTORIS GLYCOENGINEERING FOR THE SYNTHESIS OF ANTIBODY-DRUG CONJUGATES

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Antibody-drug conjugates (ADCs) have garnered considerable interest over the past decades as powerful therapeutic tools to treat a variety of diseases. Their highly specific targeting capabilities and capacity to distinguish between healthy and malignant cells are especially useful in oncology[1]. Currently, most licensed ADCs are produced using non-specific conjugation methods, based on reaction with surface exposed lysine residues or thiol group of reduced disulfides[1]. ACDs generated in such manner are often prone to aggregation, low tolerated dose, exhibit fast systemic clearance and demonstrate overall lower efficacy[2]. The conjugation of ADCs through N-glycans represents an attractive alternative as it is spatially distant from the complementarity determining regions of the antibody and the glycosylation pattern is conserved across all antibody types[3].

It is currently the case, that the majority of biopharmaceuticals are being produced by mammalian expression hosts such as Chinese hamster ovary cells. The glycosylation pattern of mammalian cells is usually heterogenous, resulting in significant batch-to-batch variation. In addition, mammalian expression systems are costly, require long fermentation times, are prone to viral infections and often yield low product titers. Consequently, yeast hosts are emerging as promising alternatives, offering the potential to overcome these limitations[4]. However, the main challenge that needs to be addressed is the discrepancies between mammalian and yeast protein glycosylation pathways. This can be resolved through the engineering of yeast strains, which allows both to mimic the human glycosylation pathway as well as to produce therapeutics with customized glycans.

The aim of this research is to develop the technology that allows precise drug loading through N-glycans that are present on the surface of the recombinant antibody. We use the yeast *Pichia pastoris* (*Komagataella phaffii*) as a novel platform for conjugation studies and employ metabolic engineering strategies to alter the native yeast protein glycosylation pathway. This study discusses the introduction of a novel enzymatic pathway in *P. pastoris* that allows desired N-glycan modification and subsequent drug conjugation.

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IN VITRO BLOOD-BRAIN BARRIER MODEL FOR EVALUATING BRAIN-TARGETING GENE EDITOR DELIVERY VECTORS

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The therapeutic application of CRISPR-Cas gene editors continues to expand, yet it is predominantly limited to blood, liver, or skin diseases. These organs are relatively straightforward to target using existing gene editor delivery systems. In contrast, the application of gene editing for neurological disorders faces significant challenges, primarily due to the inability of current delivery tools to effectively cross the blood-brain barrier (BBB). Direct injections into the brain, while capable of bypassing the BBB, pose significant risks and side effects due to direct damage to the brain tissue. Therefore, it is critical to develop systemic delivery tools that can penetrate the BBB safely and efficiently.

Central nervous system (CNS)-targeting delivery vehicles can be directly tested in animal models. However, such experiments are laborious and pose ethical issues. Due to that, there is a growing need for reliable *in vitro* BBB models that could be utilized for higher throughput vector testing. One such model can be transwell assays with brain endothelial cells that recapitulate brain capillary walls, separating the blood from the brain parenchyma.

We aim to develop and apply a reproducible and relatively easy-to-maintain *in vitro* BBB model for testing various gene editor delivery vectors. This model consists of mouse brain endothelial cell line bEnd.3 cultured on hanging inserts with porous membranes. By optimizing cell density, we established an intact endothelial monolayer, which was confirmed by examining the expression of a tight junction protein occludin during barrier formation. After establishing the *in vitro* BBB model, we tested the transcytosis of Cas9 protein and its sgRNA complexes encapsulated in lipid nanoparticles (LNPs). Future experiments will expand upon this foundation to assess the transcytosis efficiency of other gene editor delivery vectors with varying compositions and functionalities. This work is critical to developing effective delivery tools for CNS-targeted gene editing therapies.

P53 PERTURBATION DEFINES ITS CONTRIBUTION TO OXALIPLATIN RESISTANCE

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Colorectal carcinoma, a leading cause of cancer-related mortality both in men and women, frequently employs oxaliplatin-based chemotherapy (e.g., FOLFOX). However, the emergence of drug resistance significantly limits therapeutic efficacy and contributes to tumor relapse. Resistance mechanisms can be intrinsic, stemming from pre-existing alterations in resistance-associated factors, or acquired during previous rounds of therapy due to various treatment-induced adaptive responses or intrinsic tumor cell heterogeneity. In both cases, the elucidation of biological processes and pathways crucial for the development of drug resistance provides the potential for establishing the course of alternative therapy.

This study utilized oxaliplatin-resistant colorectal cell lines, which bypass cell cycle blockade and evade apoptosis after drug treatment, to investigate the underlying resistance mechanisms. Differential proteomic analysis of parental and oxaliplatin-resistant cell lines highlighted the involvement of the p53 protein in the drug response. During the selection process, oxaliplatin-resistant cells acquired mutations in the *TP53* gene, abrogating the protein's ability to bind to target sequences and, therefore, function as a transcriptional factor. Transcriptional activity of the p53 protein is necessary for cell cycle arrest and induction of apoptosis via activation of *CDKN1A* and *BAX* gene, respectively. However, experiments with the p53 knockout cell line revealed an unconventional mechanism of p53-dependent oxaliplatin toxicity involving activation of extracellular cell death pathway activation via death receptor and caspase-8 activation. Moreover, reintroducing functional p53 protein into oxaliplatin-resistant cell lines did not restore sensitivity to the drug, showing underlying mechanisms of resistance that could be attributed to mitochondrial priming of individual subclones.

Collectively, these data highlight the critical role of wild-type p53 in mediating oxaliplatin response within the FOLFOX regimen. However, drug cytotoxicity and sensitivity appear to be significantly influenced by cellular heterogeneity and intrinsic apoptotic potential, necessitating a nuanced approach to therapeutic strategies.

THE ROLE OF AUTOPHAGY IN COLORECTAL CANCER CELL CHEMORESISTANCE

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Colorectal cancer (CRC) is the third most common malignancy worldwide and has the second highest mortality among all cancer types. Despite enhancements in screening and anticancer therapy strategies, CRC remains a major clinical challenge due to tumor adaptation mechanisms that lead to chemoresistance. Resistance to chemotherapy can be intrinsic or acquired during treatment, and recent studies suggest that cancer survival mechanisms can be associated with autophagy.

Macroautophagy (hereafter autophagy) is a catabolic cellular degradation and recycling process that maintains cellular homeostasis. In normal cells, autophagy plays an oncosuppressive role by degrading misfolded proteins and damaged organelles, preserving genomic stability and taking part in anticancer immunosurveillance. When malignancy is present, autophagy exerts an elusive role, varying in different types and stages of cancer. In cancer, autophagy prevents degradation of damaged components and helps cancer cells to overcome stressful conditions, such as hypoxia, oxidative stress and nutrient deprivation, leading to the development of cancer and chemoresistance[1]. Autophagy has been recognized as a critical factor in CRC progression, influencing survival, immune escape and response to therapy[2]. Our laboratory studies of chemoresistant colorectal cancer cells revealed that chemoresistant cells upregulate the expression of core autophagy proteins (ATG7, ATG12, p62)[3,4]. Thus, we aim to evaluate the importance of these proteins to chemoresistance.

Applying lentiviral transduction for intracellular delivery of specific shRNA constructs, we have derived HCT116 cells with stable reduced expression level of core autophagy genes: *ATG7*, *ATG9A*, *ATG12*, *ATG14* and *ATG16L1*. We aim to assess the effects of silencing of different ATG proteins on autophagic flux and CRC cell chemoresistance to clinically applicable chemotherapeutic drugs 5-fluorouracil and oxaliplatin. We will uncover the importance of these ATG proteins for chemoresistance of CRC cells.

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PLASMEPSIN INHIBITORS AS XANTIMALARIAL LEADS

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Malaria is a global health concern which faces challenges due to the emergence of drug-resistant *Plasmodium* strains. This urges to bolster the antimalarial drug arsenal with drugs acting by yet unexploited mode of action. The aim of the project is to develop inhibitors of plasmepsins IX and X as anti-malarial leads with improved drug-like properties. The primary objectives of the project are to optimize metabolic stability of peptidomimetic plasmepsin X inhibitors and to identify novel non-peptidic plasmepsin IX/X inhibitor scaffolds for the development of drug-like leads.

At the time of the conference, the expression and purification of plasmepsins IX and X are ongoing, with baculovirus constructs prepared for both targets and successful expression of plasmepsin X (PMX) achieved in Hi5 insect cells. While the production of these proteins is still in progress, we are conducting fragment-based screening using thermal shift assays against alternative drug targets, specifically plasmepsins II and IV. Promising hits identified through this approach are undergoing further structural analysis via X-ray crystallography to guide inhibitor design.

DRUG REPURPOSING FOR PANCREATIC CANCER TREATMENT

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Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest cancers due to the limited number of chemotherapeutic options. Advancements in precision medicine have introduced new drug candidates to treat various cancers, but targeted treatments for PDAC remain scarce. The shortage of drugs to treat PDAC can be addressed by drug repurposing potentiated with combinatorial drug treatment strategies.

In this study, we analyzed surgical PDAC specimens, patient-derived cancer cell lines, and cancer-associated fibroblasts via global proteomics, phosphoproteomics, and kinomics to identify PDAC-specific changes in protein levels, phosphorylation, and activated kinases. We used chemical gene expression perturbation libraries to find potential chemical compounds that might revert changes of PDAC-specific protein levels. Proteomic data have led to drug predictions that target primarily receptor tyrosine kinases. Testing of the predicted drugs on patient-derived cell lines identified Afatinib and ASP2215 as key candidates. Kinomics and phosphoproteomics identified changes in the activity of a number of identical protein kinases in PDAC tumors and patient-derived cells. This allowed us to use these kinases as small molecular inhibitor targets for successful in vitro PDAC cell eradication. In addition, we observed synergistic eradication of PDAC cells using drug combinations derived from our multiproteomic data, achieving efficacy at lower doses. The analysis of mechanisms of synergy highlights SRC-targeting Saracatinib as another promising treatment candidate.

This study underscores the power of integrating multiproteomic data for drug repurposing and combinatorial therapy development for PDAC treatment, emphasizing the therapeutic potential of kinase inhibitors.

HUMAN CHORIONIC GONADOTROPIN INDUCES CHANGES IN ENDOMETRIAL EXTRACELLULAR VESICLE CARGO

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Pregnancy establishment entails a combination of embryo development and uterine differentiation, involving decidualization of the endometrial stromal compartment. The success of blastocyst implantation strongly depends on preparing the endometrium for receptivity, which accordingly requires complex molecular and morphological changes determined by a mutual cross-talk between endometrial cells and the embryo. Receptivity is also modulated by embryo-derived factors like human chorionic gonadotropin (hCG). In recent years, extracellular vesicles (EVs), nano- to micro-sized membrane-enclosed particles secreted by most cell types, have been recognized to mediate cell-to-cell communication. EVs include a range of cargoes, such as proteins, lipids, and nucleic acids, including miRNAs. The prevailing hypothesis is that EVs carry crucial signaling molecules, taking part in various biological processes, notably implantation and embryo development. For example, endometrial EVs have been shown to stimulate angiogenesis and increase migration and invasion of trophoblast cells *in vitro*. In our study, we aimed to investigate how hCG affects the cargo of EVs secreted by endometrial stromal cells (ESCs). To determine the effect hCG has on secreted EVs, non-treated and decidualized ESCs were induced with hCG for 24 hours, and then, EVs were extracted from the culturing medium by ultracentrifugation. Mass spectrometry and *Illumina* sequencing were employed to determine proteins and miRNAs in the EVs, respectively. Our results revealed that treatment with hCG in non-decidualized ESCs results in negative regulation of proteins related to oxidative phosphorylation and mitochondrion organization. In addition, upregulation of immune regulation factors (PRTN3, CXCL12) linked to neutrophil and lymphocyte activity was observed after hCG treatment. GO analysis of differentially expressed miRNAs indicated that hCG treatment in decidualized ESCs affected the expression of EVs' miRNAs that target Wnt signaling, autophagy, regulation of GTPase activity, H3 histone acetylation, and in-utero embryonic development. Specifically, hCG in decidualized ESCs upregulates the levels of hsa-miR-340-3p, hsa-miR-663a, hsa-miR-766-5p, hsa-miR-3138, and hsa-miR-3180-5p. To conclude, our findings suggest that hCG alone and combined with induced decidualization contribute to uterine environment receptiveness to implantation through cell-to-cell communication by regulating protein and miRNA levels in endometrial EVs.

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A COMPARATIVE STUDY OF THE EFFECTS OF ALPHA-SYNUCLEIN AND S100A9 ON MICROGLIAL BIOENERGETICS AND INFLAMMATORY RESPONSE

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The phenotypic plasticity and functional diversity of microglia are strongly influenced by changes in their energy metabolism. These metabolic alterations play a pivotal role in regulating neuroinflammation, particularly in response to pathological stimuli such as amyloid proteins, which are implicated in the pathogenesis of neurodegenerative diseases. Recent studies have shown that alpha-synuclein (αSyn) and another highly amyloidogenic protein – S100A9 are abundantly expressed in microglia and neurons intracellularly and deposited extracellularly in both Alzheimer's and Parkinson's disease patients. However, the precise molecular mechanisms of their neurotoxicity and the microglial metabolic changes during the inflammatory response remain to be elucidated. Thus, our research aimed to investigate and compare the effects of αSyn and S100A9 on the energy-producing pathways of the microglial cells and their immune response.

In this study, BV-2 mouse microglial cells were cultured and exposed to nanomolar concentrations of pre-aggregated recombinant amyloid proteins – αSyn or S100A9 for 24 h. Following incubation, changes in microglial energy metabolism were assessed by measuring oxygen consumption rates and glycolytic activity using a high-resolution respirometer Oroboros O2k and O2k-pH ISE-Module, respectively. The inflammatory response of microglial cells to amyloid aggregates was evaluated by measuring TNF-α levels in the cell culture medium using ELISA, nitric oxide (NO) levels were quantified using the Griess assay, and extracellular reactive oxygen species (ROS) production was measured with the Amplex Red assay.

We found that exposure of BV-2 cells to αSyn and S100A9 negatively affected mitochondrial respiration. However, the mechanisms by which they induced such effects differed. αSyn inhibited oxidative phosphorylation by decreasing oxidation of the mitochondrial complex I-linked substrates without altering uncoupled respiration. In contrast, S100A9 caused the dysfunction of the mitochondrial electron transfer system, leading to a reduction in the oxidation of both mitochondrial complex I- and II-linked substrates. Notably, BV-2 cells exhibited highly increased glycolytic activity in response to both αSyn and S100A9, suggesting a compensatory switch to anaerobic glycolysis. Moreover, S100A9 significantly increased TNF-α secretion after 24 h, while extracellular NO and H_2O_2 levels remained unchanged. In comparison, αSyn did not affect TNF-α concentration in the cell growth medium, NO levels, or ROS production after 24 h of incubation with BV-2 cells.

Overall, our data suggest that extracellular αSyn and S100A9 oligomers cause a shift in microglial energy metabolism and induce distinct inflammatory responses. These findings have important implications for defining microglial contributions to brain energy metabolism and understanding neuroinflammation in neurodegenerative diseases.

SIMULTANEOUS QUANTIFICATION OF OMEGA-3 POLYUNSATURATED FATTY ACIDS AND DERIVED ACYLCARNITINES IN HUMAN PLASMA: A NOVEL BIOMARKER DISCOVERY USING LC-MS/MS

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Omega-3 polyunsaturated fatty acids (PUFAs) are important dietary supplements, and their concentrations are commonly measured to assess PUFA intake from food and supplements. Increasing evidence indicates that acylcarnitines are markers of the intracellular fatty acid content, mitochondrial functionality, and fatty acid metabolism. However, the role of PUFA-derived acylcarnitines have not been extensively investigated.

To address the need for comprehensive monitoring of PUFAs and their acylcarnitines in human plasma, we developed and validated a novel liquid chromatography–tandem mass spectrometry (LC–MS/MS) method for the simultaneous quantification of eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), eicosapentaenoyl-L-carnitine (EPAC), and docosahexaenoyl-L-carnitine (DHAC). Matrix effects were corrected using a background subtraction approach, and analytes were extracted from blood plasma via simple protein precipitation with acetonitrile. Detection was performed in multiple reaction monitoring (MRM) mode using an electrospray ionization source. Chromatographic separation was achieved in 5 minutes using a reverse-phase C18 column and a gradient mobile phase consisting of ammonium acetate and acetonitrile.

The method demonstrated high precision and accuracy, with limits of quantification being 2 μM for EPA/DHA and 2 nM for EPAC/DHAC, and coefficient of variation (CV) and bias values below 10%. The recovery efficiency ranged from 81% to 100%. Stability tests confirmed that EPA, DHA, EPAC, and DHAC remained stable under various conditions, including up to 6 hours at room temperature and refrigeration, as well as through three freeze-thaw cycles. However, EPAC and DHAC were unstable during long-term storage. The developed method was successfully applied to plasma samples from healthy volunteers. Before fish oil administration, the plasma EPA concentration in healthy volunteers was 4.0 ± 0.5 μM, the DHA concentration was 7.1 ± 0.5 μM, the EPAC concentration was 3.2 ± 0.3 nM, and the DHAC concentration was 8.4 ± 0.8 nM. After two weeks of supplementation, EPA and DHA increased by 20% and 44%, respectively, but decreased below baseline after an additional two weeks. In contrast, EPAC and DHAC concentrations increased by 60% and remained elevated throughout the study.

This method is simple, rapid, and cost-effective, making it suitable for high-throughput analysis in clinical studies. When applied to plasma samples from healthy volunteers, the method revealed a more pronounced increase in EPAC and DHAC levels compared to EPA and DHA. These findings suggest that PUFA-derived acylcarnitines could serve as novel and sensitive biomarkers for PUFA intake.

APPLICATION OF FLUORESCENCE SPECTROSCOPY, MICROSCOPY AND DEEP LEARNING FOR STUDYING GPCR-LIGAND INTERACTIONS

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G protein-coupled receptors (GPCRs) are a major class of membrane receptors as well as drug targets - around 34% of FDA-approved drugs target one of the 800 GPCRs expressed in humans. Despite the practical significance, the function of GPCRs remains obstructively obscure to consistently deliver drugs with high efficacy with minimal side effects with a substantial percentage of drug candidates failing clinical trials. One of the most important aspects to study is the interactions between ligands, allosteric modulators, and receptors as well as downstream signalling partners such as G proteins, G protein-coupled receptor kinases and arrestins. Recent population wide genetic studies have revealed substantial variation in the GPCRs and connections between mutations, disease progression and drug suitability. While interactions with wild-type receptor interactions have been in research focus for decades, natural mutations have not received sufficient attention.

To address this problem, we have set out to screen interaction properties between naturally occurring receptor genetic variants and FDA-approved drugs and find the combinations which deviate from the norm. This could reveal patients for whom the standard treatment is ineffective, while an alternative could have reduced side effects or be even more efficacious. However, many of the previous studies have relied on already genetically modified receptors biasing the effects or used non-kinetic assays missing the importance of both association and dissociation rate on drug action. We have started our study with dopamine D_2 receptor which is a major target of antipsychotic drugs and related to diseases such as schizophenia and Parkinson´s disease.

For this end, we have developed multiple fluorescence spectroscopy and microscopy-based ligand binding approaches, which do not require additional genetic modification with tags or fluorescent proteins and are ideal for studying the effect of isolated mutations on ligand binding. The first method monitors ligand binding in real-time by measuring fluorescence anisotropy change upon fluorescent ligand binding to the receptors expressed and displayed in baculovirus particles. Another method relies on high-content fluorescence and microscopy imaging in live cells expressing the receptor of interest. The imaging is compatible with regular microplate format. To accurately quantify the receptor-bound fluorescent ligands, we have developed automatic machine learning-based workflows that allow us to quantify whole cell and cell membrane fluorescence intensity. The workflow also includes removing artefacts and anomalies from microscopy images, improving the analysis accuracy.

All of the software tools are available as free and open-source Aparecium software (https://www.gpcr.ut.ee/aparecium.html).

MOLECULAR BASIS OF TYPE II INHIBITOR DEVELOPMENT AGAINST JAK2

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Janus kinases (JAKs) are intracellular non-receptor tyrosine kinases (JAK1-3, TYK2) that interact with cell surface receptors. They activate STAT transcription factors which translocate into the nucleus to initiate gene expression. The JAK/STAT pathway is one of the most clinically relevant cytokine signalling hubs, and mutations in JAKs or their cognate receptors lead to autoimmune diseases and malignancies.

All the JAKs have a unique C-terminal tandem kinase domain consisting of an inactive regulatory pseudokinase domain followed by a typical tyrosine kinase domain. FERM-SH2 domains in the N-terminus, have no intrinsic catalytic activity, but they link JAKs to their membrane-bound cytokine receptors.

All clinically approved JAK2 inhibitors (e.g. Ruxolitinib) are known as type I since they target the conserved ATP-binding site of the protein, when the enzyme is in its active conformation. Despite the therapeutic benefits, they lack specificity and after prolonged exposure, drug resistance mechanisms emerge. Contrary, type II inhibitors stabilize the inactive kinase domain conformation, reducing mutant burden and being effective against type I refractory diseases like B-cell acute lymphoid leukaemia (B-ALL).

JAK2 mutations play a significant role in various blood-borne malignancies, particularly in myeloproliferative neoplasms (MPNs), a group of chronic diseases with no curative treatments that often progress to acute myeloid leukaemia (AML). The most common mutation found is JAK2-V617F. Another point mutation, JAK2-L884P, located outside the active site, reduces the potency of type II inhibitors like CHZ868 and BBT594 by increasing the flexibility of the kinase domain's allosteric pocket, leading to resistance especially if other mutations like R683G occur.

Despite intensive research since 1990s, the development of potent and selective JAK2 inhibitors that would target regions outside the highly conserved ‘type I’ pocket have been hampered by the lack of detailed structural data and robust assay methods.

The Structural Immunology research group has determined the resistance mechanism of the L884P mutation by integrating data of co-crystal structures, molecular dynamics (MD) simulations, virtual screening, *in vitro* and *in cella* testing of the compounds. Furthermore, several JAK2 type II compounds that can bypass the acquired L884P resistance have been identified.

SYNERGISTIC EFFECTS OF CANNABIDIOL AND EPIGENETIC MODIFIERS ON HIGH-GRADE ENDOMETRIAL CANCER CELL PROLIFERATION, SURFACE MARKERS, AND STEMNESS GENE EXPRESSION

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High-grade endometrial cancer is an aggressive malignancy with limited treatment options, particularly in cases resistant to conventional therapies such as chemotherapy and hormonal treatment. Recurrence and poor prognosis are common, highlighting the urgent need for novel therapeutic approaches. Epigenetic modifications play a crucial role in cancer progression, and targeting them with histone deacetylase inhibitors (HDACi) and DNA methyltransferase inhibitors (DNMTi) has shown promise in restoring normal gene expression patterns. Additionally, cannabinoids, such as cannabidiol (CBD), have demonstrated potential anti-tumor effects through mechanisms affecting proliferation, apoptosis, and cellular plasticity.

In this study, we investigated the effects of CBD in combination with HDACi and DNMTi on KLE endometrial carcinoma cells. The KLE cell line was treated with HDACi like sodium butyrate, EGCG, BML-210, also with DNMTi like zebularine, and cannabidiol. Cell cycle analysis indicated cycle arrest in G1 phase after treatment with cisplatin and HDACi and in S phase after – paclitaxel and zebularine. Combination with cannabidiol reduced viability of the cells and increased apoptosis. Migration assay highlighted that combination with cannabidiol reduced migration even more compared to epigenetic inhibitors used individually. We observed changes in surface markers, notably, upregulation of CD9. Moreover, we discovered changes in the expression of genes and proteins responsible for stemness like NANOG, OCT-4, NOTCH-1, LIN28A, SOX-2, KLF4.

Our results demonstrated that combination of CBD with epigenetic modifiers significantly inhibited cell proliferation while inducing only a modest increase in apoptosis. Furthermore, we observed alterations in cell surface properties and notable changes in the expression of stemness-related genes, suggesting a potential impact on tumor cell plasticity. These findings provide new insights into the potential of combining cannabinoids with epigenetic therapy as an alternative approach for High-grade endometrial cancer treatment, warranting further investigation into its clinical applicability.

CHANGES OF EXPRESSION OF B7 FAMILY PROTEINS IN CHEMORESISTANT COLORECTAL CANCER CELLS

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Colorectal cancer is one of the most common malignancies worldwide and the third leading cause of cancer-related deaths. Chemoresistance remains an unresolved problem in the anti-cancer treatment. It can be caused by a variety of reasons: changes in drug transport and metabolism, modification of drug targets, activation of DNA repair, changes in cellular death or survival signaling.

Immune checkpoint inhibitor therapy is one of the potential approaches to address the problem of chemoresistance. This therapy acts by different mechanisms than chemotherapy – it restores the immune system's ability to recognize cancer cells by blocking the immune checkpoint proteins that are overexpressed on the surface of cancer cells and inhibit their recognition. Cancer cells that are resistant to the initiation of the cell death may activate molecular pathways that not only confer chemoresistance but may also modulate the expression of immune checkpoint proteins.

In this study we have evaluated the changes in the expression of eight B7 family immune checkpoint proteins in the chemoresistant colorectal cancer cells. The changes in expression of these proteins after treatment with 5-fluorouracil or oxaliplatin were also investigated. We have determined that the levels of several B7 family protein transcripts are differentially expressed in HCT116 and DLD1 cells which have acquired chemoresistance to 5-fluorouracil. Treatment with 5-fluorouracil or oxaliplatin also modulates the levels of B7 protein-coding transcripts in HCT116, DLD1 or SW620 cells.

BIOPHYSICAL CHARACTERIZATION OF TAU FILAMENT-ANTIBODY INTERACTIONS FOR IMMUNOTHERAPY STRATEGIES

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Introduction: Alzheimer's disease and tauopathies pose clinical challenges and unmet medical needs in prevention, diagnosis, and treatment. Their hallmark is pathological tau aggregation, forming neurofibrillary tangles, therefore making tau a promising therapeutic target. Despite ongoing efforts to develop anti-tau immunotherapies, the precise mechanisms of action and effects on tau aggregation pathways remain poorly understood. A deeper understanding of antibody interactions could guide the development of more targeted and effective therapies. Advances in *in vitro* methodologies now enable the preparation of disease-like tau filaments, allowing biophysical characterization of antibody binding and functional effects[1].

Aim of the study: We aimed to establish a biophysical toolbox to characterize antibody binding and its effects on tau aggregation pathways, using humanized monoclonal antibodies Bepranemab, which targets residues 235–250 near the microtubule-binding region (MTBR), and DC8E8 (AX004), which recognizes four binding sites within the MTBR.

Materials and methods: Using bacterial transformation, recombinant *tau-441* was expressed in an *E. coli* system. Purification was performed using nickel affinity and gel filtration chromatography. Tau fragment dGAE(297-391), which aggregates spontaneously, was used to seed the formation of full length tau into disease-specific filaments. Binding of biotin-labeled monomeric tau and filaments to antibodies was assessed via ELISA, while surface plasmon resonance (SPR) was used to determine binding kinetics.

Results: We successfully purified dGAE and *tau-441* and achieved full-length tau seeding using aggregated dGAE, forming disease-specific filaments. ELISA confirmed high binding affinity of Bepranemab and AX004 to tau monomers, which was validated by SPR (Bepranemab: $k_a = 4.49 \times 10^6$ (1/Ms), $k_d = 2.25 \times 10^{-3}$ (1/s); AX004: $k_a = 1.49 \times 10^6$ (1/Ms), $k_d = 3.2 \times 10^{-3}$ (1/s)). On tau filaments immobilized on a CM5 chip, AX004 retained strong binding ($k_a = 3.99 \times 10^5$ (1/Ms), $k_d = 1.08 \times 10^{-3}$ (1/s)), while Bepranemab showed no significant binding. To account for potential binding site occlusion due to fibril immobilization, biotinylated *tau-441* was used in ELISA, only AX004 exhibiting binding.

Conclusions: The successful expression and purification of tau-441 and dGAE and tau seeding enabled the formation of disease-specific filaments, facilitating biophysical analysis of antibody interactions. Applied methods confirmed AX004 binding to both monomers and filaments, while Bepranemab bound only to monomers, suggesting its epitopes are masked in filamentous tau, providing insights into epitope accessibility and antibody selectivity.

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METABOLIC FATE OF ACETYLCARNITINE IN MICE AND HUMANS: INSIGHTS INTO BIOAVAILABILITY AND EXCRETION

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Objectives. Acetylcarnitine, widely used in food supplements as a source of carnitine and acetyl groups, is marketed for its potential benefits in neurological disorders. This study aimed to clarify the bioavailability, distribution, metabolism, and elimination pathways of acetylcarnitine through investigations in both murine models and healthy human volunteers.

Materials and Methods. In murine studies, [^{13}C]-acetylcarnitine was administered intravenously and orally at doses of 20 and 200 mg/kg. In the clinical study, healthy volunteers received a single oral dose of 1500 mg acetylcarnitine. Blood and urine samples were collected at baseline and specified intervals throughout the study. Quantification of acetylcarnitine and L-carnitine concentrations in biological samples was conducted by ultrahigh-performance liquid chromatography-tandem mass spectrometry (UPLC–MS/MS).

Results. Oral administration of acetylcarnitine in mice at a dose of 200 mg/kg resulted in a bioavailability of only 8.6%. Administration of [^{13}C]-acetylcarnitine induced the washout of endogenous unlabeled carnitine and unlabeled acetylcarnitine from tissue stores, leading to significant increases in plasma concentrations by 26% and 139%, respectively. Furthermore, acetylcarnitine administration markedly increased urinary excretion of carnitine and acetylcarnitine, accounting for up to 50% of the administered dose. Plasma levels of medium- and long-chain acylcarnitines also increased following acetylcarnitine intake. In human subjects, acetylcarnitine exhibited even lower bioavailability than in mice. Additionally, substantial part of the ingested dose was metabolized by gut microbiota into trimethylamine that later was metabolised to trimethylamine N-oxide by the liver enzymes. Consistent with murine data, acetylcarnitine supplementation in humans stimulated the renal excretion of carnitine and its derivatives.

Conclusions. The oral bioavailability of acetylcarnitine is minimal (1-2%) in both mice and humans. Supplementation induces the release of endogenous carnitine, acetylcarnitine, and other acylcarnitines from tissues, promoting their subsequent urinary excretion. These findings offer novel insights into the in vivo regulation of acetylcarnitine and carnitine homeostasis.

SIGNIFICANCE OF B7 FAMILY PROTEINS FOR COLORECTAL CANCER CELL SENSITIVITY TO CHEMOTHERAPY DRUGS

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Colorectal cancer is one of the leading types of cancer in terms of both the number of incidence and mortality worldwide. Despite the widespread practice of chemotherapy, the unresolved problem of chemoresistance encourages to look for alternative ways of colorectal cancer treatment. Immunotherapy has recently become one of the promising alternatives. Despite numerous improvements in this field, more research is still required to fully address its shortcomings.

Immune checkpoint inhibitors are one of the types of immunotherapy. They block the immune checkpoint proteins thus preventing inhibitory signaling from the cancer to immune cells. This inhibition returns the ability for the immune system to recognize tumor cells. It is known that in many cases of cancer there is an increased expression of these proteins. Furthermore, immune checkpoint proteins, including the ones belonging to B7 family, may have additional functions for cancer cells that are not related to immune suppression.

The aim of our study is to examine how the silencing of different immune checkpoint proteins belonging to B7 protein family affects chemoresistance of colorectal cancer cells. Silencing was achieved by introducing shRNA coding sequences targeting specific B7 protein transcripts into the genome of colorectal cancer cells HCT116. The efficiency of this method was evaluated on both transcript and protein levels. We have found that the silencing of several B7 family proteins increases sensitivity of colorectal cancer cells to chemotherapy drugs, suggesting the role of these proteins in chemoresistance.

HUMAN TRYPSIN-ISOENZYME INHIBITORS FROM FINNISH CYANOBACTERIA

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Proteases play important roles in all living organisms. About one third of the over 500 known human proteases are serine proteases. These include trypsins, which are among the best characterized proteases. Among three different human trypsin-isoproteases, trypsin-3 appears to play an important role in the metastatic spread of cancer. Recently selective targeting of trypsin-3 has been shown to reduce the cancer cell invasion. However, due to high sequence and functional similarity, it is difficult to produce selective trypsin-3 inhibitors. Cyanobacteria produce potent inhibitors of trypsins. Here we tested the selective inhibition of three human trypsin-isoproteases using crude extracts of 505 strains, belonging to 19 different genera of cyanobacteria isolated from Baltic Sea and Finnish lakes. Extracts of 88 strains (17.4%) showed strong inhibition of one or more trypsin-isoproteases. The strains producing these inhibitors belonged especially to *Microcystis*, *Nodularia*, *Nostoc* and *Planktothrix* genera. Extracts of 13 strains showed selective inhibition activity. Of these, 3 preferentially inhibited trypsin-2 and -3 and 10 trypsin-1 and -2. Up to now, we have isolated and characterized trypsin inhibitors among aeruginosin family of cyanobacterial peptides (suomilide and varlaxins) and a dipeptide called radiosumin C. The most potent of these inhibited human trypsins with subnanomolar IC_{50} values. Interestingly, these peptides showed different selectivity profiles for inhibiting different human trypsin-isoproteases. Suomilide also inhibited the invasion of aggressive and metastatic PC-3M prostate cancer cells without effecting the cell proliferation. These results show that cyanobacteria are rich source of protease inhibitors with interesting selectivity profiles. The inhibitory peptides we have isolated and characterized may serve as leads for development of selective and potent trypsin inhibitors.

INHIBITION OF *ESCHERICHIA COLI* SERYL-TRNA SYNTHETASE: FRAGMENT-BASED DISCOVERY OF NEW ANTIBACTERIAL AGENTS

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Introduction: The misuse and overuse of antibiotics, incorrect prescriptions, and extensive use in agriculture have contributed to the development of antibacterial resistance (ABR), which has become a global public health concern. ABR occurs when bacteria no longer respond to antimicrobial treatment, making infections difficult, if not impossible, to treat. This increases the risk of disease spread, severe illness, and even death. Hence, there is an increasing need to identify new antibacterial drug targets, as existing ones have progressively become redundant. Aminoacyl-tRNA synthetases are a group of enzymes essential for protein biosynthesis and serve as attractive antibacterial drug targets, as inhibiting a member of this group disrupts protein production and viability of the bacteria.

Aim of the study: We aimed to identify molecular fragments that inhibit *Escherichia coli* seryl-tRNA synthetase (*Ec*SerRS) as a discovery platform for novel antibacterial drug development.

Materials and methods: Using bacterial transformation, recombinant *Ec*SerRS was expressed in an *E. coli* system. Purification was performed using nickel affinity and gel filtration chromatography. Protein activity was assessed using isothermal titration calorimetry and an enzymatic assay. To identify potential binding fragments, a fragment screen was conducted using a thermal shift assay (TSA) with the *Maybridge Ro3* fragment library (1000 compounds). To validate fragment binding to the enzyme, nuclear magnetic resonance (NMR) spectroscopy was performed.

Results: We successfully produced pure *Ec*SerRS with 70–75% enzymatic activity. Fragment screening with TSA identified six fragments that bound to the protein, stabilizing it with an increase in denaturation temperature of 1.2–1.4°C, and eight fragments that stabilized the protein with a denaturation temperature increase of more than 2°C. NMR results confirmed binding of three fragments.

Conclusions: The successful expression, purification, and characterization of *Ec*SerRS enabled the identification of multiple fragment compounds that bind and stabilize the enzyme, with NMR confirming fragment interactions. These findings lay groundwork for further development of antibacterial inhibitors targeting *Ec*SerRS.

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EVALUATION OF CARDIOPLEGIC SOLUTIONS FOR PRESERVING CARDIAC CELLS IN ISOLATED RAT HEART ASSAYS

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Introduction. Cardioplegic solutions are employed during heart surgery to halt myocardial contraction. Their main purpose is to minimize myocardial oxygen demand by inducing electro-mechanical quiescence and cooling of the heart, thereby reducing ischemic effects. This process helps prevent degenerative changes, decreases the metabolic demands of the cardiac muscle, and protects cardiac myocytes from apoptosis and cell death [1]. Ischemic injury in the heart can be characterized by various molecular alterations within cells. BCL-2 family proteins play a crucial role in regulating mitochondrial apoptosis, either facilitating or preventing cell death [2]. In addition, FOS proteins have been associated with cell transformation, differentiation, proliferation [3]. The BNIP2 gene interacts with various proteins to protect cells from externally induced cell death [4]. Meanwhile, HIF1α serves as a key regulator of cellular and systemic homeostasis in response to hypoxia [5].

Aim. To assess the effects of three different cardioplegic solutions on immature rat heart tissues, we analyzed gene expression associated with the cell cycle, proliferation, apoptosis resistance, and hypoxia response.

Methods. The animals were sacrificed, and the heart was removed through a median sternotomy, then transferred to a cold perfusion buffer. Within 5 min, the heart was mounted on a Langendorff system and perfused retrogradely. Cardioplegia was administered using St. Thomas (CP1), Custodiol HTK (CP2), or del Nido (CP3) solutions, with re-plegia intervals of 30 min (CP1), 90 min (CP3), or a single dose (CP2). As a positive control, fully perfused heart tissue was used (K-PRF). Ventricular biopsies were homogenized in liquid nitrogen to extract total RNA, which was then reverse-transcribed and analyzed for gene expression using TaqMan assays.

Results. CP3-treated tissue had an average 50% lower BAX/BCL2 ratio than CP1 and CP2 tissue, however, these changes were not statistically significant. Following CP treatment, HIF1α gene was significantly overexpressed CP3 group compared to, CP1, and CP2 groups ($P<0.05$). In all cases, FOS expression was significantly decreased compared to the K-PRF after CP1 and CP2 treatment. After 2 and 4 h of incubation with CP3 solution, FOS gene expression remained substantially higher compared to CP1 ($P<0.05$) and CP2 ($P<0.05$). Similar results were observed in BNIP2 gene expression. Based on gene expression, CP3 treated cardiac tissue was comparable to K-PRF heart tissue in all timestamps.

Conclusions. The CP3 solution exhibited the most effective cell-protective properties, comparable to heart perfusion. However, further studies are required to assess the electrophysiological and metabolic changes in heart tissue.

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PLANT VIRUS-LIKE PARTICLES AS A NOVEL VACCINE PLATFORM FOR A-GAL SYNDROME

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Allergy is a hyperimmune response triggered by specific allergens, leading to adverse health effects. A severe manifestation is systemic anaphylaxis, a life-threatening reaction caused by allergen-IgE interactions that induce mast cell and basophil degranulation. Food allergies (FAs) are increasingly prevalent, affecting up to 10% of industrialized populations, particularly in urban areas. Despite advancements such as oral immunotherapy for peanut allergies, FA management primarily relies on allergen avoidance, significantly impacting quality of life and imposing economic burdens. The U.S. FDA identifies nine major allergens responsible for 90% of FA reactions, with efforts ongoing to identify additional allergens globally.

A recently recognized FA, α-Gal Syndrome (AGS), involves an IgE-mediated reaction to galactose-alpha-1,3-galactose (α-Gal), a carbohydrate found in mammalian meat and products. AGS was discovered during cetuximab drug trials when patients exhibited IgE reactivity to α-Gal on the Fab portion of cetuximab. This syndrome is linked to tick bites, particularly from Amblyomma americanum and Ixodes scapularis, which may introduce α- Gal either from previous blood meals or intrinsic biosynthesis. AGS challenges traditional immunological understanding, as carbohydrates were long considered weak allergens due to their T-cell-independent nature.

Humans and Old-World primates lack α-Gal due to an evolutionary loss of the α1-3 galactosyltransferase gene. However, humans produce anti-Gal antibodies (1%-5% of total Ig), likely induced by gut microbiota containing α-Gal-expressing bacteria. These antibodies exhibit cytotoxic activity against pathogens, protozoa, and certain viruses. Currently, AGS has no specific treatment; management relies on avoiding tick bites and mammalian-derived foods. Symptom control includes antihistamines and corticosteroids, highlighting the urgent need for long-term therapeutic solutions.

Advances in plant virology and genetic engineering enable the development of plant virus-based vaccines for infections, cancer, autoimmune diseases, and allergies. This study aims to develop a therapeutic vaccine targeting the α-Gal disaccharide and assess its immunogenicity. α-Gal (synthetic, in house produced) was chemically coupled to plant virus-derived virus-like particles (VLPs) using EDC-Sulfo-NHS chemistry. Selected plant coat proteins with surface-exposed lysine residues included potato virus M (PVM), potato virus Y (PVY), eggplant mosaic virus (EMV), and cowpea chlorotic mottle virus (CCMV). UV spectroscopy confirmed successful α-Gal conjugation, and Western blot analysis using anti-α-Gal monoclonal antibody (M86) validated antigen recognition. Integrity analysis demonstrated structural stability comparable to unmodified VLPs, supporting their suitability for further immunogenicity testing in animal models. These findings highlight the potential of plant virus-derived VLPs as effective carriers for α-Gal-based allergy vaccines.

CALORIMETRY FOR MECHANISTIC INSIGHTS INTO LIPOLYTIC ENZYMES IN PHYSIOLOGICAL CONDITIONS

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Cardiovascular diseases (CVD) are the leading cause of death worldwide, with hypertriglyceridemia (HTG) as an independent and causal risk factor. HTG can also cause hepatic steatosis and acute pancreatitis. It arises from genetic, lifestyle, and medical factors like obesity and diabetes, affecting millions globally. Pancreatic lipase (PL) and lipoprotein lipase (LPL) regulate triglyceride metabolism, making them crucial drug targets for HTG treatment. PL inhibitors reduce dietary fat absorption in the intestine, while LPL activators lower circulating triglycerides. Measuring PL and LPL activity is essential for diagnosing pancreatitis and HTG causes. Both PL and LPL function in highly crowded complex environments where a multitude of factors can influence their activity. Stemming from this fact, and considering the broad substrate specificity of both lipases, many assays have been developed for determining lipase activity. However, most approaches utilize water-soluble synthetic substrates for continuous measurements, otherwise sacrifices in sensitivity and throughput must be made to accommodate more physiological-like conditions.

Here we demonstrate a calorimetric method that enables real-time monitoring of lipase activity in physiological-like or complex substrate environments. We propose that this approach could be used to reveal new insights into how various endogenous regulators or drug candidates modulate lipase activity. We show that PL activity can be measured with picomolar sensitivity in conditions that contain crucial components of intestinal substrate emulsions. Exogenous LPL activity can also be measured at picomolar concentrations in undiluted human plasma. It is also possible to monitor endogenous LPL activity by simply injecting post-heparin plasma into Intralipid, VLDL or undiluted plasma. This approach ameliorates the need to inhibit post-heparin hepatic lipase as is the case for fluorescent or colorimetric substrates.

We applied the calorimetric method in combination with other biophysical methods to elucidate how intestinal ANGPTL4 affects PL activity through interactions with substrate emulsions. Analysis of over 50 plant extracts has also revealed new natural inhibitors of PL, as well as demonstrated how some known inhibitors can have vastly different effects depending on the substrate system used. In the case of LPL, we have shown how exogenous LPL activity can greatly vary between individuals by analyzing blood serum from vegans or omnivores. Mechanistic studies of LPL using calorimetry have revealed how albumin acts as an LPL oligomerization regulator and how an apolipoprotein C-II mimetic peptide can be an efficient LPL activator. Finally, our measurements have also revealed how people with lipodystrophy tend to exhibit lower exogenous LPL activity.

CHARACTERIZING THE INTERACTION OF FULL AND FAB FRAGMENTED ANTIBODY DC11 WITH TAU FILAMENTS

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Introduction: Alzheimer's disease and other tauopathies present significant healthcare challenges, with a pressing need for effective tools and therapies for prevention, diagnosis, and treatment. These diseases are characterized by the aggregation of pathological tau protein, which forms neurofibrillary tangles in the brain, making tau a key target for therapeutic intervention. The administration of monoclonal antibodies has been shown to effectively reduce tau pathology and/or enhance cognitive and motor functions in animal models. However, detailed insights into their mechanisms of action, the specific changes they induce in the tau assembly pathway, and their pathological significance remain limited. A deeper understanding of these mechanisms could pave the way for the development of more precise and effective immunotherapies.

Aim of the study: This project seeks to uncover the mechanistic basis of how DC11 full and fragmented antibodies bind to pathological tau and explore potential strategies for developing more effective therapies for tauopathies.

Materials and methods: Recombinant Tau-441 was expressed in an *E. coli* system through bacterial transformation and was purified using nickel affinity and gel filtration chromatography. Aggregation of Tau-441 was induced by seeding it with the dGAE(297– 391) Tau fragment. Full and fragmented DC11 antibody binding to the assembled disease-like tau filaments was be validated using an ELISA assay, while ThT fluorescence was used to measure the aggregation kinetics of dGAE-seeded full-length tau.

Results and discussion: Gaining a comprehensive understanding of tau biology and its interactions with therapeutic antibodies requires considering a wide range of structural and kinetic factors, along with their responses to various environmental conditions. By shifting the focus from in-depth analysis of a single patient-derived *ex vivo* sample to the rapid evaluation of multiple disease-like recombinant samples under different conditions, this research will drive progress in tau immunotherapy. This *in vitro*-based approach is crucial for designing more effective therapies. Additionally, the methods developed in this project could be applied to other protein aggregation disorders, providing proof-of-concept for tools and strategies to characterize diverse protein-antibody complexes.

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SELECTIVE MULTI-MICRORNA-BASED GENE SILENCING IN BREAST CANCER CELLS

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We investigated the effectiveness of a technology that allows for the efficient silencing of three proteins in the branched-chain amino acid degradation pathway (dihydrolipoamide S acetyltransferase (DLAT), branched-chain amino acid transaminase 2 (BCAT2), and dihydrolipoamide branched-chain transacylase E2 subunit (DBT)) simultaneously. Different vectors with self-amplification, multi-miRNA, and exosome motifs were constructed. Plasmids were isolated from bacteria and transfected into breast cancer cells (BCC). Real-time PCR from isolated BCC cell RNA, as well as Western blotting of proteins, were performed. The expression of DLAT, BCAT2 and DBT genes was significantly reduced after transfection of cells with a plasmid with the complete tested RNA system. The expression of all three test proteins was inhibited in cells transfected with the full-construct SF13 plasmid.

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THE EFFECTIVENESS OF NYSTATIN AGAINST *CANDIDA* YEAST UNDER VARIOUS ENVIRONMENTAL CONDITIONS

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Candida yeasts are commensals in the human body, however, once the immune system is weakened, they can become opportunistic pathogens, causing superficial or disseminated and even fatal diseases. The infections caused by the genus *Candida* yeast are very hard to treat for there are only four known classes of antifungals and the cells can acquire multidrug resistance to all of them [1]. To effectively fight these infections, finding new antifungal drugs or improving the efficiency of those already known is necessary.

The aim of this study was to increase the effect of nystatin against *C. albicans* and *C. glabrata* yeasts under various environmental conditions. For this research real-time analysis methods were used. The binding of lipophilic phenyldicarbaundecaborane (PCB^-) ions to the yeast cells, the monitoring of the respiration rate, and the release of intracellular K^+ were measured in thermostated cuvettes, using O_2 sensing and ion-selective electrodes. It was determined that nystatin is the most effective in an acidic environment – the highest binding of PCB^- was observed when the pH of the incubation buffer was 2.5-3. In buffer solutions of higher acidity, the yeast cells were inactivated. The quickest inhibition of yeast respiration by nystatin also was monitored in an acidic environment. A surprising finding was that at pH 3, *C. albicans* wild-type cells do not release any intracellular K^+, while at pH 6 a slow continuous leakage of this cation was observed. Moreover, it was observed that when the yeast cells were incubated in the presence of phenolic acids, the cells bound a high amount of PCB^-. This observation suggests that phenolic acids might have antifungal properties. However, such an increase in the binding of PCB^- was observed only in acidic buffer solutions, while at pH 6 the phenolic acid did not affect *C. albicans* wild-type cells. Other weak acids, like acetic, were ineffective even in acidic environments.

These findings broaden our understanding of the efficiency of nystatin and how phenolic acids could be used as antifungal agents.

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COMPARISON OF DI-ERENT EXPRESSION SYSTEMS FOR TIPARP/PARP7 BIOSYNTHESIS

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Introduction: Cancer is a leading cause of death worldwide. In 2022, there were 20 million recorded cases of cancer, and it was responsible for 9.7 million deaths, therefore highlighting the urge for new medication[1]. One possibility is related to DNA repair in cancer cells due to recent discoveries on PARP functions and the development of PARP inhibitors. Most of them are focused on PARP1 and PARP2, but the newest information urges to shift the focus on TIPARP. Research presents that TIPARP negatively aIects tumour immunoreactivity via inhibiting the TBK1 cell signalling pathway and others, therefore suggesting that TIPARP inhibitors could be a potential new drug for several cancers[2].

Aim of the study: This study focuses on comparing diIerent methods and finding the most eIicient way of TIPARP synthesis *in vitro* as a base for further drug discovery.

Materials and methods: The main methods included molecular cloning, cell-free gene expression, recombinant protein expression in *E.coli*, protein expression in insect cell lines such as Hi5 and subsequently purification was done using diIerent chromatography metods Expression tests were conducted via BugBuster® kit.

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THE EFFECT OF HYPOXIA AND IONIZING RADIATION ON PARP GENE EXPRESSION IN COLORECTAL CANCER CELLS

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Colorectal cancer is the second leading cause of cancer-related deaths worldwide. Radiotherapy, often combined with chemotherapy, is a common treatment strategy for this disease. However, its effectiveness is limited by therapy resistance and damage to adjacent normal tissues. Targeting specific molecular pathways could enhance treatment efficacy.

Poly (ADP-ribose) polymerase (PARP) proteins are of particular interest in cancer therapy. The human PARP family consists of 17 proteins that regulate various cellular processes, such as DNA repair and transcription, by catalyzing ADP-ribosylation reactions and through catalysis-independent mechanisms. While several PARP inhibitors are already used in clinical settings, they have not yet been approved for the treatment of colorectal cancer.

Our previous studies have shown that the expression of several *PARP* genes increases in 3D colorectal cancer cell models after exposure to fractionated dose ionizing radiation. A similar effect was observed in rectal cancer tumor samples after patients underwent chemoradiotherapy. Both 3D cell models and the tumor microenvironment exhibit hypoxia. Therefore, this study aimed to determine whether the expression of these genes is linked to hypoxia.

Hypoxia levels in clinical samples were assessed based on the expression of hypoxia signature genes. We then examined whether hypoxia levels correlated with increased *PARP* expression. For cell models, colorectal cancer cell lines HT29 and DLD1 were cultured under normoxic or hypoxic conditions and exposed to ionizing radiation. *PARP* gene expression was then assessed.

GENOME-GUIDED DISCOVERY OF ANTIMICROBIAL COMPOUNDS FROM ACTINOBACTERIA STORED AT MICROBIAL STRAIN COLLECTION OF LATVIA

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The rise of antibiotic resistance has become a serious public health issue. To combat it, the discovery of novel, bioactive compounds is necessary. Actinobacteria is a Gram+ phylum of bacteria that has been historically proven to be a prolific source of antibiotics. Up to 75% of known antimicrobials today have been isolated from Actinobacteria. From Actinobacteria, *Streptomyces* genus is the one most research. Due to the increasing number of natural product rediscovery, nowadays natural products research has turned to lesser characterised bacteria or to bacteria from unexplored biotopes.

Microbial Strain Collection of Latvia holds more than 1700 different strains of microorganisms collected throughout the years at the University of Latvia. It also has more than 70 different strains of Actinobacteria, isolated from the Baltic region. Sixteen of these strains were genome sequenced, and the taxonomy based on the 16S sequence was determined. Four strains belonged to *Kitasatospora* genus, one to – *Achromobacter* sp., but the rest belonged to seven *Streptomyces* species. To give more insight into the evolutionary relationship between these Actinobacteria strains, phylogenetic analysis, using core genome alignment was performed. Additionally, to gain identify potential biosynthetic gene clusters encoding antimicrobial compounds, the BGCs were predicted using antiSMASH software. BGC analysis revealed diverse polyketide synthases (PKS), nonribosomal peptide synthetases (NRPS), and other secondary metabolite pathways.

To functionally validate antiSMASH findings, the bioactivity of these Actinobacteria was screened for antibacterial and antifungal activity against clinically relevant pathogens, as well as, plant fungal pathogens. Further studies will focus on characterization of the rest of the Actinobacterial collection and metabolome analysis using LC-MS to characterize these bioactive metabolites.

NANOPORE SEQUENCING-BASED DIRECT DETECTION OF SYNTHETIC DNA MODIFICATIONS

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Epigenetic modifications play a crucial role in gene regulation, imprinting, and chromatin organization. 5-methylcytosine (5mC) is the most prevalent DNA modification in eukaryotes, forming distinct methylation patterns that help regulate gene expression, cell differentiation, healthy function, and disease. However, the tiny, chemically inert methyl group is not readily detectable, which complicates sensitive methylation studies and requires alternative markers.

The mTAG (methyltransferase-directed Transfer of Activated Groups) technique enables site-specific covalent biomolecule modification using engineered methyltransferases and synthetic cofactor S-adenosyl-L-methionine (AdoMet) analogues [1]. Unlike natural modifications, the resulting synthetic markers can be selectively detected through the chemical attachment of reporters, such as fluorophores for optical detection or biotin for affinity-based assays [2,3]. However, there is still room for increased resolution, sensitivity, speed, and relative simplicity in synthetic DNA modification detection.

Nanopore sequencing offers a direct approach to detecting synthetic and natural modifications by measuring unique ionic current shifts as the DNA strand is pulled through a nanopore. Our study explores the possibility of detecting synthetically modified cytosine bases in DNA, introduced via the mTAG method. Raw signal analysis indicated distinct signal shifts upon reading a synthetically modified C and several bases around it in the sequence. These complex signal variations required fine-tuning of the basecalling model for improved accuracy in recognizing the primary modified base as C. Furthermore, we trained a modification prediction model to recognize the synthetic modification with high sensitivity and relatively low false-positive rates.

Our findings support nanopore sequencing as a powerful tool for the direct detection of synthetic DNA modifications, offering an alternative to traditional biochemical assays. Combined with the ability to detect natural modifications, this methodology could open avenues for further applications in epigenetic analysis, synthetic biology, and precision epigenomics.

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OMICS AND SYSTEMS BIOLOGY

OS-1

FUNGAL AND BACTERIAL MICROBIOTA OF DIFFERENT DEVELOPMENTAL STAGES HONEY BEE DRONES

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Honey bees, *Apis mellifera*, are economically and ecologically important insects that ensure biodiversity in the natural environment and contribute to human well-being. They also form an environment for a distinct microbiota. Worker bees transfer bacteria and fungi from plants to all hive members. The microorganisms living on different hive substrates interact with each other and function together as a superorganism. The microorganisms impact the metabolism, development, and evolution of honeybees. The core microflora protects the hive environment from undesirable and pathogenic invaders. Honey bee drone microbiota could indicate the overall microbial status of the hive. Therefore the aim of this study was in-depth characterisation of microbial communities-inhabiting honey bee drones.

During this study, the bacterial and fungal communities present on honey bee drones were revealed by Next Generation Sequencing of the PCR-amplified V3-V4 region of the 16S rRNA gene and ITS2 region of rDNA. Significant differences in microbial amplicon sequence variants (ASV) among honey bee drones at different development stages were observed. The highest number of bacterial ASV was exhibited at the larva stage, while the lowest number was found in the eggs and pupa stage. Fungal ASV counts were notably higher on mature honey bee drones and larvae than on eggs and pupae. The bacterial community on eggs and larva stage honey bee drones was shown to be prevalent by *Acetobacteraceae*, while on pupae stage *Lactobacillaceae* and *Orbaceae* were observed at similar levels. More than 80% of bacteria distributed on adult honey bee drones belonged to *Lactobacillaceae* family. Among taxonomically differentiated fungal microbiota, *Saccharomycetaceae* dominated on adult honey bee drones, while eggs and pupa were almost sterile. Only small traces of *Cladosporiaceae* were detected on the first, and *Saccharomycetaceae* were observed on the last ones. The diversity of fungal microorganisms was highest on adults, while bacterial – on larva stage honey bee drones. Potentially pathogenic to honeybees and humans, also beneficial and relevant for biocontrol microorganisms were identified.

Resolving the microbial composition of honey bee drones is highly relevant for the evaluation of overall hive health. The revealing of the biocontrol potential of particular microorganisms will aid in developing targeted approaches for disease management. This study was funded by the Research Council of Lithuania (project no. S-MIP-24-55).

EFFECT OF CRISPR-CAS GENE DELETIONS ON COEVOLUTION DYNAMICS OF *PSEUDOMONAS AERUGINOSA* AND DMS3VIR LYTIC PHAGE

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CRISPR-Cas systems are often used in biotechnology and are best known for their antiviral activity, however their other functions in bacteria are not as well characterized. It has been shown that CRISPR-Cas systems can impact biofilm formation, quorum sensing and pathogenicity [1]. Bacteriophages have also been used to treat highly antibiotic resistant *Pseudomonas aeruginosa* [2]. To examine the importance of CRISPR-Cas for phage resistance in *P. aeruginosa*, coevolution experiments of three different *P. aeruginosa* PA14 strains lacking *Cas1* (Cas1), *Cas2/3* (Cas2/3) or the entire CRISPR-Cas system (CRKO) and a wild type (WT) strain with a virulent bacteriophage DMS3*vir* were conducted. Phage and bacteria co-cultures were passaged daily in minimal media for 21 days or until complete phage clearance. Bacteriophage and bacterial concentrations were measured by counting plaque forming units of serial dilutions of phage and estimated via an optical density calibration curve, respectively. Obtained results revealed that WT and Cas1 were able to fully clear the phage infection in 4-5 days, while Cas2/3 and CRKO were able to clear most the phage in the same timeframe but maintained a low level of infection for the rest of the 21 days. DNA and RNA sequencing is planned for all 4 strains.

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ANTIMICROBIAL RESISTANCE PATTERNS IN DOGS WITH PERIODONTAL DISEASE

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Canine *(Canis lupus familiaris)* periodontitis is a severe oral infection affecting majority of populations older than 2 years old characterized by microbial colonization and tissue destruction. This study aimed to investigate the antibiotic resistance patterns of bacteria isolated from the alveoli of extracted teeth in dogs with advanced periodontitis.

Traditional bacteriological plating methods and next generation sequencing technologies were performed to analyse the microbiota and antimicrobial resistance. The predominant bacteria detected by NGS were *Porphyromonas gulae, Prevotella spp., Tannerella forsythia, Porphyromonas crevioricanis, Porphyromonas cangingivalis.* Traditional plating methods most frequently identified *Pasteurella spp., Streptococcus spp. and Neisseria spp*.

Antibiotic resistance testing performed by E-test on isolated bacteria revealed clinical resistance to ampicillin, doxycycline, sulfamethoxazole-trimethoprim, ciprofloxacin and chloramphenicol. Genetic markers for resistance to beta-lactams (*OXA347*), tetracyclines (*TetM*, *TetQ*, *TetW*, *Tet32* and *TetO*) aminoglycosides (*AadE*, *AadS*), macrolides (*ErmF*, *Erm39, Mef(En2)*) and colistin (*Mcr1*) were detected using next generation metagenomic sequencing.

The presence of antimicrobial resistance to critically important antimicrobial classes for humans and animals' bacteria in dogs with periodontal disease highlights the potential to public health risk, because these bacteria can be transmitted to humans through dog bites or even daily contact. Although the study highlights the importance of antimicrobial susceptibility testing before prescribing antibiotics for dogs, it should be kept in mind, that some of the pathogens located in periodontal pockets are still unculturable. By this reason, etiological agents detected solely by classical bacteriological methods may be underestimated, whereas incorrect prescription of antibiotics for periodontal disease treatment can increase resistance problem in small animal medicine. Prophylaxis of periodontal disease is a key element keeping an appropriate oral health in animals, thus preventing the necessity for radical treatment and excessive usage of antibiotics.

IN VITRO ESTABLISHMENT AND CHARACTERIZATION OF A CAR-EXPRESSING CELL MODEL SYSTEM

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B-cell lymphomas are commonly treated with chemotherapy, radiotherapy, and stem cell transplantation. However, some cases are resistant to therapy, leading to poor outcomes. The introduction of CAR-T therapy has significantly improved prognosis. CAR-T cells are genetically modified T cells that express a chimeric antigen receptor (CAR), enabling them to recognize and eliminate cancer cells expressing specific surface biomarkers. CAR-T therapy is used for chemotherapy-resistant B-cell lymphomas that overexpress CD19, which is the primary CAR-T target. While highly effective, CAR-T therapy has limitations, including autologous cell origin and low viability. Developing universal CAR-T cells requires a deeper understanding of CAR signaling and cytotoxic mechanisms, which can be studied using model systems.

In this study, we used the Jurkat T-cell leukemia line, engineering it to express a CAR construct consisting of FMC63, CD8, CD28, 4-1BB, and CD3-ζ domains. The resulting J-CAR cells were co-cultured with the CD19-expressing B-cell lymphoma line SU-DHL-4 to study CAR activation.

Co-culturing the cells revealed increased cell death, prompting further investigation. We found that immune response activation and cytotoxicity depend on T-cell machinery, the CAR intracellular domain, and CD19 expression in target cells. High-throughput phosphoproteomic analysis showed activation of stress-related kinases in J-CAR cells, and inhibitor assays confirmed the necessity of Erk kinase in CAR signaling.

Further analysis of J-CAR and SU co-cultures revealed increased expression of *TNFSF10* and *GZMB* genes in activated J-CAR cells. Notably, a significant portion of dead cells in the culture were J-CAR, not SU, suggesting that while CAR activation induces cytotoxicity, J-CAR cells undergo self-cytotoxicity due to their inability to regulate cytotoxic protein expression.

An unexpected finding emerged when SU cells were co-cultured with non-cancerous hTERT-RPE1 cells. CAR-expressing R-CAR cells inhibited SU cell growth; remarkably, this process did not require CAR signaling in the effector cell. This discovery suggests a potential new approach for CAR-based therapies.

Our study highlights key mechanisms of CAR signaling and cytotoxicity, offering insights for optimizing CAR-T therapy. The model system we developed enables the identification of new therapeutic strategies and potential limitations of CAR-T cell therapy.

MICROBIOTA OF HEALTH-RELEVANT WILD BERRIES

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Lingonberries (*Vaccinium vitis-idaea* L.), rowanberries (*Sorbus aucuparia* L.), and rosehips (*Rosa canina* L.) are attractive examples of functional foods due to their high content of bioactive substances and positive effects on human health. These wild berries are rich in phytochemical bioactive substances that have a positive impact on the treatment of inflammatory and cardiovascular diseases, exhibit antioxidant, antiviral, and antibacterial effects, modulate the human gut microbiota, reduce the risk of developing diabetes and cancer, strengthen the immune system and reduce the likelihood of developing depression. Nutrition experts encourage the consumption of minimally processed berries; therefore, it is important to assess the microbiological contamination associated with them to ensure food safety.

This study presents a large-scale metagenomic analysis of bacterial and fungal communities found on lingonberries, rosehips, and rowanberries. Following DNA isolation, DNA fragments of the ITS2 rRNA gene region and the V3-V4 region of the 16S rRNA for each sample were individually amplified and subjected to high-throughput Next Generation Sequencing. Significant differences in the taxonomic composition of prokaryotic and eukaryotic microbiota were observed. It was determined that representatives of the *Enterobacteriaceae* bacterial families predominated on rosehips, *Methylobacteriaceae* – on lingonberries, and *Sphingomonadaceae* – on rowanberries. Among fungal microorganisms, Dothioraceae prevailed on rosehips and Exobasidiaceae – on lingonberries. Various fungal families were observed at similar levels on rowanberries. Molecular analysis of isolated cultivable yeasts revealed that the highest yeast diversity was distinguished on lingonberries. Our findings uncover that wild berries support unique microbial ecosystems, which may significantly contribute to their health-promoting properties. The identification of both beneficial and potentially pathogenic microorganisms highlights the importance of microbial diversity in determining the safety and nutritional value of the berries. The research was funded by the Research Council of Lithuania (LMTLT) (S-PD-22-85).

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