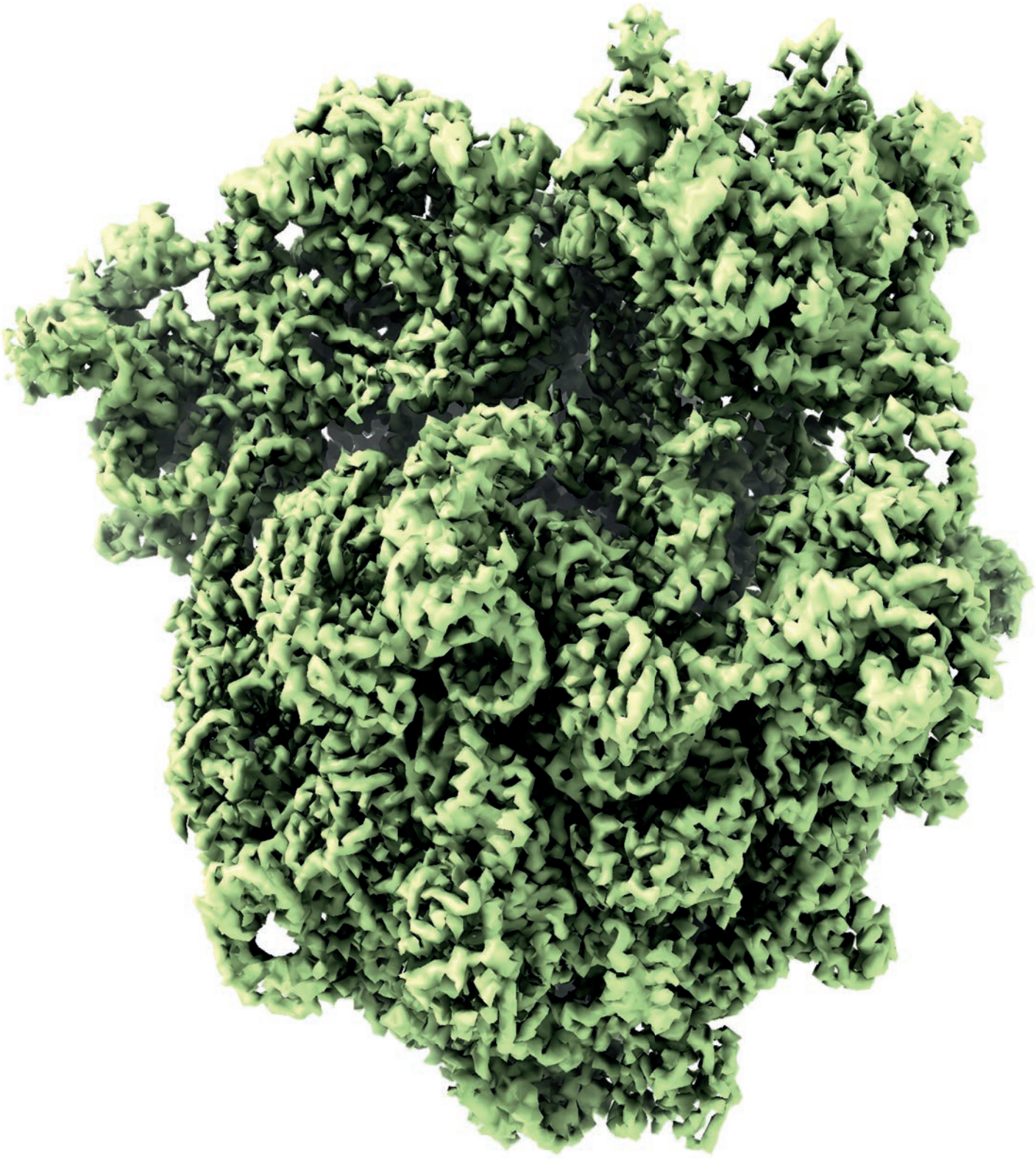
Life Sciences Center



Annual Report 2021

CONTENTS

FOREWORD

Year 2021 marked the fifth anniversary of establishment of the Life Sciences Center at Vilnius University. Even though the COVID-19 pandemic was still threatening to interfere with the academic activities a range of important academic milestones were achieved by our community at Vilnius University Life Sciences Center (VU LSC).

In 2021, our community grew up to new record heights amounting to over 1500 members (students and staff) with 290 PhD holders and 134 PhD candidates. We were excited to welcome 9 new PhD degree researchers from foreign universities who along with over 20 international graduate students chose to develop their academic careers at the VU LSC. This demonstrates a continuously increasing potential of our Center to compete for young talents internationally.

For the first time, our student admission numbers at the VU LSC surpassed 400, out of which 287 were admitted to BSc and 117 to MSc programmes. The Molecular Biotechnology programme, which we started just a year ago, exhibits a significant popularity among academic entrants making it one of three largest MSc programmes at the VU LSC. This attests to the strengthening of our academic positions not only in the field of natural sciences but also in that of technological ones.

In 2021, we continued assisting the government of Lithuania in fighting the COVID-19 pandemic. Our Temporary COVID-19 Testing Laboratory provided the Ministry of Health with numerous services. Along with testing clinical samples we developed, introduced and implemented nationwide the environmental testing of SARS-Cov-2 in the pre-school educational institutions. This helped the Ministry of Health to identify and prevent outbreaks of COVID-19 in kindergartens and elementary schools.

In 2021, we started to practically implement the agreement between the Vilnius University Life Sciences Center and the European Molecular Biology Laboratory (EMBL). The Ministry of Education, Science and Sport allocated 6 Million EUR to start the academic activities at the recently established VU LSC-EMBL Partnership Institute for Genome Editing Technologies. An international call for the group leader positions was announced and by the end of 2021, we employed 6 outstanding young researchers who joined our community bringing in new research ideas aiming at the development of genome editing tools. The inaugural meeting, which was attended by the Director General of EMBL Prof. Edith Heard, the Minister of Education, Science and Sport Dr Jurgita Šiugždinienė and Rector of Vilnius University Prof. Rimvydas Petrauskas marked the official beginning of academic life at the VU LSC-EMBL Partnership Institute. The internationalization and development of the VU LSC was highly acknowledged by the International Advisory Board, which also pointed out the increasing needs of our wider involvement in competition for international funds for research.

Year 2021 was marked with a large number of national and international awards to our scientists and students. This year we celebrated the recognition of women scientists at the VU LSC. Joana Smirnoviene was awarded the L'Oreal Baltic program *For Women in Science* fellowship to carry out research leading to the development of novel therapeutics against cancer and obesity. The parliaments of the Baltic countries in recognition of pioneering discoveries of genome editing tools awarded the Baltic Assembly Science Prize to Virginijus Šikšnys. Member of the community Gediminas Niaura was awarded the Lithuanian Science Prize. Numerous academic awards were received by our students: Tomas Šneideris and Kristina Šnipaitienė for the Best Doctoral Thesis, Giedrė Skliutė, Denis Baronas and Justina Žvirblytė for the Best Master Thesis. Two doctoral students, Lorenzo Camisi and Jonas Juozapaitis, made history by winning the Gold Medal in International Directed Evolution Competition, which was organized for the first time in 2021. The members of the Vilnius-Lithuania iGEM team won the gold medal in 2021 for the project aimed at fighting the spread of infectious amoebiasis. These and all other rewards witness solid leadership and recognition of our academic community nationally and internationally.

Year 2021 was the last full year of my appointment as the Director of the Life Sciences Center. In May 2022, the management of the Center will be taken over by Daumantas Matulis and his new administration team. At this point, I wish to thank all our academic community: professors, researchers, students and support staff for the privilege I had to lead such a great team of exceptionally talented people. The people who are committed to excellence in science and studies, the people who are dedicated to maintaining principles of academic freedom, collegiality and inclusiveness, openness to the world and non-discrimination at the VU LSC. I am confident that our community at the Life Sciences Center will continue expanding horizons of scientific knowledge, providing world-class education for talented students and professional expertise for the benefit of society and the world.

Gintaras Valinčius
Director

RESEARCH DESCRIPTIONS

Biomolecular Structure & Function

Cell & Molecular Biology

Ecosystems & Biodiversity

Molecular Biotechnologies

Molecular Mechanisms of Disease

Neuro-biology

Genome Editing Technologies

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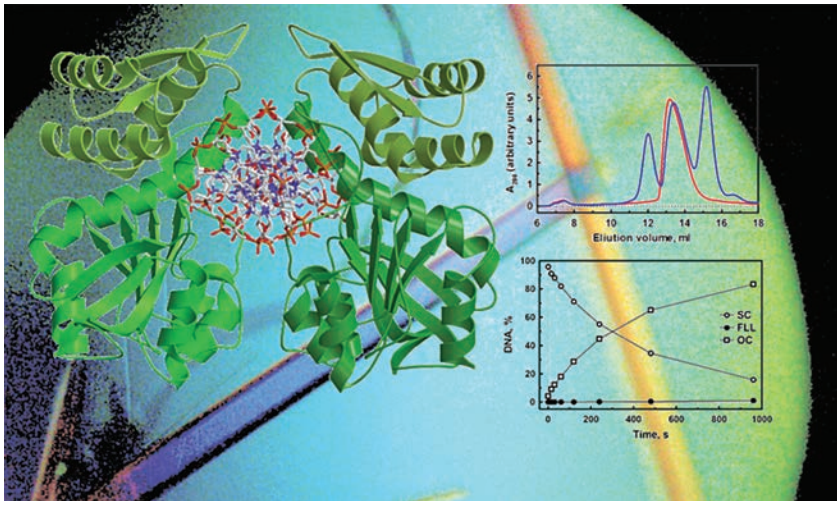

Antiviral Defense Systems in Bacteria

Phages are the most abundant organisms in the biosphere and the major parasites of bacteria. They infect bacteria in order to replicate and usually kill bacteria when the replication is completed. In response to the phage threat, bacteria developed multiple defence barriers for countering and fighting viral attacks. In the Department of Protein-Nucleic Acids Interactions we aim to understand the structure-function relationships of enzymes and enzyme assemblies that contribute to the bacteria defence systems that target invading nucleic acids. We are particularly interested in the molecular machinery involved in the CRISPR-Cas function and the structural and molecular mechanisms of other antiviral defence systems including prokaryotic Argonautes, BREX, toxin-antitoxin systems and others. We are using X-ray crystallography, mutagenesis and functional biochemical as well as biophysical assays to acquire more information on these systems.

CRISPR-Cas has been recently discovered as a prokaryotic antiviral defence system that hijacks short fragments of invasive DNA as spacers and subsequently uses them as templates to generate specific small RNA molecules that combine with Cas proteins into effector complexes that trigger the degradation of foreign nucleic acid. In this respect, CRISPR-Cas systems constitute an adaptive microbial immune system that provides an acquired resistance against invaders. CRISPR systems are very diverse, and we aim to understand the molecular and structural mechanisms of immunity provided by different CRISPR-Cas systems.

In recent years, we have focused on different aspects of CRISPR-Cas and other antiviral defence systems in bacteria, in collaboration with Dr. Kiana Aran (Keck Graduate Institute), Dr R. Seidel (Universität Leipzig), Dr J. Young (Corteva), Dr C. Venclovas (Vilnius University), Drs. K. Makarova and E. Koonin (NIH).

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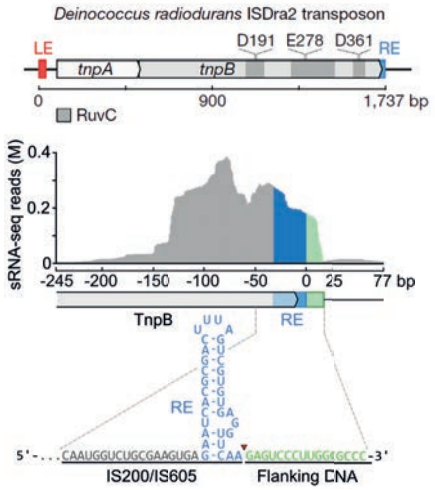
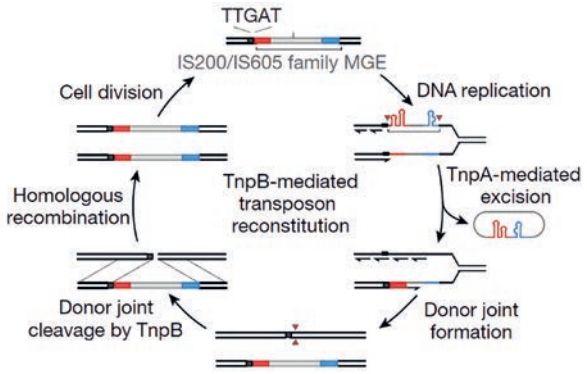
1. Karvelis, T., Druteika, G., Bigelyte, G., Budre, K., Zedaveinyte, R., Silanskas, A., Kazlauskas, D., Venclovas, Č., Siksnys, V. Transposon-associated TnpB is a programmable RNA-guided DNA endonuclease. *Nature*. 2021, 599(7886): 692–696. doi: 10.1038/s41586-021-04058-1.
2. Bigelyte, G., Young, J. K., Karvelis, T., Budre, K., Zedaveinyte, R., Djukanovic, V., Van Ginkel, E., Paulraj, S., Gasior, S., Jones, S., Feigenbutz, L., Clair, G. S., Barone, P., Bohn, J., Acharya, A., Zastrow-Hayes, G., Henkel-Heinecke, S., Silanskas, A., Seidel, R., Siksnys, V. Miniature type V-F CRISPR-Cas nucleases enable targeted DNA modification in cells. *Nat Commun.* 2021, 12(1): 6191. doi: 10.1038/s41467-021-26469-4.
3. Balderston, S., Taulbee, J. J., Celaya, E., Fung, K., Jiao, A., Smith, K., Hajian, R., Gasiunas, G., Kutanovas, S., Kim, D., Parkinson, J., Dickerson, K., Ripoll, J. J., Peytavi, R., Lu, H. W., Barron, F., Goldsmith, B. R., Collins, P. G., Conboy, I. M., Siksnys, V., Aran, K. Discrimination of single-point mutations in unamplified genomic DNA via Cas9 immobilized on a graphene field-effect transistor. *Nat Biomed Eng.* 2021, 5(7): 713–725. doi: 10.1038/s41551-021-00706-z.
4. Songailiene, I., Juozapaitis, J., Tamulaitiene, G., Ruksenaite, A., Šulčius, S., Sasnauskas, G., Venclovas, Č., Siksnys, V. HEPN-MNT Toxin-Antitoxin System: The HEPN Ribonuclease Is Neutralized by OligoAMPylation. *Mol Cell.* 2020, 80(6): 955–970.e7. doi: 10.1016/j.molcel.2020.11.034.

Transposon-Associated TnpB Is Functional Progenitor of CRISPR-Cas Nucleases

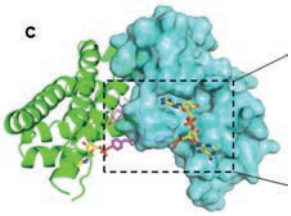
Transposition has a key role in reshaping genomes of all living organisms. Insertion sequences of IS200/IS605 and IS607 families are among the simplest mobile genetic elements and contain only the genes that are required for their transposition and its regulation. These elements encode *tnpA* transposase, which is essential for mobilization, and often carry an accessory *tnpB* gene, which is dispensable for transposition. Although the role of TnpA in transposon mobilization of IS200/IS605 is well documented, the function of TnpB has remained largely unknown. A bioinformatic analysis indicated that TnpB might be a predecessor of the CRISPR–Cas9/Cas12 nucleases. However, no biochemical activities have been ascribed to TnpB. Here we show that TnpB of *Deinococcus radiodurans* ISDra2 is an RNA-directed nuclease that is guided by an RNA, derived from the right-end element of a transposon, to cleave DNA next to the 5′-TTGAT transposon-associated motif. Together, this study expands our understanding of transposition mechanisms by highlighting the role of TnpB in transposition and experimentally confirms that TnpB is a functional progenitor of CRISPR–Cas nucleases (Karvelis et al. *Nature*. 2021, 599(7886): 692–696).



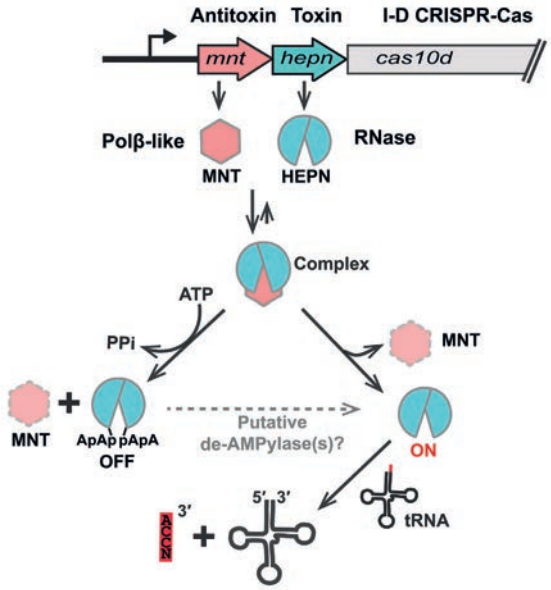


Novel Toxin-Antitoxin System Related to I-D CRISPR-Cas System

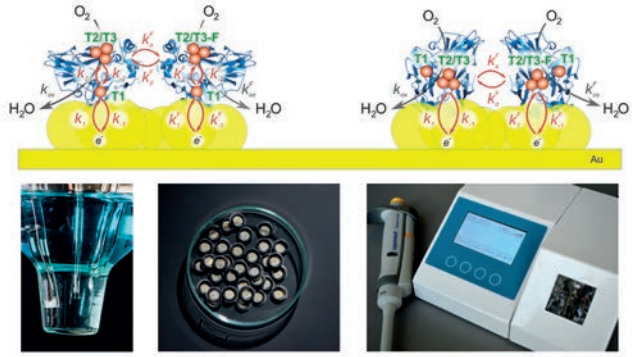
HEPN-MNT toxin-antitoxin (TA) system is encoded in the vicinity of a subtype I-D CRISPR-Cas system in the cyanobacterium *Aphanizomenon flos-aquae*. Using biochemical and structural methods, we showed that HEPN acts as a toxic RNase, which cleaves off 4 nt from the 3′ end in a subset of tRNAs, thereby interfering with translation. Surprisingly, we find that the MNT (minimal nucleotidyltransferase) antitoxin inhibits HEPN RNase through covalent di-AMPylation (diadenylylation) of a conserved tyrosine residue, Y109, in the active site loop. We propose that the HEPN-MNT system functions as a cellular ATP sensor that monitors ATP homeostasis and, at low ATP levels, releases active HEPN toxin. The I-D CRISPR-Cas system present in *A. flos-aquae* contains a putative Cas3-like ATPase-helicase; thus, the HEPN-MNT TA system could become activated due to additional ATP degradation by CRISPR-Cas3 in response to phage infection. In this case, I-D CRISPR-Cas ATPase-controlled activation of HEPN RNase in *A. flos-aquae* would be analogous to activation of the auxiliary Csm6 RNase by cyclic oligoadenylate produced by the type III CRISPR-Cas system in response to phage infection. However, the exact mechanism of the HEPN-MNT system action and its possible crosstalk with the CRISPR-Cas system remain to be established (Songailiene et al. *Mol. Cell.* 2020, S1097-2765(20) 30834-0).



Crystal structure of di_ AMPylated HEPN ribonuclease



Proposed mechanism of action of *A. flos-aquae* HEPN-MNT toxin-antitoxin system



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Bioelectrochemical Systems in Biosensors and Bioreactors

Mediated and direct electron transfer (ET) coupling of enzymes to electrodes is important in realizing bioelectrocatalysis, which is often exploited as a basic principle of biosensors, biofuel cells, and other bio-based devices. These technologies exploit the inherent enzyme substrate specificity, for example, enzyme-based biosensors excel in direct measurement of single compound in presence of interfering materials in complex media such as blood. On the other hand, if the power density generated by enzyme-based electrode is high enough, biofuel cells can be constructed, where bioelectrodes selectively oxidise and reduce abundant fuel (i.e., glucose and oxygen) and provide electric power for implantable devices. The fragile nature of proteins dictates that the electrochemical properties of such biodevices degrade over time. Therefore, several techniques are developed to protect the biomolecule and extend the working period of device. The shortcoming could be avoided whatsoever by adsorbing live, whole cells on electrodes at the expense of reduced power density.

Our team is proficient at constructing bioelectrochemical systems by wiring oxidoreductases to gold and carbon-based electrode surfaces [1, 2]. A. Laurynėnas contributed to developing *ProteinGAN*, a self-attention-based variant of the generative adversarial network that can 'learn' natural protein sequence diversity and enables the generation of functional protein sequences [3]. We are developing bioreactor systems, where wasteful saccharide substrates are selectively oxidised and high-value oxidation products are produced. For such approach, we utilize bi-enzymatic reaction with biosensor-based microprocessor-controlled substrate dispensing. To obtain a self-regulating system, the fluid dispensing and sensor devices are coordinated by an advanced algorithm embedded in microcontroller-based electronic system. All the custom components were designed and produced by our team. We are thoroughly studying the molecularly imprinted polymers based on polypyrrole and polyaniline preparations for sensor electrode application [4], the approach should help in finding new ways to discover new supramolecular systems for small biomolecule detection.

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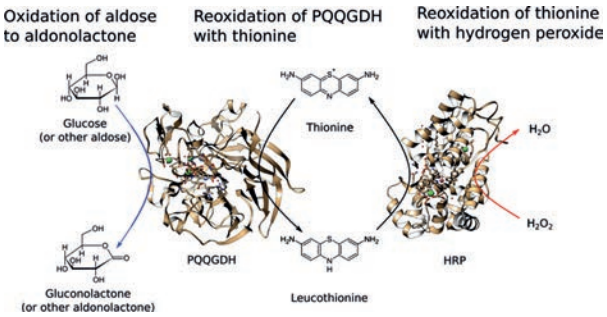
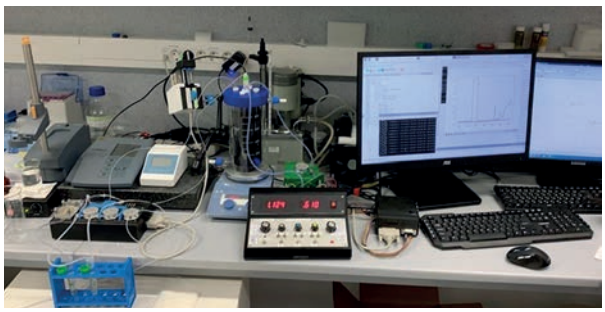
4. Bagdžiūnas, G. Theoretical design of molecularly imprinted polymers based on polyaniline and polypyrrole for detection of tryptophan. *Mol. Syst. Des. Eng.* 2020, 5: 1504.

Efficient Bi-Enzymatic Synthesis of Aldonic Acids

We have developed a new scheme for synthesis of aldonic acids based on PQQ-dependent dehydrogenase. The used enzyme has wide substrate specificity and high catalysis rate. The enzyme is dehydrogenase and reacts with oxygen very slowly. The mediators can be used instead to regenerate the oxidized form of the enzyme. In our scheme, a stable, efficient and recoverable mediator was used in the enzyme reoxidation scheme coupled with heme peroxidase or catalase. The terminal electron acceptor in such a scheme may be oxygen, but the most efficient was hydrogen peroxide. To avoid the inactivation of the enzymes with high concentrations of hydrogen peroxide, the dozing of the compound was controlled.

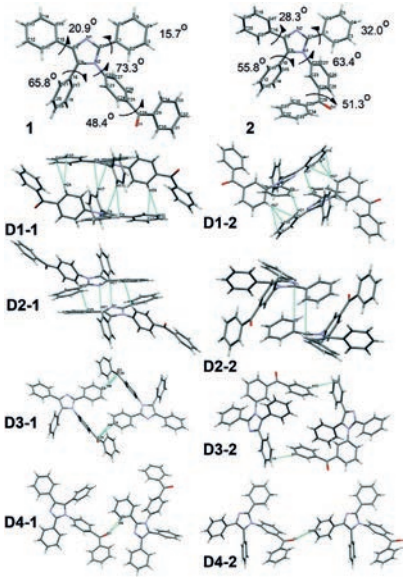
To avoid the hydrogen peroxide-induced enzyme inactivation, the addition of hydrogen peroxide to the reactor mixture was performed in very small doses by using either syringe or sensitive peristaltic pumps developed by our team. The rate of dosage was controlled by analysing the data of our custom highly-sensitive sensors' system, all combined into microcontroller-coordinated control algorithm (RCL grant No. 01.2.2-LMT-K-718-01-0019). The results are being used in preparing an EPO patent application.





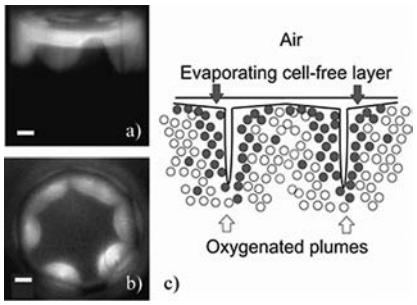
Isomeric Organic Compounds Based on Triphenylimidazole and Benzophenone

Creation of molecularly imprinted polymers (MIPs) as the supramolecular systems with tailor-made binding sites complementary to template molecules in shape, size and functional groups is our priority work for the creation of piezoelectric and electrochemical sensors. This year, we developed two new derivatives based on the triphenylimidazole and benzophenone moieties to achieve isomeric phenyl(4-(2,4,5-triphenyl-1*H*-imidazol-1-yl)phenyl)methanone and phenyl(3-(2,4,5-triphenyl-1*H*-imidazol-1-yl)phenyl)methanone *via* the Debus–Radziszewski condensation reaction from commercially available compounds. Structures of these derivatives from single-crystal analysis of these isomeric compounds were applied to improve theoretical methods for modelling of the photophysical properties and charge carrier motilities in the presence of an external electric field. The study will help in finding new ways to discover new supramolecular systems for small biomolecule detection [4] (RCL grant No. S-MIP-20-45).

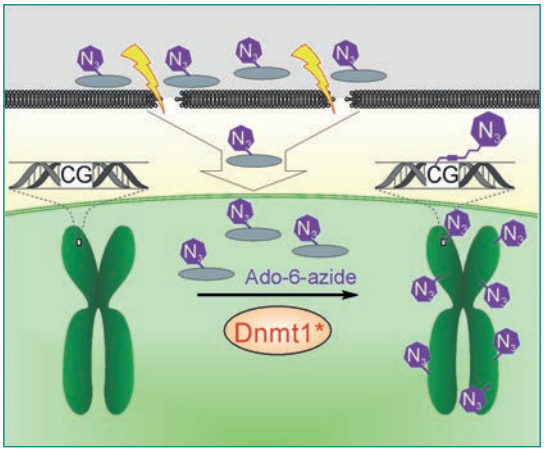


Self-Organization of Bacteria

Bioanalytical systems can be constructed by using whole-cell biosensors, where bacteria are grown on electrode surfaces. We use bioluminescence imaging to record images of liquid mixed cultures of the *lux*-gene reporter *E. coli* and other bacteria in microtiter plate wells and in vertical Hele-Shaw cells. The self-organization of *E. coli* cells, as detected by bioluminescence imaging of bacteria suspensions, can be modelled by the Keller–Segel equations of chemotaxis with logistic cell kinetics. The analysis of mutants regarding their ability to self-organize shows that the observed formation of the chemotaxis patterns is related neither to chemosensory and chemotaxis signal transduction systems nor to dynamics of flagellar motors of bacteria. Mathematical modelling indicates that the



spatio-temporal patterns in the suspensions of *E. coli* form due to phoretic interactions between oscillating cells of high metabolic activity (RCL grant No. S-MIP-17-98).



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Biological Modification of DNA and RNA

Epigenetic Modifications of DNA and RNA in Mammals

In recent years, epigenetic phenomena have become a major focus in studies of embryonic development, genomic imprinting and complex human diseases. One of the best-understood epigenetic mechanisms is enzymatic DNA methylation. In the mammalian genome, cytosines in CpG dinucleotides are often methylated to 5-methylcytosine (m5C), which is brought about by combined action of three known AdoMet-dependent DNA methyltransferases (DNMTs). DNA methylation profiles are highly variable across different genetic loci, cell types and organisms, and are dependent on age, sex, diet and disease. Besides m5C, certain genomic DNAs contain detectable amounts of 5-hydroxymethylcytosine (hmC) and lower levels of 5-formylcytosine and 5-carboxylcytosine (caC), which are produced by the oxidation of m5C residues by TET oxygenases. However, many details of how these modifications are established at specific loci and how they control cellular events remain obscure [3,5].

More than 160 chemically distinct covalent modifications have been detected across various RNA species in prokaryotic and eukaryotic cells. One of the most abundant and important RNA modifications is methylation of the 2'OH group. miRNAs, piRNAs and siRNAs are small non-coding RNA molecules that control gene activity in a homology-dependent manner. Biogenesis of miRNAs and siRNAs in plants involves a methylation step catalysed by the HEN1 methyltransferase, whereas piRNAs are similarly modified in animals [1,2].

Following our long-standing interest in mechanistic studies of DNA MTases, we turned our focus on advancing DNA and RNA modification analysis and its applications for studies of epigenetic mechanisms [4]. Our current ERC-supported studies seek to gain in-depth understanding of how the DNA methylation patterns are established by the three known DNMTs during differentiation and development. Here, our efforts are devoted to devising single-cell methodologies that permit precise determination of where and when the methylation marks are deposited by the individual DNMTs inside living cells (see Figure above).

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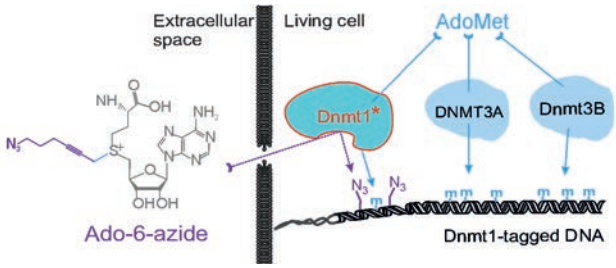
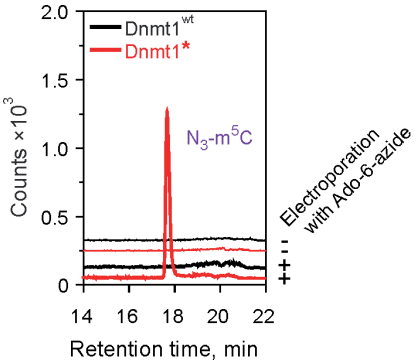
Chemical Tracking of DNMt1 Catalysis in Live Cells

DNA methylation in vertebrates is brought about by three DNA methyltransferases (DNMT1, DNMT3A and DNMT3B) whose catalytic interactions and temporal interplay in establishing genomic methylation during cell speciation are poorly understood due to limitations of available techniques. To achieve selective tracking of the catalytic contribution of an individual DNMT enzyme, we used structure-guided engineering of mouse Dnmt1 to enable selective catalytic transfer of extended bioorthogonal moieties containing a functional azide group onto DNA from a synthetic cofactor analog, Ado-6-azide, *in vitro*. We then engineered the *Dnmt1* locus in mouse embryonic stem cells (mESCs) to install the engineered codon, which, following pulse-internalization of the Ado-6-azide cofactor by electroporation, permitted selective azide-tagging of Dnmt1-specific genomic targets *in cellula*. The deposited azide groups were exploited as "click" handles for reading adjoining sequences and precise mapping of the chemically tagged methylation sites in the genome. Altogether, we demonstrate that this new general approach, Dnmt1-TOP-seq, enables selective high-resolution temporal tracking of the Dnmt1 catalysis in live mammalian cells paving the way to such studies of other biologically important Ado-Met-dependent methyltransferases in a wide range of eukaryotic systems (Stankevičius et al. *Mol. Cell*, doi: 10.1016/j.molcel.2022.02.008).



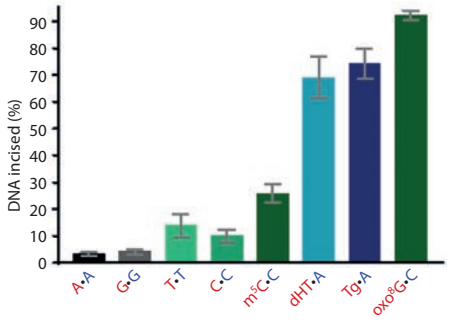


Top – strategy for selective Dnmt1-directed bioorthogonal pulse-tagging of endogenous DNA targets in a living cell; m, methylated cytosine

Bottom – HPLC-MS/MS analysis of genomic cytosine modification in Dnmt1^{wt} and Dnmt1* mESCs after electroporation with Ado-6-azide cofactor analogue

Alleviation of C:C Mismatches in DNA by the *Escherichia coli* Fpg Protein

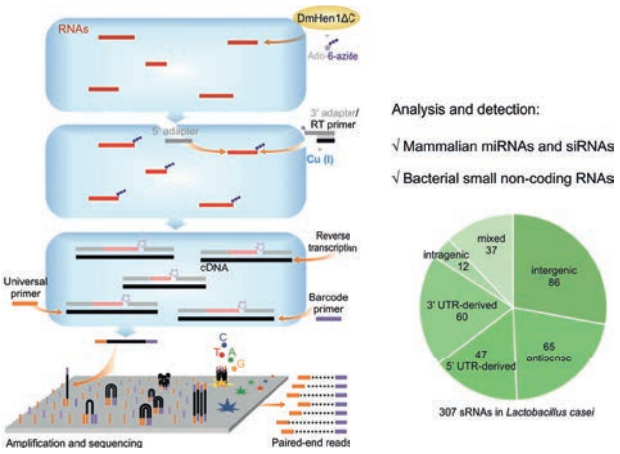
DNA polymerase mis-insertions, if not corrected by proofreading or the mismatch repair (MMR) function, may result in all possible non-cognate base pairs in DNA generating base substitutions. The most thermodynamically unstable base pair, the C:C mismatch, destabilizes adjacent base pairs, is resistant to correction by MMR in *E. coli*, and its repair mechanism remains elusive. In a joint effort with the group of Svein Bjelland at University of Stavanger, Norway, we present here *in vitro* evidence that C:C mismatches can be processed by base excision repair initiated by the *E. coli* formamidopyrimidine-DNA glycosylase (Fpg) protein. The catalytic rate at C:C is 2.5–10 fold slower than for its primary substrate 8-oxoguanine (8-oxoG:C), but approaches those for 5,6-dihydrothymine dHT:C and thymine glycol (Tg:C). We hypothesize that Fpg plays a role in resolving C:C in particular, but also other pyrimidine:pyrimidine mismatches, which increase survival at the cost of some mutagenesis (Tesfahun et al. Front. *Microbiol.* 2021, 12: 608839).



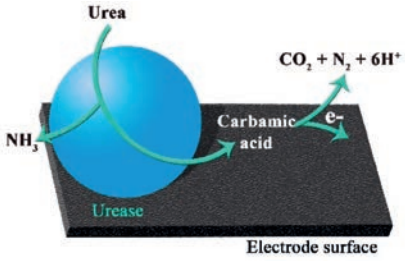
Fpg-directed incision at unmethylated and methylated cytosine when placed opposite C and T in DNA as compared to other mismatches in DNA

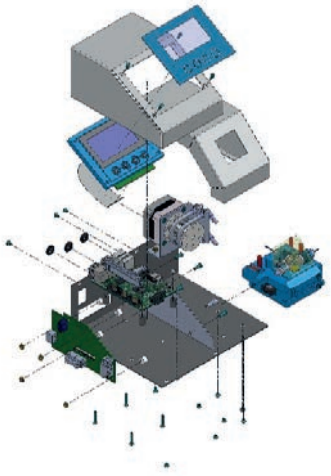
Methyltransferase-Directed Orthogonal Tagging and Sequencing of miRNAs and Bacterial Small RNAs

Targeted installation of chemical tags on biopolymers provides an orthogonal means for their visualisation, manipulation and sequence analysis. Although high-throughput RNA sequencing is a widely used method for transcriptome analysis, certain steps, such as 3′ adapter ligation, remain challenging due to biases of RNA ligases. Here, we remedy this limitation by adapting DmHen1 RNA 2′-O-methyltransferase for chemo-enzymatic click tethering of a 3′ sequencing adapter that supports cDNA production by reverse transcription of the tagged RNA. Using this new approach, we identified miRNAs and prokaryotic small non-coding sRNAs in probiotic *Lactobacillus casei*. We found that methyltransferase-Directed Orthogonal Tagging and RNA sequencing, mDOT-seq, avoids misdetection of unspecific RNA species, thus providing a better accuracy and advancing analysis of eukaryotic and prokaryotic ssRNAs (Mickutė et al, *BMC Biology*, 2021, 19: 129; EP3271478B1, US11008605B2).



Left – schematic of the mDOT-seq analytical procedure
Right – genomic distribution of ssRNAs identified in *Lactobacillus casei*





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Electrochemical Biosensors for Real-World Applications

Biosensors are handy devices, which can rapidly detect and measure a variety of specific compounds. Those devices can significantly improve the quality of life for patients suffering from a variety of diseases, by helping the medical personnel to diagnose diseases faster and more accurately, as well as help to evaluate other significant factors. However, to develop a biosensor operating with adequate performance for real-world applications is a tedious task. Real media samples are complex, making an accurate detection difficult. For example, human blood, the most clinically relevant sample, is composed of thousands of different compounds with unique properties, a variety of blood cells and countless number of proteins. To discern a single type of molecule from this entire composition, a biosensor should be specifically designed and engineered. Typically, the surface of the electrochemical biosensor is covered with compound-specific enzyme, unique membranes are designed to reject the interfering compounds, complex electronics and mathematical analysis models are used to increase the signal-to-noise ratio. Our department designs such biosensors capable of analysing various peculiar media obtained from nature. The significance of some of analytes we are interested in are not yet realized, e.g., glutamate concentration in mice brain media or release of glucose as a stress factor in fish tanks.

Our department has been working with the development of biosensors for real-world applications involving devices for clinical practice, accumulating sizeable competencies in this field. In recent works, we have demonstrated a new type of nanomaterial-based glucose biosensors, which could operate with high precision and accuracy in clinically relevant fluids [1]. This type of biosensors could be miniaturized and implanted in patients' bodies and be used for real-time glucose monitoring of glucose. We have solved the long-standing question, as we carried out an in-detail study of catalytic properties of citrate-capped gold nanoparticles and protein conjugates and showed that nanoparticles do not increase catalytic activity of enzymes [2]. We continued developing biosensors for low-level glucose measurements for monitoring the acclimation of juvenile fish kept in tanks [3]. Clinically relevant projects in our department are carried out in cooperation with VU Hospital *Santaros klinikos* and are related to the diagnosis and prognosis of clinical outcome of patients undergoing renal replacement therapy and with certain conditions, e.g., acute pancreatitis.

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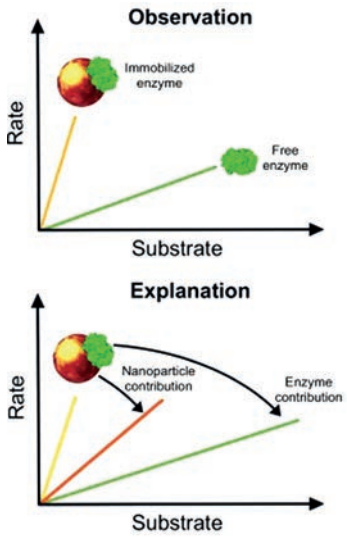
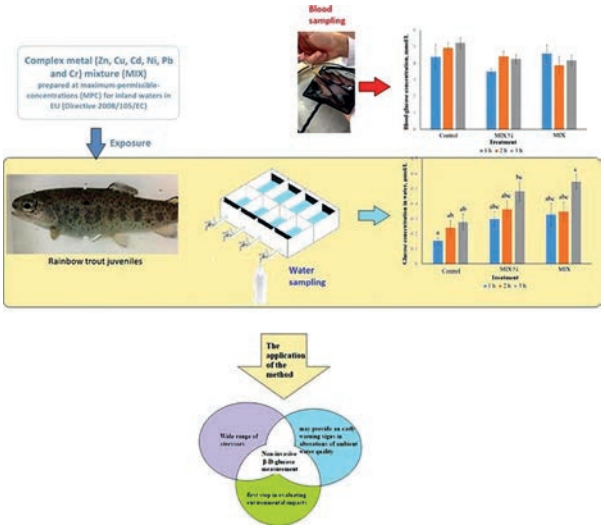
Catalytic "Acceleration" of Enzymes on Gold Nanoparticles

Many papers reported catalytic "acceleration" of enzymes, when immobilized on gold nanoparticles. Gold nanoparticles are often considered as an inert and safe nanomaterial, and are widely used for various purposes, e.g., experiments with humans are being conducted *in vivo*. We have carried out an in-detail study of catalytic properties of citrate-capped gold nanoparticles and gold nanoparticle-protein conjugates using three model proteins – enzymes glucose oxidase and catalase, and catalytically inactive protein bovine serum albumin.
Our research demonstrated that protein/enzyme-AuNP conjugates are complex catalytic systems. AuNPs are not "inert" nanomaterial and should not be considered as such – AuNP can catalyse a variety of chemical reactions with rates, which could be comparable or even exceed enzymatic catalysis. Experimental results demonstrating atypical catalytic performance of enzymes on nanoparticles should be interpreted with additional care, and a widely propagated view of "inert gold nanoparticles" should probably be reconsidered [2].



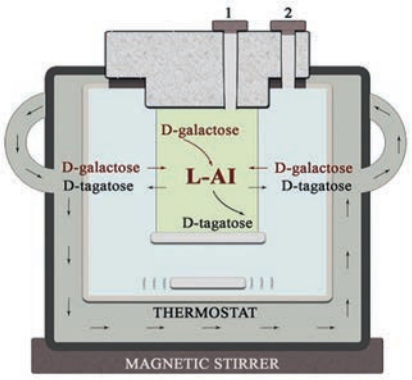
Biosensors for Fish Stress Level Control

Recently, our long-lasting glucose biosensor technology has been upgraded for measuring nanomolar concentrations of glucose in fish tanks. This has been proven statistically relevant in determining levels of stress experienced by rainbow trout juveniles. The developed biosensor has proved useful in investigation of acclimation duration (up to 4 h) on behavioural characteristics of taxonomically and functionally different fish species (trout, salmon, etc.) [3]. In future, glucose measurement in water using an appropriate biosensor could be a useful tool for assessing environmental risk for assessing different contaminant exposure and effects (RCL grant No. 09.3.3-LMT-K-712-19-0110).



Tagatose Biosensors Based on Immobilized Fructose Dehydrogenase

Thermally reduced graphene oxide (TRGO) nanoparticles were used as a basis for design of bioelectrocatalytic systems for reliable D-tagatose monitoring in a long-acting bioreactor where the valuable sweetener D-tagatose was enzymatically produced from a dairy by-product D-galactose [4]. For this goal, D-fructose dehydrogenase (FDH) from *Gluconobacter industrius* was immobilized on these electrode nanomaterials by forming three amperometric biosensors: AuNPs coated with 4-mercaptobenzoic acid (AuNP/4-MBA/FDH) or AuNPs coated with 4-aminothiophenol (AuNP/PATP/FDH) monolayer, and a layer of TRGO on graphite (TRGO/FDH) were created. Operational stability of the biosensors indicated that detection of D-tagatose could be performed during six hours without loss of sensitivity (European Regional Development Fund Grant No. 01.2.2-CPVA-K-703-03-0010).



This study extends the utilization of TRGO and carbon-based biosensor electrode applications, which the group usually utilizes for urea measurement in urine and in spent dialysate, as a tool for evaluation of dialysis adequacy (RCL grant No. 01.2.2-LMT-K-718-01-0025).

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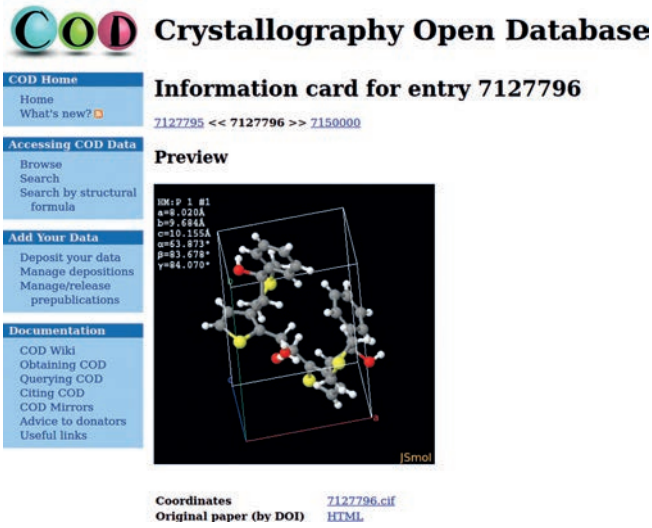

Crystallography and Molecular Modelling

Modelling matter at atomic level is important for structural biology, material science, physics and (bio)chemistry. These methods become increasingly important with the growth of available computing power, availability of large amounts of high quality, machine-readable computer data and advent of new methods such as machine learning. Our approach to molecular modelling consists of organizing available data into well-defined, curated machine-readable open databases, and then using these databases for scientific inferences applying thoroughly documented, reproducible computation procedures.

The main collection of data that we maintain is the Crystallography Open Database (COD). Over 15 years of development, the COD supervised by the international Advisory Board (of which S. Gražulis and A. Merkys are members) was transformed into the world's largest open access small molecule crystal data collection. Containing currently close to half a million records, the COD is widely used by researchers worldwide (the two seminal publications together attracted over 1000 citations), and form basis for extracting scientific knowledge from measurement data. This collection is augmented by well-established databases such as PDB, PubChem, ChEMBL and others.

To perform reproducible computations, our group develops and maintains software tools that are capable of utilizing the Crystallographic Information Framework. These tools are routinely used to ensure the syntactic and semantic validity of data in the COD as well as other projects. Our group also routinely collaborates with the International Union of Crystallography and has contributed to the development of the CIF2 file format and the DDLm dictionary definition language.

Current project is "Chemical annotation in the Crystallography Open Database (COD)", 2020–2022 (S-MIP-20-21, project leader – Dr A. Merkys).

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Chemical Annotation of Crystallographic Data

Crystallographic data is not immediately usable for chemistry, as X-ray crystallography does not detect atomic charges, bond types or the presence of lone electrons in radicals. All such information needs to be inferred either manually or using heuristics implemented as computer programs. Crystallographic reports usually are accompanied by additional chemical information – systematic chemical names or connectivity details – albeit mostly in forms not suited for automated overlay. Nevertheless, graph-based algorithms could be employed to establish the missing linkage between crystallographic data and chemical annotations (project leader – A. Merkys).

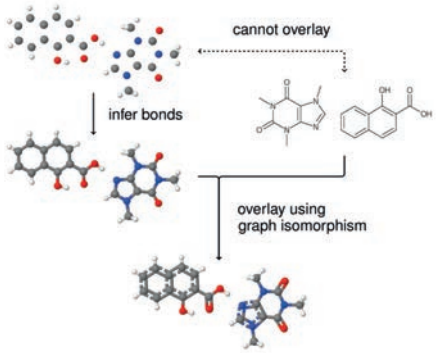


Fig. 1. Scheme of the automated pipeline used to annotate crystallographic data with chemical attributes.

Derivation of Chemical Information from Crystallographic Data

The emergence of new interdisciplinary fields has stipulated the need to establish a greater connectivity between scientific data from different research areas. One strategy of relating crystallographic data to other fields such as chemistry or material science relies on generating chemical descriptors of molecules from their crystallographic structures and using these descriptors to identify the chemical compounds that the crystals encompass. To facilitate the cross-linking of the COD with other open resources, our group has developed an automated pipeline capable of extrapolating chemical data such as atom connectivity, bond orders and atom charges from crystallographic models, thus enabling the generation of chemical descriptors, which were later used to link a large portion of the COD to the PubChem database. The derived chemical data can also be retrieved directly from the COD in various well-known cheminformatics file formats (project leader – A. Vaitkus).

Fig. 2. Chemical information derived from the COD can be conveniently explored and analysed using the DataWarrior open source cheminformatics program.

Molecular Geometries in Macromolecular Structures

Identifying the probable positions of the protein side-chains is one of the protein modelling steps that can improve the prediction of protein-ligand, protein-protein interactions. In our research, we are trying to approach rotamer library generation problem by scanning for side-chain conformations and calculating potential energy values instead of pooling occurrences of angles only from the structural data (PDB). This ability to capture unobserved angles in the structural data was implemented in rotag software. By comparing our method to the already existing rotamer libraries, it was shown that the method produces more diverse and accurate distributions of side-chain dihedral angles (project leader – A. Grybauskas).

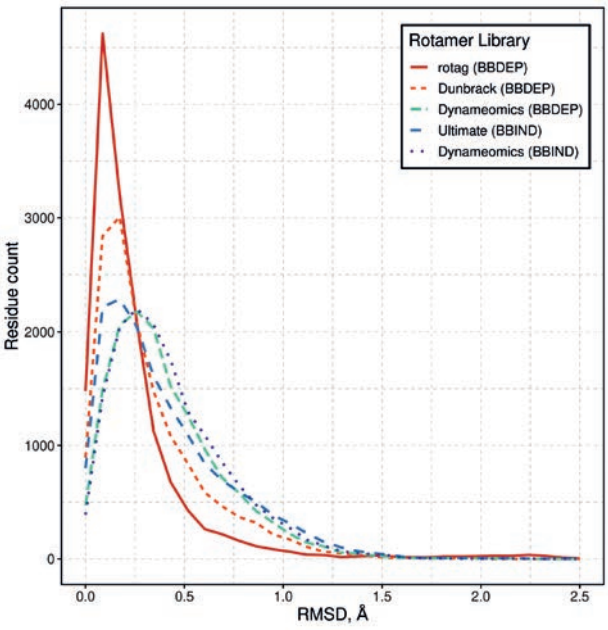
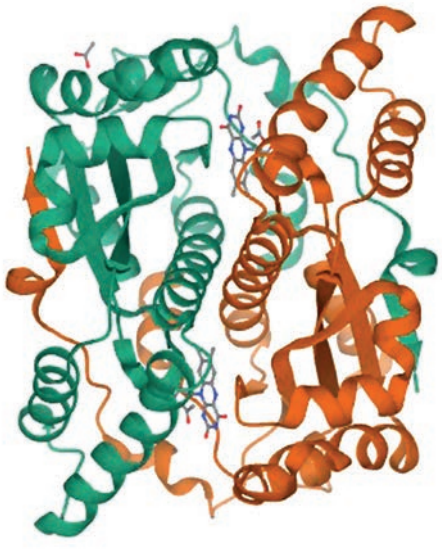


Fig. 3. The best-case RMSD distributions of rotamer libraries compared to the experimental structural data.



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Mechanisms of Flavoenzyme Redox Reactions

Flavoenzymes containing flavinmononucleotide (FMN) or flavinadenindinucleotide (FAD) in their active centres, are able to transform single-electron transfer into a two-electron one. They play important roles in biological oxidation-reduction, hydroxylation, transhydrogenation, antioxidant protection and redox signalling, and participate in biodegradation of toxic environmental pollutants and manifestation or neutralization of therapeutic activity/cytotoxicity of drugs or xenobiotics. Frequently, flavoenzymes are considered as drug targets. Taken together, these factors foster the permanent interest in the studies of flavoenzyme catalysis and its application in biomedicine, industries, and environmental protection. During last two decades, our studies were concentrated on the following issues: 1) the mechanisms of electron/hydride transfer in catalysis of flavoenzymes electrontransferases and transhydrogenases; 2) single- and two-electron reduction of quinones, nitroaromatics and other redox active organic compounds by mammalian, microbial or parasite flavoenzymes and their impact on their cytotoxicity. These studies were accompanied by intensive synthesis of above compounds; and 3) studies of prooxidant xenobiotics as inhibitors and subversive substrates for antioxidant mammalian or parasite FAD/SS and FAD/SS/SeS-containing enzymes.

Our main activities in 2018–2021 were as follows: a) characterization of interaction mechanism of quinones, nitroaromatics and aromatic N-oxides with possible target enzymes in bacteria (*Rps. palustris* ferredoxin:NADP+ oxidoreductase, collaboration with Dr D. Seo, Kanazawa University, Japan), mammalian cells (neuronal NO synthase, collaboration with Dr J.-L. Boucher, Université Paris Descartes, France), and parasites (*Plasmodium falciparum* ferredoxin:NADP+ oxidoreductase, collaboration with Dr A. Aliverti, Universitá degli Studi di Milano, Italy); b) characterization of the mechanisms of two-electron reduction of quinones and nitroaromatics compounds by *Plasmodium falciparum* NADH:ubiquinone reductase-2 (collaboration with Dr E. Davioud-Charvet, Universite de Strasbourg, France), and by *E. coli* nitroreductase-A (collaboration with Dr D. F. Ackerley, Victoria University of Wellington, New Zealand); c) evaluation of nitroaromatic compounds as inhibitors for *Trypanosoma congolense* trypanothione reductase in the context of development of antitrypanosomal agents (collaboration with Dr M. A. Comini, Institut Pasteur Montevideo, Uruguay); d) continuation of synthesis, quantum mechanical, electrochemical, enzymatic and cytotoxicity studies of new polynitrobenzenes, nitrofurans, nitrothiophenes, and aromatic N-oxides (EU Structural Funds, Global Grant Measure, Grant No. 09.3.3-LMT-K-712-01-0058, 2018–2021). In 2021, we synthesized a number of nitroaroamatic compounds with bioreductively activating leaving groups and mitochondriotropic substituents. With an aim of repurposing of nitroaromatic drugs, we examined enzymatic reactions and mammalian cell cytotoxicity of nitroaromatic antiandrogens and antiparasitic drugs. We were continuing the studies of a potent antiplasmodial agent, 1,4-naphthoquinone plasmodione, and its derivatives (Lithuanian-French Programme "Gilibert" , No. S-LZ-19-4).

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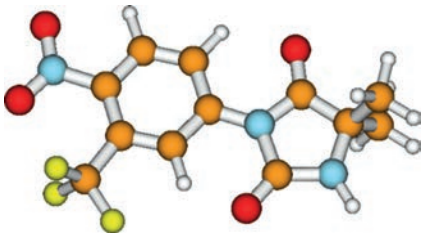
Role of Redox Cycling in Side-Action of Nitroaromatic Antiandrogens

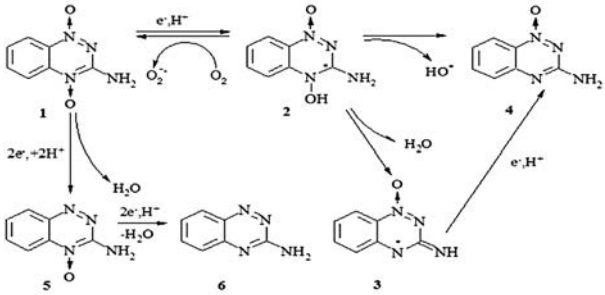
The studies of the single-electron reduction of nitroaromatic antiandrogens nilutamide, flutamide and their derivatives (n=5) by NADPH:cytochrome P-450 reductase (P-450R) and adrenodoxin reductase/adrenodoxin complex (ADR/ADX) revealed that their single-electron reduction potentials (E^1_7) vary between -0.38 V and -0.41 V. These values correlated with the heats of formation of their free radicals calculated by quantum mechanical methods. The studies of MH22a murine hepatoma cells demonstrated the prooxidant character of cytotoxicity of above compounds, which correlated with that of model nitroaromatics possessing similar E^1_7 and log D values (Nemeikaitė-Čėnienė et al. *Free Rad. Res.* 2021, 55: 246–254).



Structure of nilutamide showing the 370 torsion angle of nitrogroup (generated by Molden)

Enzymatic Redox Reactions and Cytotoxicity of Aromatic *N*-oxides

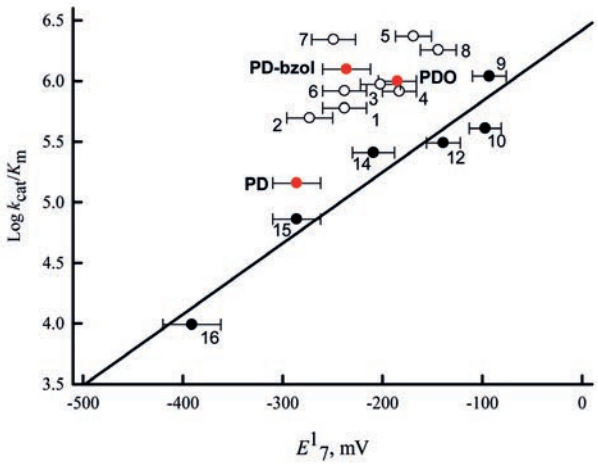
3-Amino-1,2,4-benzotriazine-1,4-dioxide (tirapazamine, TPZ) is a hypoxia-selective anticancer agent. However, some of its derivatives are strongly toxic to oxic cells as well. We found that the aerobic cytotoxicity of a number of TPZ derivatives is well above that of quinones with similar E^1_7 values, although their reactivity towards single-electron transferring flavoenzymes is similar. This was attributed to the potentiation of cytotoxicity of TPZ derivatives by cytochromes P-450 and NAD(P)H: quinone oxidoreductase (NQO1). The contribution of NQO1, which reduces TPZ derivatives in a mixed single- and two-electron way, is statistically significant (Nemeikaitė-Čėnienė et al. *Int. J. Molec. Sci.* 2019, 20: 4602; *Int. J. Molec. Sci.* 2020, 21: 8574). We also derived a 6-fold resistant to TPZ subline of MH22a cells with a 5-3-fold increased amount of antioxidant enzymes aldehyde dehydrogenase and carbonyl reductase.



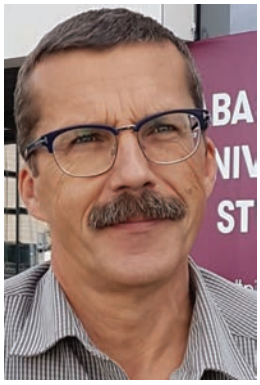
Scheme of single-electron reduction, redox cycling, and formation of metabolites of tirapazamine (1)

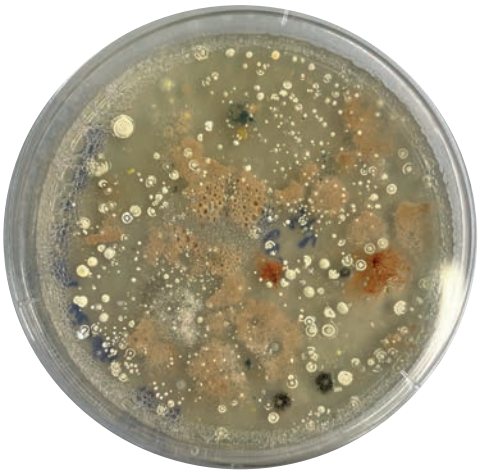
Single-Electron Reduction Potentials and Enzymatic Reactions of Plasmodione Derivatives

Plasmodione (3-[4′-(trifluoromethyl)benzyl]-2-methyl-1,4-naphthoquinone) is a potent antiplasmodial agent, whose activity is partly related to its single-electron enzymatic reduction and redox cycling. We determined the E^1_7 values of plasmodione and its derivatives (n=11). Their reactivity towards single-electron transferring mammalian flavoenzymes P-450R, ADR/ADX and NO synthase followed the same dependence on E^1_7 as that of model quinones. However, *P. falciparum* ferredoxin:NADP+ oxidoreductase (PfFNR) was specific towards plasmodione derivatives which was attributed to their higher lipophilicity (Cichocki et al. *ACS Infect. Dis.* 2021, 7: 1996–2012).



The reduction rate constants of plasmodione derivatives (red and blank circles) and model quinones (solid circles) by *Pf*FNR





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Microbial Diversity as a Source of New Biocatalysts

Modern biotechnology is based on the application of enzymes derived predominantly from microorganisms. Both genetic and biochemical microbial diversity is an immense source of different proteins and biocatalysts. The analysis and exploration of said diversity is one of the main aims of our group. The studies are concentrated on several fields. The first one is related to the isolation of N-heterocyclic compound-utilizing microorganisms and the investigation of the catabolic pathways of these compounds in individual bacteria. Unique oxidoreductases active towards various aromatic compounds have been characterized, genetically modified and applied for development of biocatalytic processes [1, 2]. Screening for novel enzymes and construction of desired biocatalysts are also carried out by applying metagenomic techniques combined with the selection systems and bioinformatics, or by using the deep-learning approaches [3, 4].

Various differently modified nucleotides play a crucial role in various biological processes. Also, the modified nucleotides are used as promising building blocks for programmable changes of nucleic acids. The biosynthetic pathways of many modified nucleotides including various thionucleotides are well understood, but the catabolism or salvage of those compounds are only scarcely studied. For the first time, the structural features of the TudS enzyme have been identified and a mechanism of desulfuration of thiouracils and thiouridines has been proposed [5].

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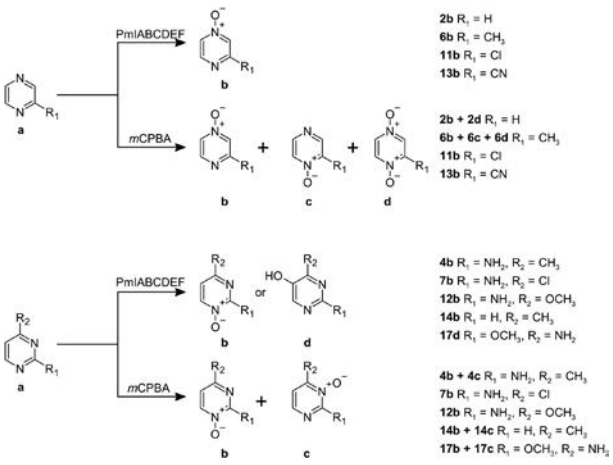
Regioselective Biocatalytic Synthesis of Aromatic *N*-oxides by Using Soluble Di-iron Monooxygenase PmlABCDEF Produced in *Pseudomonas* Species

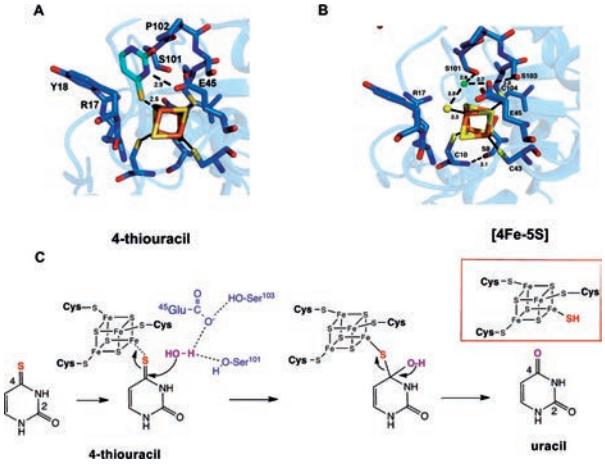
The most common methods for aromatic *N*-oxides (ArN→O) synthesis such as oxidation with *meta*-chloroperoxybenzoic acid (mCPBA) or hydrogen peroxide are hazardous and possess some serious limitations. In this study, we describe a new whole-cell biocatalysis system for ArN→O synthesis based on production of soluble di-iron monooxygenase PmlABCDEF. By substituting *E. coli* host cells with certain *Pseudomonas* strains, we simplified the biotransformation procedure and vastly increased the productivity. Various pyridine, pyrazine and pyrimidine derivatives could be converted into appropriate *N*-oxides on a preparative scale in the simple shake-flask cultivation. This improved synthesis method was used to yield specific pyrazine and pyrimidine oxidation products that were difficult to obtain by employing a standard mCPBA-based technique. (Petkevičius et al. *Microb. Biotechnol.* 2021, 14: 1771).



Representation of the synthesis pathways for pyrazine and pyrimidine *N*-oxides utilizing different catalysts
The upper arrow presents biocatalysis method based on production of soluble di-iron monooxygenase PmlABCDEF in *Pseudomonas* strains, while the lower arrow displays oxidation using mCPBA

TudS Is [4Fe-4S]-Containing Enzyme that Converts 2-Thiouracil and 4-Thiouracil into Uracil

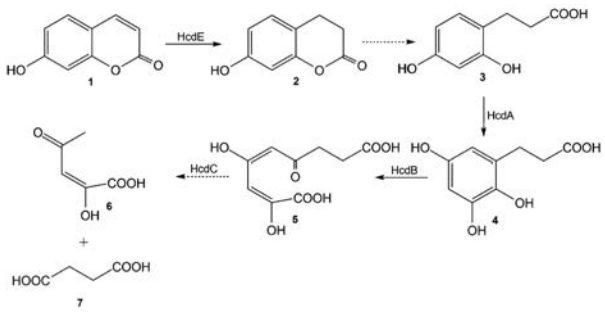
The TudS protein catalyses efficient desulphuration of thiouracil to uracil and has been identified following the recent discovery of novel genes encoding proteins from the Domain of Unknown function 523 (DUF523) family. By using a high-resolution X-ray crystallography, it has been shown that TudS is a [4Fe-4S]-containing enzyme that promotes the direct transfer of a sulphur atom from its sulphurated substrate to the [4Fe-4S] cluster forming a [4Fe-5S] cluster. A mechanism for its formation has been proposed. The present work provides a strong support to a novel function of iron-sulphur clusters in biology, namely as sulphur transfer agents. (Zhou et al. Angew. *Chem. Int. Ed.* 2021, 60: 424).



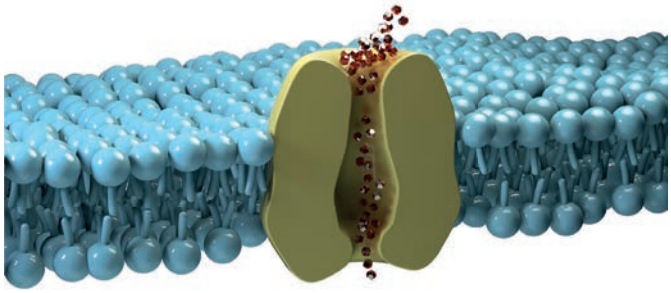
Proposed reaction mechanism for 4-thiouracil desulphuration by TudS involving the formation of a [4Fe-5S] intermediate
A – model of the 4-thiouracil/TudS complex; B – crystal structure of the [4Fe-5S] intermediate; C – proposed catalytic mechanism of TudS

First Step of Biodegradation of 7-Hydroxycoumarin in *Pseudomonas mandelii* 7HK4 Depends on Alcohol Dehydrogenase-Type Enzyme

Coumarins are well known secondary metabolites widely found in various plants. However, the degradation of these compounds in the environment has not been studied in detail, and, especially, the initial stages of the catabolic pathways of coumarins are not fully understood. A soil isolate *Ps. mandelii* 7HK4 is able to degrade 7-hydroxycoumarin via the formation of 3-(2,4-dihydroxyphenyl)propionic acid. To elucidate an upper pathway of the catabolism of 7-hydroxycoumarin, 7-hydroxycoumarin-inducible genes *hcdD*, *hcdE*, *hcdF*, and *hcdG* were identified by RT-qPCR analysis. The DNA fragment encoding a putative alcohol dehydrogenase HcdE was cloned, and the recombinant protein catalysed the NADPH-dependent reduction of 7-hydroxycoumarin both *in vivo* and *in vitro*. Thus, in contrast to the well-known fact that the ene-reductases usually participate in the reduction of the double bond, an alcohol dehydrogenase catalysing such a reaction has been identified (Krikštaponis et al. *Int. J. Mol. Sci.* 2021, 22: 1552).



The proposed catabolic pathway of 7-hydroxycoumarin in *Pseudomonas mandelii* 7HK4 bacteria
(**1**) 7-hydroxycoumarin; (**2**) 7-hydroxy-3,4-dihydrocoumarin; (**3**) 3-(2,4-dihydroxyphenyl)propionic acid; (**4**) 3-(2,3,5-trihydroxyphenyl)propionic acid; (**5**) (2*E*,4*E*)-2,4-dihydroxy-6-oxonona-2,4-dienedioic acid; (**6**) (*E*)-2-hydroxy-4-oxopent-2-enoic acid; (**7**) succinic acid; HcdA – 3-(2,4-dihydroxyphenyl) propionic acid 1-monooxygenase; HcdB – 3-(2,3,5-trihydroxyphenyl)propionic acid 1,2-dioxygenase; HcdC – putative (2*E*,4*E*)-2,4-dihydroxy-6-oxonona-2,4-dienedioic acid hydrolase; HcdE – 7-hydroxycoumarin reductase. The dashed arrows indicate the hypothetical reactions



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Protein Structure and Interactions in Phospholipid Membranes

The molecular organisation and function of biological membranes are essential to understanding of living processes in general and the development of various biotechnological processes including molecular medicine in particular. Membrane proteins (MPs) represent almost 60% of pharmaceutical targets. However, despite their fundamental role, only 2% of the protein of known structure are that of MPs, and such lack of knowledge seriously affects understanding of the membrane protein functions slowing down the development of new diagnostic tools and therapies. The major difficulties and challenges for structural and functional studies of MP's arises from their instability outside a lipid bilayer environment, where specific hydrophobic and other molecular forces keep the protein in its native and active conformational state. Therefore, considerable efforts are directed towards the development of simplified but biologically relevant model membrane systems to study molecular processes in membranes.

Our group is specializing in the development of tethered bilayer membrane (tBLM) models. tBLMs are solid-supported phospholipid bilayers anchored to a surface via hydrophobic interactions between the molecular anchors and hydrophobic sheet of the membrane. The molecular anchors are synthetic thiolipids or silanes covalently attached to a metal or metal-oxide surfaces. The anchors may contain hydrophilic fragments separating thiol/silane group and the glycerol backbone of the lipid, thus ensuring 1–2 nm thick water-reservoir between tethered bilayer and solid support. Alternatively, bilayers with no water sub-phase can be engineered. Recently, we developed an affordable and reproducible methodology for tBLM assembly using multilamellar vesicle fusion. We showed that such tBLMs are capable of reconstituting transmembrane proteins retaining their biological function. Membrane reconstituted proteins (peptides, oligomers) may be probed by the surface specific techniques, including surface plasmon resonance, vibrational spectroscopies and atomic force microscopy. Fine structural details revealing the molecular geometry of tBLMs are evaluated by the neutron reflectometry. Functional properties membranes with reconstituted protein complexes are accessible by the electrochemical impedance spectroscopy (EIS). The theoretical framework of EIS developed in our group allows a detailed analysis of protein membrane interactions as well as applications of tBLMs for bioanalysis.

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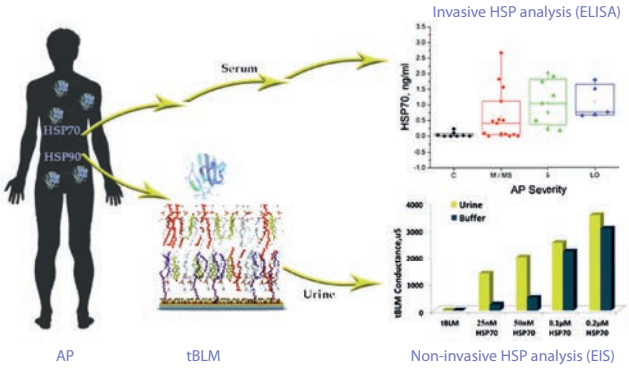
Tethered Lipid Membranes for Non-Invasive Bioanalysis of Acute Pancreatitis

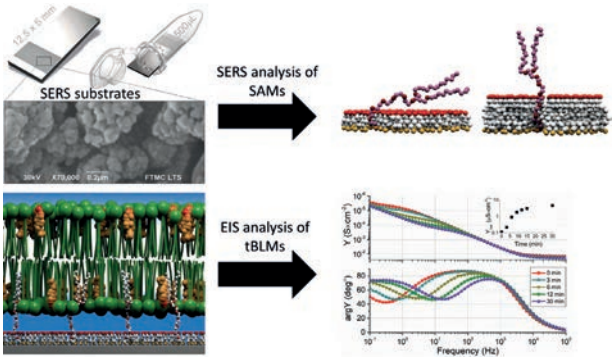
When cells undergo stress in Acute Pancreatitis (AP), heat shock proteins (HSPs) response is activated. Our multidisciplinary study shows that the heat shock proteins HSP90 and HSP70 are expressed during the development of AP, which affects the course of AP. The understanding of the disease development mechanisms involving HSPs interaction with cell membranes may provide new clues for the clinical prevention and therapy solutions in AP. Tethered bilayer lipid membranes (tBLMs) have been developed to investigate HSP's interaction with membrane that can be probed by electrochemical impedance spectroscopy (EIS). The results revealed that HSP70 and HSP90 interact with the membrane via different mechanisms: HSP70 shows the damage of the membrane, while HSP90 increases the insulation properties of tBLM. Herein, we are presenting an alternative, simple electrochemical technique without any immunoprobes for monitoring HSPs action on tBLM. This project



is a part of collaboration with Dr Julija Razumienė's group from the Institute of Biochemistry at the Life Science Center of Vilnius University. It was published in Budvytyte et al. *Biomedicines.* 2021, 9(7): 755.

Tethered Bilayer Lipid Membranes on Silver Substrates

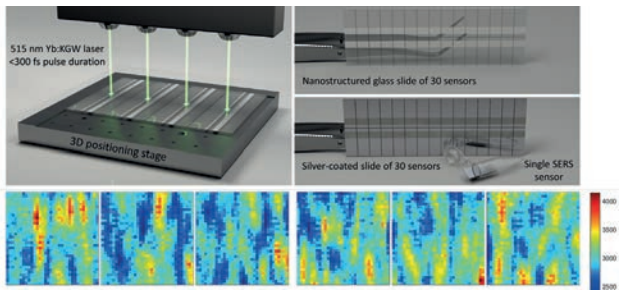
The assembly of functional tBLMs on silver substrates was accomplished for the first time. The suitability of mixed-component self-assembled monolayers (SAMs) for tBLM formation was assessed by electrochemical impedance spectroscopy (EIS) and vibrational spectroscopy. We found that using different-length backfiller molecules it is possible to control the orientation of anchor molecules WC14 on the surface, thus ensuring control of the properties of tethered bilayer membranes. EIS attested the formation of well-insulating tBLMs if 3-mercapto-1-propanol (3M1P) is used as a backfiller. An increase in the length of the backfiller led to increased defectiveness of tBLMs, however, presumably proper adjustments of the SAM composition can improve properties of tBLMs when other backfillers are utilized. All tBLMs assembled on silver substrates responded to the pore-forming cholesterol-dependent cytolysin, vaginolysin in a manner consistent with the functional reconstitution of toxins into phospholipid bilayers.



Our experiments demonstrate the biological relevance of tBLMs assembled on silver surfaces and indicate their utility as biosensing elements for the detection of pore-forming toxins in liquid samples. The results were published in Aleknavičienė et al. *Electrochim. Acta.* 2021, 389: 138726, and Aleknavičienė et al. *Molecules.* 2021, 26: 6878.

Novel Surface Enhanced Raman Spectroscopy Substrates for Bioanalysis

In this work, we aimed at fast and scalable manufacturing of low-cost SERS substrates for the spectroscopic analysis of biomolecules. Using ultrashort-pulse laser-induced plasma-assisted ablation (LIPAA) of soda-lime glass we accomplished the task of developing homogenous SERS-active substrates covered with nanostructures of around 100 nm in diameter on 1–3 μm size dendrimers. The average enhancement factor (EF) evaluated using thiophenol monolayer was 3.0×10^5, while the surface activity was found to be consistent at any given point of substrates. Relatively modest EF was explained by amorphous, rounded-shaped features on soda-lime glass formed during the LIPAA-induced melting and subsequent solidification. That resulted in a lesser amount of hot spots but increased surface homogeneity. The use of soda-lime glass to fabricate SERS substrates



promises a cheaper and scalable alternative to more widely used sapphire and other material substrates opening prospects for low-cost routine SERS testing of biomolecules with high reproducibility in biochemistry, medical, forensic, and environmental sciences. The study was published in Aleknavičienė et al. *Appl. Surf. Sci.* 2022, 571: 151248.

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Protein Structural Bioinformatics

Proteins typically function as three-dimensional (3D) structures, often through interaction with each other and/or with other macromolecules. Protein 3D structure is also the most conserved property of evolutionary related proteins. Therefore, the knowledge of structures of individual proteins and their complexes is essential for understanding their evolution, function and molecular mechanisms. However, the experimental determination of protein structure is slow, expensive and not always successful. Not surprisingly, computational prediction of 3D structure of proteins and their complexes has become an important alternative to experiments. For example, thanks to recent advances of deep learning methods the protein structure prediction problem for individual proteins has largely been solved. However, many other computational problems such as the prediction of 3D structures for protein-protein and protein-nucleic acid complexes remain challenging. More efficient methods for the analysis and prediction of protein binding sites are also in great demand. In addition to providing predictive information, computational methods are indispensable in the annotation and analysis of experimentally solved structures of macromolecules and their complexes.

Our team addresses a broad range of protein-centred research topics that can be collectively described as Computational Studies of Protein Structure, Function and Evolution. There are two main research directions:

1) Development of computational methods for detection of protein homology and for modelling, analysis and evaluation of 3D structure of proteins and protein complexes. In recent years, we have developed several new methods addressing these research topics. All of the software packages implementing these methods are freely available at our web site (https://bioinformatics.lt/software).

2) Application of computational methods to biological problems. In this research direction, we are using computational methods for discovering general patterns in biological data, structural/functional characterization of proteins and their complexes, design of novel proteins and mutants with desired properties. Over the years, our major focus has been on studies of DNA replication and repair systems in viruses, bacteria and eukaryotes. More recently, we have entered a highly dynamic CRISPR-Cas research field and have already made important contributions in elucidating structural and mechanistic properties of CRISPR-Cas systems and their evolutionary relationships.

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Analysis of Results in CASP14, CAPRI and SARS-CoV-2 Global Modelling Experiments

CASP and CAPRI global experiments establish state-of-the-art in predicting 3D structures of proteins and protein complexes. In 2020, we took part in both CASP14 and CAPRI experiments running in parallel. We also participated in a global effort to predict structures of SARS-CoV-2 proteins and provide the best models for experimentalists. In these three experiments, our focus was on testing the performance of our protein assembly modelling protocol and on the ability to select the most accurate models. Some of the key components in our approaches were the latest versions of PPI3D and VoroMQA, developed in our group. The PPI3D web server enables searching, analysing and modelling protein complexes, whereas VoroMQA allows estimation of protein structure quality. In 2021, independent assessors and individual groups were involved in detailed analysis of outcome of these experiments. According to CAPRI assessment, our group ('Venclovas') shared the top spot with two other groups. In CASP14 our group was second, but our models of protein complexes still had the best-predicted intersubunit interfaces. In SARS-CoV-2 protein modelling experiment we devised a procedure for selecting the best models. Our results in these three global experiments were reported in a special issue of Proteins. (Dapkūnas, Olechnovič & Venclovas. *Proteins*. 2021, 89: 1834–1843; Lensink et al. Proteins. 2021, 89: 1800–1823; Kryshtafovych et al. *Proteins*. 2021, 89: 1987–1996).

VoroContacts: Software Tool for Analysing Contacts in Macromolecular Structures

The knowledge of interatomic interactions is critical for understanding the formation and functioning of macromolecular structures and their binding to other partners and ligands. Interatomic contacts are often defined using popular, yet simplistic, distance criteria. A more consistent way to describe both contacts within structures and contacts between structures and the solvent is through the contact surface area (CSA). Following this idea, we developed VoroContacts, a versatile tool for computation and analysis of interatomic CSAs and solvent accessible surface areas (SASAs) using Voronoi structure tessellation. VoroContacts server enables users to ask a large variety of questions related to interactions within and between biological macromolecules as well as their interactions with ligands and solvent. Typical applications might include calculation of SASA and buried surface area, detection and annotation of interactions in protein–protein, protein–DNA/RNA or protein–ligand complexes. VoroContacts is available at https://bioinformatics.lt/wtsam/vorocontacts (Olechnovič and Venclovas. *Bioinformatics*. 2021, 2021, 37:4873–4875).

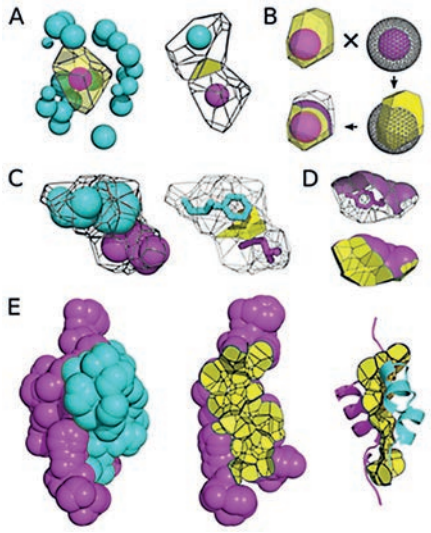
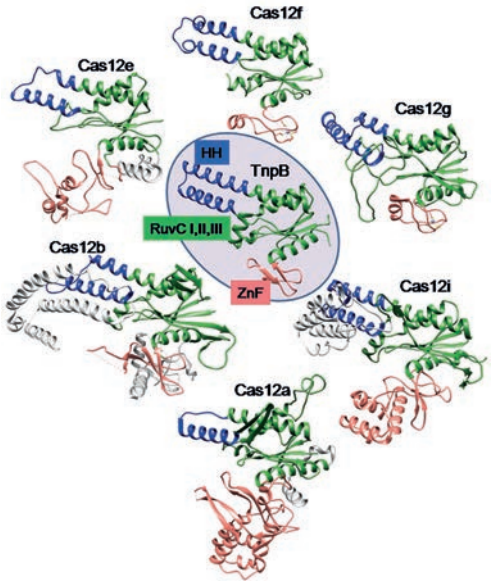


Illustration of contact surfaces at different scales: (A) atom-atom, (B) atom-solvent, (C) residue-residue, (D) residue-solvent, (E) protein-protein

Exploring Origins of Type V CRISPR-Cas Systems

Cas12 proteins (Type V CRISPR-Cas effectors) along with Cas9s are commonly used in genome editing and other biotechnologies. Although previously it was suggested that TnpB proteins encoded by prokaryotic IS200/605 transposons may be related to Cas12s, both structure and function of TnpBs remained unknown. In a joint experimental and computational study, we aimed to characterize TnpBs in detail. Using sensitive sequence searches and computational structure modelling we showed that TnpB and Cas12 family proteins have similar domain composition, including a conserved RuvC endonuclease-like motif (see the figure). Moreover, we found that TnpB corresponds to the minimal structural core of Cas12 family members. At the same time, biochemical experiments revealed that ISDra2, a TnpB representative from *D. radiodurans*, just like Cas12 proteins, is a programmable RNA-guided DNA endonuclease. Thus, it could be concluded that TnpB is an evolutionary ancestor of Cas12. Importantly, because of small size and huge variety, TnpBs open up new possibilities for genome editing and other applications (Karvelis et al. *Nature*. 2021, 599:692–696).



Comparison of RuvC regions in TnpB and Cas12 proteins

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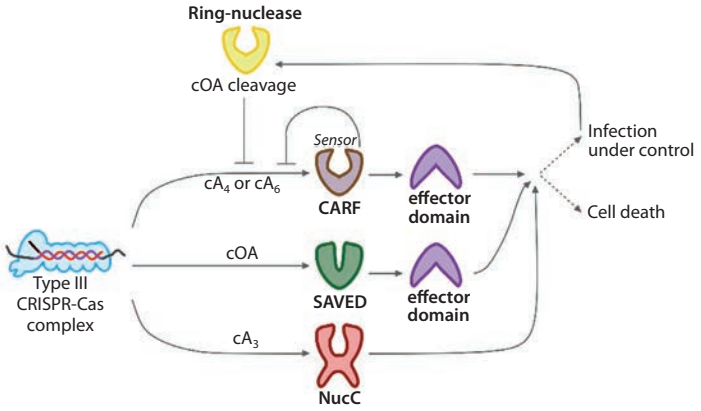

CARF/SAVED proteins are predicted to be involved in second messengers signalling pathway. cOA – cyclic oligoadenylates

Signalling in Prokaryotic Antiviral Defence

Cyclic mono- and di-ribonucleotides are widely employed for controlling various biological processes by eukaryotes and especially prokaryotes. Purine ribonucleotides serve not only as building blocks for RNA, universal currency of energy and components of coenzymes such as NAD(P)+, FAD or CoA, but are also assembled into signalling molecules. Both prokaryotes and eukaryotes employ cAMP and cGMP as key second messengers in a variety of biological processes, including quorum sensing, energy homeostasis, neuronal signalling, and muscle relaxation. Prokaryotes also use a variety of cyclic dinucleotides – c-di-AMP, c-di-GMP, and 3'3'-cGAMP – as signalling molecules for biofilm formation, virulence and regulation of bacterial cell cycle. Recent studies in bacteria have reported that various small cyclic molecules are used as signalling messengers in antiviral defence systems of type III CRISPR-Cas, CBASS, Pycsar and Thoeris. As these systems are widespread and abundant, this suggests that signalling is widely used in prokaryotic antiviral defence.

Our group has pioneered by elucidating the interference mechanism of the type III CRISPR-Cas immunity [1–4]. We revealed that to provide immunity against invading nucleic acids in prokaryotes, type III CRISPR-Cas system combines transcription-dependent DNA degradation by crRNA guided Csm or Cmr complex [1,3] with the cyclic oligoadenylates (cOA)-dependent immunity pathway [2]. In response to the viral RNA binding, the Csm/Cmr complex synthesizes cA_n molecules of various ring size (n=2–6) [2,4]. The cA_4 or cA_6 acts as a signalling molecule that binds to the CARF domain sensor of the stand-alone Csm6 or Csx1 proteins and allosterically activates the non-specific ribonucleolytic activity of their HEPN effector domains [2,4]. Further bioinformatics analysis revealed that the sensor CARF and SAVED (a divergent version of CARF) domains are found fused with diferent enzymatic or non-enzymatic effector domains of type III CRISPR-Cas and CBASS associated proteins (see Figure above). We aim to understand the molecular and structural mechanisms by which CARF/SAVED domain-containing proteins are involved in bacterial immunity and possibly other cell processes.

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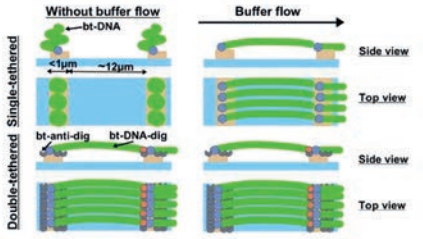
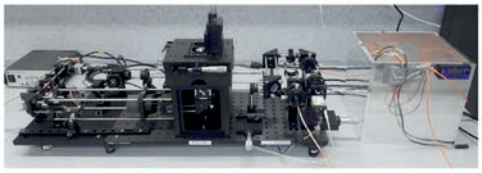
Soft DNA Curtains



Our iteration of miCube microscope



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Single-Molecule Studies of Protein and DNA Interactions

Real-time monitoring of single-proteins on nucleic acid (NA) substrates is an essential tool for mechanistic studies of NA-interacting proteins. Over the past 20 years, a number of such methods emerged: tethered particle motion, optical or magnetic tweezers, flow-stretch assays, and various combinations of these single-molecule (SM) methods. DNA Curtains is a next-generation high-throughput biophysical approach. The technological design of original DNA Curtains is implemented solely with two fundamental components – SLBs and nanobarriers to the lateral diffusion of lipids. Nevertheless, they confer limiting factors that create the need for the development of innovative strategies for assembling DNA Curtains.

Recently our team developed an alternative assay to the original DNA Curtains that we entitled the Soft DNA Curtains. We fabricated streptavidin (sAv) line-features on the modified coverslip surface that can be utilized to assemble stably immobilized biotinylated DNA arrays [1]. The application of hydrodynamic buffer flow allows extension of the immobilized DNA molecules along the surface.

This year we published a follow-up article in Langmuir on the Oriented Soft DNA Curtains [2]. In this work, we fabricated the uniformly oriented double-tethered DNA Curtains using heterologous labelling of the DNA by biotin and digoxigenin. In addition to that, we increased stability of the immobilized DNA molecules using a more stable alternative to sAv called traptavidin.

The main goal of our research is to apply the developed platform for studies of DNA targeting mechanisms of diverse CRISPR-Cas systems family, novel molecular-tools – prokaryotic Argonaute proteins, and various restriction endonucleases. Since 2017, we have received two grants for these studies from the Lithuanian Research Council (LRC): S-MIP-17-59 and S-MIP-20-55. Also, we received funding for a post-doc project (LRC. Nr.09.3.3-LMT-K-712-19-0113) dedicated for pAgo studies.

With the help of our recent grant from Vilnius University Science Fund (MSF-JM-10/2021) we started development of a home-made fluorescence microscope (https://github.com/samhitech/microEye) dedicated for super-resolution studies [https://www.nature.com/articles/s41592-021-01313-1?proof=t%29Nature].

This year we also received funding for improvement of Soft DNA Curtains stability (01.2.1-MITA-T-851-01-0163) as well as for Soft RNA Curtains platform (01.2.1-MITA-T-852-01-0218). These projects will help us to make our platform more attractive. In addition, it will allow us to study the RNA-interacting proteins that are important, e.g., for virus infection.

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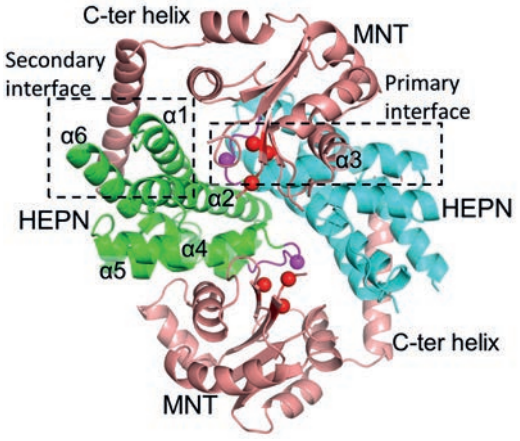

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Structural Biochemistry

Protein X-ray crystallography and cryo-EM are the primary techniques for elucidation of three-dimensional protein structures, which are critical for understanding macromolecule mechanism and function.

We perform protein crystallization using Oryx8 and Gryphon crystallization robots, monitor crystal growth in automatic Rigaku Minstrel DT UV stations and collect X-ray diffraction data either on an in-house Rigaku MicroMax-007HF X-ray diffractometer fitted with a Dectris Pilatus 3R 200K-A detector or during data collection sessions in DESY synchrotron, Hamburg. A 200 kV Cryo-EM Glacios microscope was recently installed at the Life Sciences Center.

Our research combines two major directions:

1) Structural characterization of prokaryotic proteins and protein complexes involved in bacterial antiviral defence. In order to survive under a constant pressure of phage infection, bacteria have developed a great variety of defence mechanisms. Currently, we study components of various bacterial antiviral systems, including:

(i) restriction endonucleases (REases), components of Restriction-Modification systems that protect host bacteria by cleaving bacteriophage DNA, constitute a large and highly diverse family of proteins, which differ in their activity regulation, DNA recognition and DNA cleavage mechanisms. We study orthodox Type II enzymes (PfoI [1], Kpn2I, AgeI, BsaWI), ATP-dependent REases (NgoAVII, CglI), and REases specific for methylated DNA sequences (LpnPI, EcoKMcrBC [2], EcoKMcrA [3], the latter in collaboration with Prof. M. Bochtler group from the International Institute of Molecular and Cell Biology in Warsaw);

(ii) components of the CRISPR-Cas adaptive immunity systems (Cas1-Cas2, Cas6, Cascade);

(iii) bacterial toxin-antitoxin systems, e. g. the MNT-HEPN system from *A. flos-aquae* cyanobacteria [4];

(iv) prokaryotic argonaute proteins and other novel antiviral systems.

2) Crystallographic studies of protein-inhibitor complexes, which are primarily focused on inhibitors of human carbonic anhydrases (hCAs) developed in the Department of Biothermodynamics and Drug Design. hCAs are present in 12 active isoforms in all human tissues. Some isoforms are important therapeutic targets. Design of isoform-specific inhibitors of hCA is a complex project that combines comprehensive thermodynamic description of the protein-ligand interaction with structural characterization. We perform structural characterization of hCA complexes with newly designed inhibitors in order to correlate the binding modes with the thermodynamic parameters of interaction.

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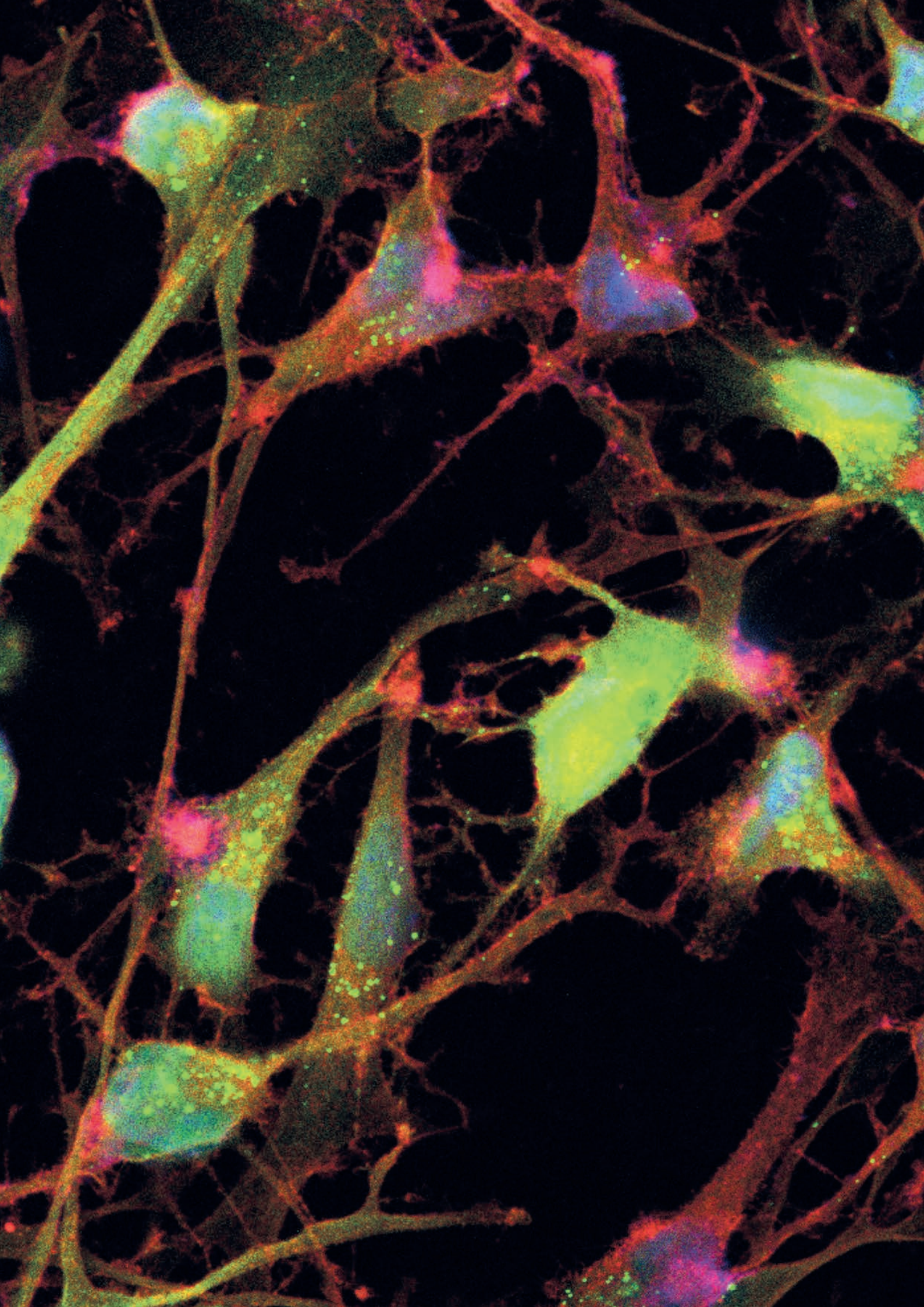

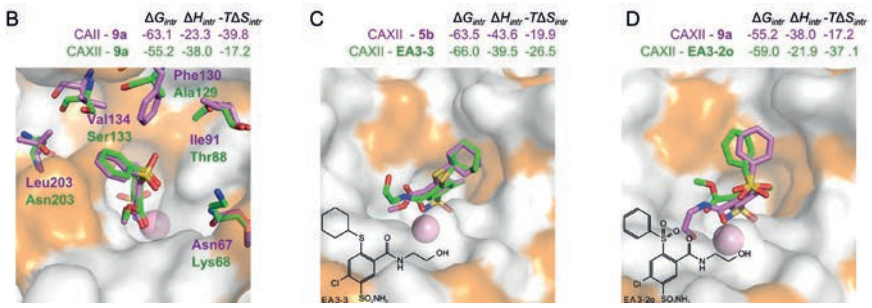

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Structure and Thermodynamics for Drug Design

We measure the energies of protein–ligand binding and apply structure–thermodynamics correlations to design compounds of greater affinity and selectivity for target proteins. We attempt to improve the structure–activity relationships and the principles of rational drug design. We are primarily focused on the human family of twelve catalytically active carbonic anhydrase (CA) isozymes as a disease protein-target. These enzymes have essentially the same fold and a similar shape of the active site suitable for the testing of isozyme selectivity. Sometimes structurally similar compounds exhibit highly different affinities. Deep understanding of the underlying forces that determine the affinity and selectivity is one of the main goals of our laboratory.

In an effort to design CA isozyme-selective compounds, we have assembled a database of 1092 chemical compounds binding to CA isozymes, including

(a) the X-ray crystallographic structures of 180 CA-compound complexes,
(b) the thermodynamics of CA-compound interaction, including the standard observed and intrinsic dissociation constant, Gibbs energy, enthalpy, entropy, volume, and heat capacity changes upon binding,
(c) the kinetics of CA-compound binding, including the on- and off-rates.

Our group of over 30 researchers and students include molecular biologists, biochemists, organic chemists, biophysicists, physicists, computer modellers, biologists and medical doctors. Organic chemists design and synthesize novel compounds, molecular biologists clone, express (both in bacterial and in human cell cultures), and purify various target proteins (Hsp90, HDAC isozymes, functional proteins of COVID-19 coronavirus, etc.), biothermodynamicists determine the energies of binding (by isothermal titration calorimetry, thermal shift assay, and enzyme inhibition assay), crystallographers determine the X-ray crystallographic structures of protein-compound complexes, *in silico* modellers perform compound docking, while cell biologists perform drug-candidate compound studies in biological systems including cancer cell cultures, zebrafish, and mice. Together with medical doctors, we are developing the inhibitors of anticancer target protein CAIX for tumour visualization and treatment.

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Design of CAIX-Selective EA Compounds

Among the twelve catalytically active carbonic anhydrase isozymes present in the human body, the CAIX is highly overexpressed in various solid tumours. The enzyme acidifies the tumour microenvironment enabling invasion and metastatic processes. Therefore, many attempts have been made to design chemical compounds that would exhibit high affinity and selective binding to CAIX over the remaining eleven catalytically active CA isozymes to limit undesired side effects. It has been postulated that such drugs may have anticancer properties and could be used in tumour treatment.

Here we have designed a series of compounds, methyl 5-sulfamoylbenzoates, which bear a primary sulfonamide group, a well-known marker of CA inhibitors, and determined their affinities for all twelve CA isozymes. Variations of substituents on the benzenesulfonamide ring led to compound 4b, which exhibited an extremely high *observed* binding affinity to CAIX; the K_d was 0.12 nM. The *intrinsic* dissociation constant, where the binding-linked protonation reactions have been subtracted, reached 0.08 pM. The compound also exhibited more than 100-fold selectivity over the remaining CA isozymes. The X-ray crystallographic structure of compound 3b bound to CAIX showed the structural position, while several structures of compounds bound to other CA isozymes showed structural reasons for compound selectivity towards CAIX. Since this series of compounds possesses physicochemical properties suitable for drugs, they may be developed for anticancer therapeutic purposes (Zaksauskas et al. *Int J Mol Sci.* 2021, 27).

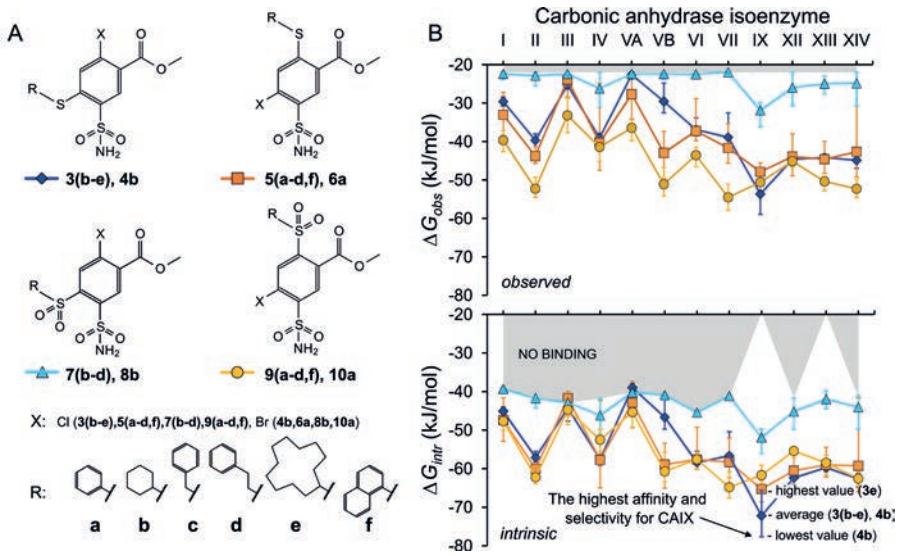

Fig. 1. (**A**) Chemical structures of the four groups of compounds—methyl halo 2- or 4-substituted-5-sulfamoylbenzoates containing chlorine or bromine and substituents listed at the bottom of the panel. (**B**) Averaged change in the standard observed (upper panel) or *intrinsic* (lower panel) Gibbs energy for compounds belonging to one of the four groups: **3(b–e)**, **4b**—blue rhombs, **5(a–d,f), 6a**—orange squares, **7(b–d), 8b**—cyan triangles, and **9(a–d,f), 10a**—yellow circles. The bars show the margin between the strongest and weakest affinity of compounds in that group for the indicated CA isozyme. The *intrinsic* binding affinity of compounds **3(b–e), 4b** to CAIX is highlighted: the blue rhombus is the average of all five interactions, the bar above shows the weakest CAIX interaction with **3e**, and below is the strongest with **4b**. Compounds in this group have the highest selectivity and affinity for CAIX.

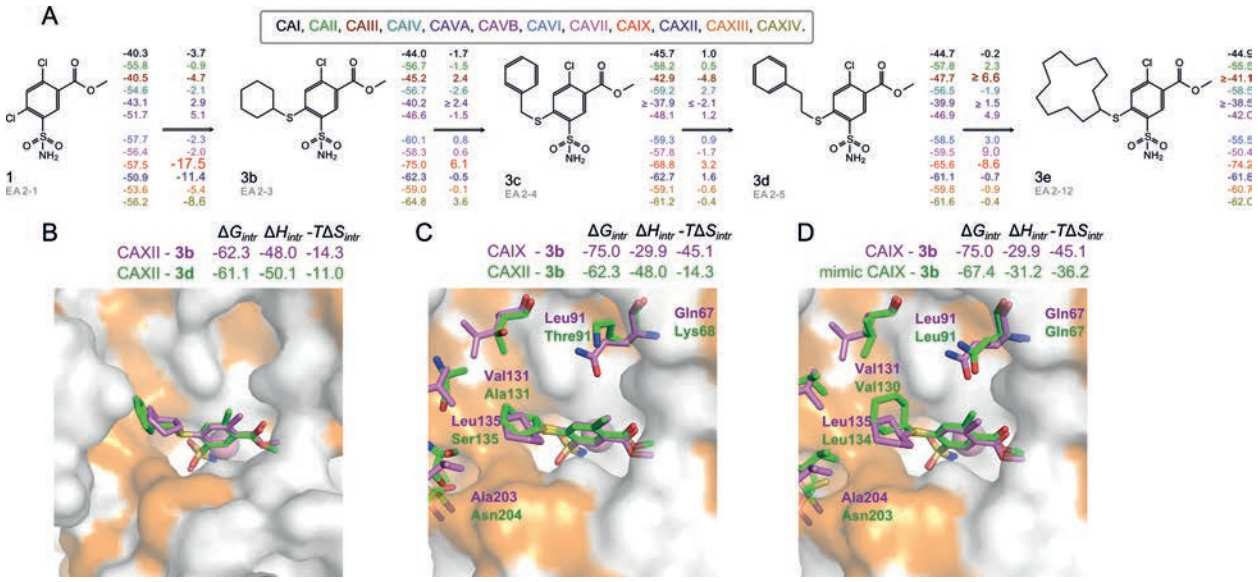

Fig. 2. Correlation of compound chemical structures with the changes in standard intrinsic Gibbs energy upon binding and the comparison of the crystal structures between CA isozymes. (**A**) Differences of ΔG_{intr} between neighbouring compounds are listed on the connecting arrows. All values have units of kJ/mol. Colours represent CA isozymes. (**B**) Compounds **3b** (magenta, PDB ID: 7PP9) and **3d** (green, PDB ID: 7PUW) bound to CAXII. (**C**) Compound **3b** bound to CAXII (green, PDB ID: 7PP9) and CAIX (magenta, PDB ID: 7POM). (**D**) Compound 3b bound to CAIX (magenta, PDB ID: 7POM) and mimic-CAIX (green, PDB ID: 7Q0C). Zinc ion is shown as a pink sphere. The notable nonconservative residues between active sites are shown in the "stick" mode. The protein surface of the CA active site is coloured orange for hydrophobic residues (V, I, L, F, M, A, G, and P) and grey for the residues with charged and polar side chains (R, D, N, E, Q, H, K, S, T, Y, W, and C).

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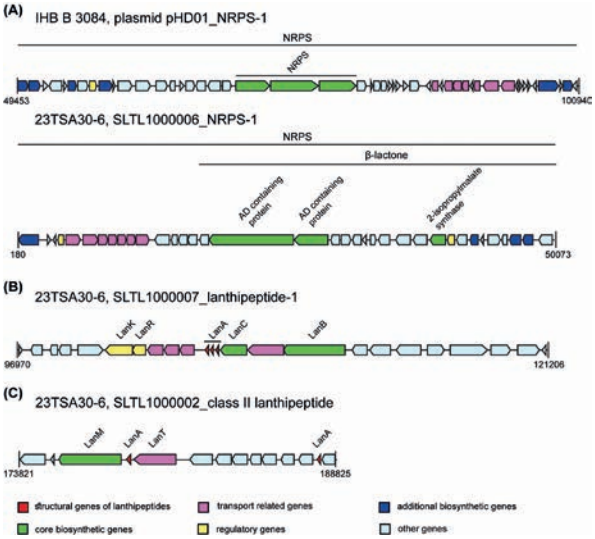

Applications of Molecular Microbiology of Prokaryotes in Biotechnology and Biopharmacy

Prokaryotes represent the largest source of biotechnologically relevant products in nature. New species of prokaryotes are continuously described, and new strains of the "old" species are also continuously isolated. It is known that every new bacterial strain adds dozens of new genes to the genome of its own species, and at least some of these new genes can be exploited for the development of novel, biotechnologically relevant products.

Prokaryotes developed a range of enzymes that degrade polysaccharides, producing oligosaccharides. Different bioactivities useful for human health were reported for oligosaccharides; they are also used as prebiotics in functional food. The enzymatic production of these compounds is the most promising.

Prokaryotes also developed a whole range of structural proteins, and some of them (collagen-like proteins, for example) can be used for the construction of biomaterials with the desirable properties for regenerative medicine.

Most bacteria produce antimicrobial compounds of different nature: volatile compounds, bacteriocins, antibiotics. In practice, they can be used for both the prevention and treatment of infections. Screening for novel antimicrobial compounds is regarded to be the most promising strategy for overcoming the problem of antimicrobial resistance.

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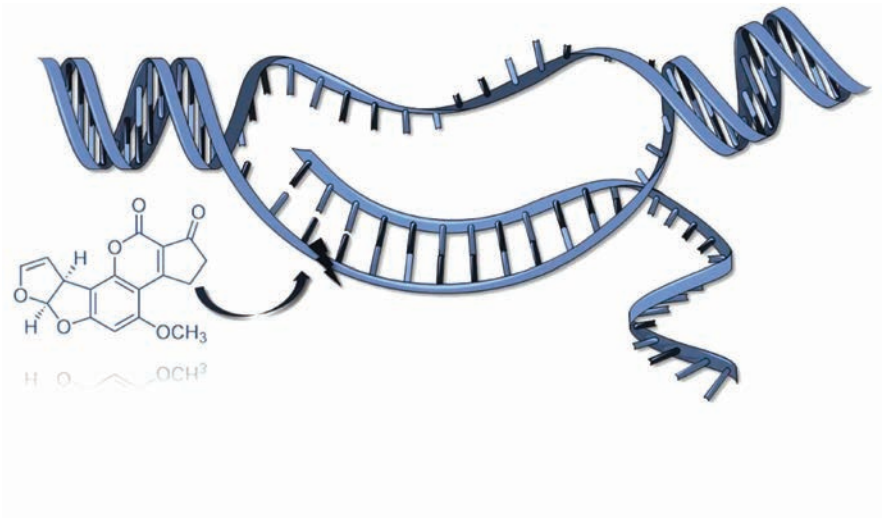

Genotoxicity of Anthropogenic and Natural Factors

Bioactive molecules from natural sources play an important role in the development of nutraceuticals and pharmaceuticals. Nowadays, bioactive natural products are the sources for >80% of active compounds in foods and >30% of drugs. However, the plants may also produce natural toxic, mutagenic and/or carcinogenic compounds. The increasing demand for plant-derived natural products in cosmetics, medicine and products from the food industry requires a more systematic and comprehensive evaluation of their benefits and possible adverse effects, e.g. such as genotoxicity. However, until now, only a small part of plant species has been screened for their biological activities and genotoxic properties. There is a strong need for a more systematic and comprehensive evaluation of the phytochemical composition and genotoxicity of plant extracts using various genotoxicity assays covering different DNA damage endpoints.

Recent studies have confirmed the usefulness of biomonitoring chromosome damage in groups exposed to genotoxic agents by finding an increased risk of cancer in subjects with high levels of chromosome aberrations and thus proving the chromosome aberration assay as a reliable indicator of cancer risk. The monitoring of chromosome damage in radiation-exposed workers remains important to estimate the genotoxic risk associated with chronic exposure to low doses.

UV lasers have provided completely new possibilities for surgery and therapeutic treatments and are increasingly applied in medicine. A number of studies performed in the field of laser treatment and surgery have proved that there are femtosecond laser pulses that have advantages as compared with the longer duration pulses. Although the employment of femtosecond lasers as medical tools opens new possibilities for eye and skin treatment and surgery, the impact of their use on genetic material is not yet fully understood.

We use different methods of genotoxicity assessment (cytogenetic tests, the Ames test, the Comet assay) to investigate the genotoxic action of anthropogenic and natural factors. We investigated the genotoxicity of different plant products and showed that some natural compounds from different plant species are relatively safe and have good potential for use in the food industry. We are studying the effects of ionizing radiation on human chromosomes. Our former and recent study established a link between the incidence of chromosome aberrations and the risk of cancer. We studied the possible harmful impact of the brand-new 206 nm femtosecond laser Pharos on bone marrow, skin and corneal cells. Our investigations demonstrated that the DNA-damaging effect of laser irradiation was mostly dependent on the wavelength, but the influence of other parameters was also revealed.

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Applied and Environmental Microbiology

Although the potential of microbial degradation is ubiquitous, many organic contaminants are not or often only poorly transformed in natural environmental conditions, thus, organic, and other waste treatment and recycling is an important topic. Therefore, the enhancement of natural microbiological degradative activities at contaminated sites is one of the challenges of the present research group. Through exploitation of advances conventional and molecular biology techniques, search, identification, and characterization of microbes or microbial enzymes active towards fatty substances or aromatic compounds are done. Microbial enzymes, especially those exerting activity against ester bonds have a broad range of applications in modern biotechnology. Lipolytic enzymes are among the most industrially relevant and widely used in biocatalysis, both at academic and industrial levels due to their immense versatility regarding catalytic behaviour and great stability in different reaction media. Nevertheless, for the industrial implementations, immobilized enzymes are preferred over their soluble forms. Ecologically inspired method of immobilization of lipolytic enzymes on industrial waste products as carriers are developed by the group.

Another emerging topic is alternative antibacterial compounds such as bacterial ribosomally synthesized peptides with antibacterial activity (bacteriocins). These natural compounds have considerable diversity with respect to their size, structure, mechanism of action, inhibitory spectrum, immunity mechanisms and targeted receptors. In the era of antibiotic resistance, bacteriocins are suggested as a potential alternative to antibiotics in clinics and as food preservatives against spoilage and pathogenic microorganisms.

The group is also participating in a research regarding safe bacterial biofilm control method development for European Space Agency (ESA). In collaboration with the Institute of Photonics and Nanotechnology, Faculty of Physics (Vilnius University), a novel natural photosensitizers-based antimicrobial photoinactivation (API) technology that is safe for the use in the confined, closed-loop systems such as spacecraft is being developed. Furthermore, the group is currently adapting the API technology and creating the prototype that could be used for the destruction of phytopathogenic microorganisms infecting strawberries. The project is being implemented under the project of Lithuanian Agency for Science, Innovation and Technology.

Yeast β-glucans, a diverse group of polysaccharides, exhibiting immunostimulating activity, and algal pigments, which, besides their health benefits, have great commercial value in nutraceutical, cosmetic and pharmaceutical industries, are among the research group's topics as well.

Enzymes and antimicrobial compounds and systems that are analysed by our research group, are attractive both biotechnologically and in basic research. Some of the competences are achieved not only by introducing publications but also by participating in scientific projects co-financed by ESA, EU funds and collaborating with the regional waste treatment company for the pilot study of biogas production from municipal waste.

Some of our PhD students defended doctoral theses describing the identification of new bacterial lipolytic enzymes and post-translationally modified bacteriocins.

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Resilient bacterial biofilms play an important role in human infections and endanger material integrity not only in confined facilities such as hospitals and food settings on Earth but also in spacecraft, which is inhabited by a changing microbial consortium originating from life-supporting devices, equipment collected in pre-flight conditions and crewmembers. Bacterial biofilms are characterized by faster formation and acquisition of resistance to chemical and physical control methods, making most decontamination methods unsafe. Thus, biofilm control methods that are safe for confined, closed-loop systems are in high demand. Visible-light irradiation technology – antimicrobial photoinactivation (API) based on natural photosensitizers (PSs) such as riboflavin (RF) and chlorophyllin (Chl) – were developed and tested by the research group in collaboration with Institute of Photonics and Nanotechnology, Faculty of Physics of Vilnius University; and the technology showed promising results. During the ESA API research project, effects and applications of API on the planktonic bacteria and formed biofilms, their integrity and viability were determined. API technology was effective to have a bacteriostatic effect on both, Gram-positive and Gram-negative bacteria (Fig. 1). Furthermore, API based technology showed some prominent advantages over the chlorhexidine and UV irradiation that are usually used, e.g., in spacecraft. API belongs to a multitarget process (Fig. 2), therefore, bacteria do not develop resistance, and the process exhibits a very rapid microbial killing as well.

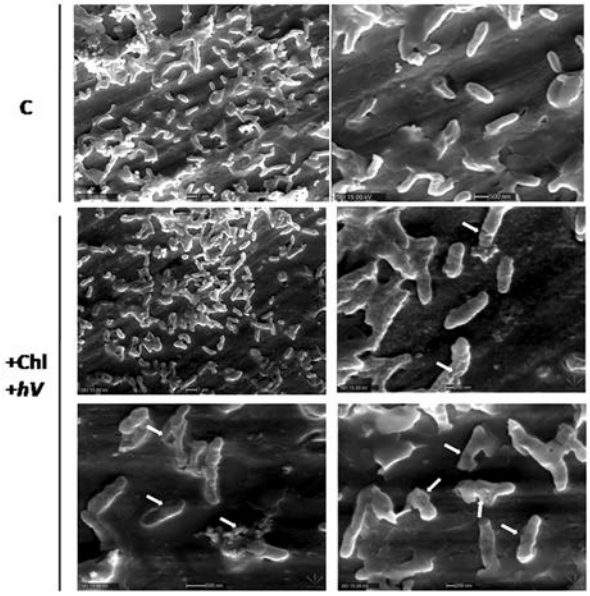

Fig. 1. SEM illustrating the effect of Chl-API on one of Gram-negative bacteria biofilm used in the work as a model microorganism. **C** – control, untreated biofilms; **+Chl *+hV*** – biofilms treated with Chl and irradiated by 405 nm light (dose ~60 J/cm^2).

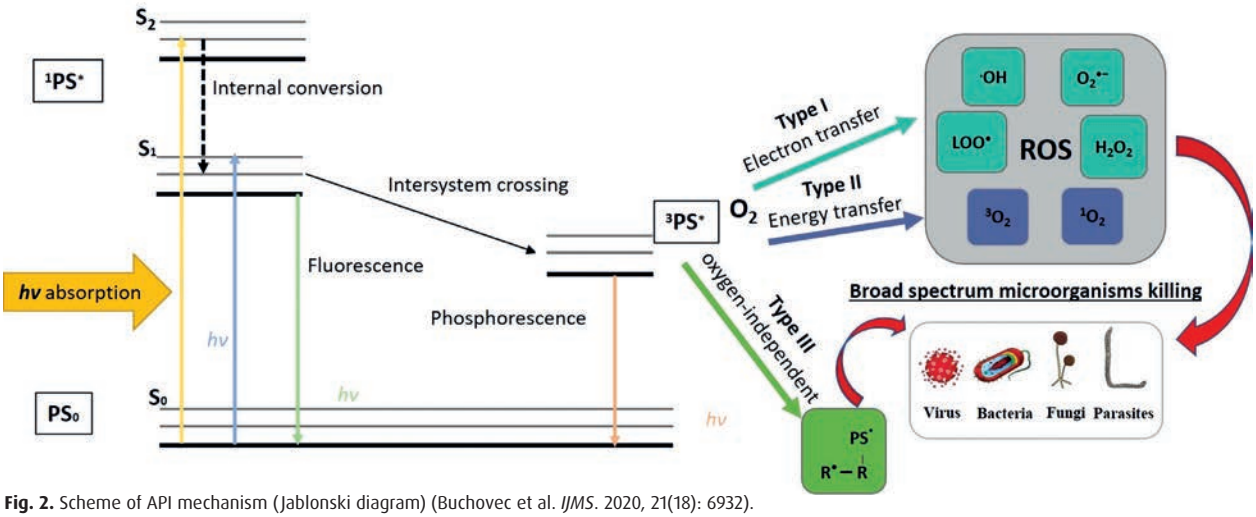

Fig. 2. Scheme of API mechanism (Jablonski diagram) (Buchovec et al. *IJMS*. 2020, 21(18): 6932).

In our studies, we aimed to synthesize and characterize new antibacterial peptides. We analysed various genomes of thermophilic bacteria species and identified gene clusters encoding potential bacteriocins. One of the identified gene clusters in genome of *Parageobacillus thermoglucosidasius* encodes lacticin-like bacteriocin. It is a leaderless bacteriocin and does not contain post-translational modifications. We have cloned bacteriocin precursor gene into a vector and performed its heterologous expression in *Escherichia coli* cells to obtain active antibacterial peptide. The synthesized bacteriocin, which we entitled geobacillin 6 (Geo6), was purified by chromatographic methods. Subsequent analysis of bacteriocin showed that it has broad antibacterial spectrum against closely related thermophilic species (Fig. 3). Unfortunately, it has no antibacterial activity against mesophilic pathogenic bacteria. In addition, this 6 kDa molecular weight peptide is stable even at 100°C temperature.

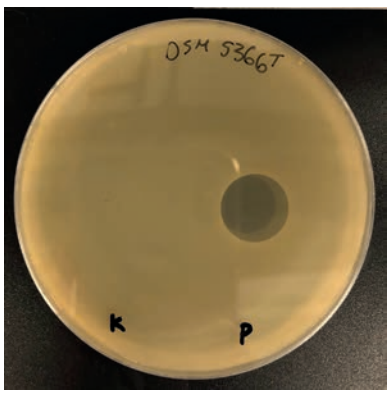

Fig. 3. Antibacterial activity of Geo6. NB-agar medium inoculated with sensitive strain *Geobacillus thermoleovorins* DSM 5366.

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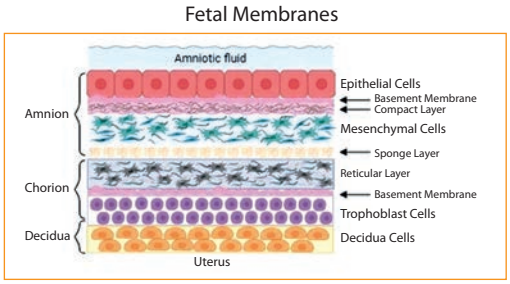

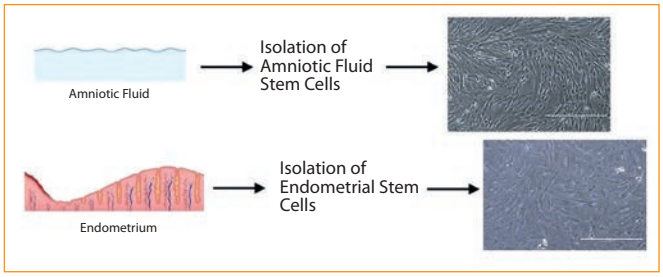

Functioning and Epigenetic Mechanisms of Human Stem Cells

Epigenetic regulation, when influenced by DNA and histone modifications as well as microRNA expression, causes variances in gene expression and cell phenotype. It has a great influence on the development and functioning of stem cells. These changes could cause cancer and other diseases. An understanding of regulatory and epigenetic molecular mechanisms of stem and cancer cell functioning is the main interest for developing new tools in regenerative medicine as well as novel epigenetic therapeutics. Many factors influence the regulation of stem cell, cancer stem cell and cancer cell proliferation, differentiation and apoptosis, including intracellular signalling molecules, transcription factors and epigenetic events. However, the epigenetic and other regulatory mechanisms, governing stem and cancer cell identity, as well as fate determination are still not well-understood.

One of our research objects in the Department of Molecular Cell Biology is human amniotic fluid-derived stem cells (AFSCs). AFSCs are a valuable, easily obtainable alternative of stem cells for cell therapy and regenerative medicine. Although this field has gained much research attention, differentiation capacity of AFSCs and epigenetic regulation leading to AFSCs fate determination are still poorly characterized. Therefore, in our previous research studies we have investigated the differentiation potential of AFSCs and assessed epigenetic factors involved in tissue-specific differentiation. Moreover, we are continuing to extend our research towards the improvement of stem cell qualities and their potential.

Another object that is being extensively researched is human endometrium-derived stem cells. These cells similarly to amniotic fluid stem cells represent typical, with mesenchymal stem cells associated properties, such as the ability to self-renew, high proliferative activity and multilineage differentiation potential. Our studies focus on the characterization of endometrium derived stem cells, which includes compiling epigenetic and gene expression profiles, as well as assessing proliferative activity during long-term cultivation.

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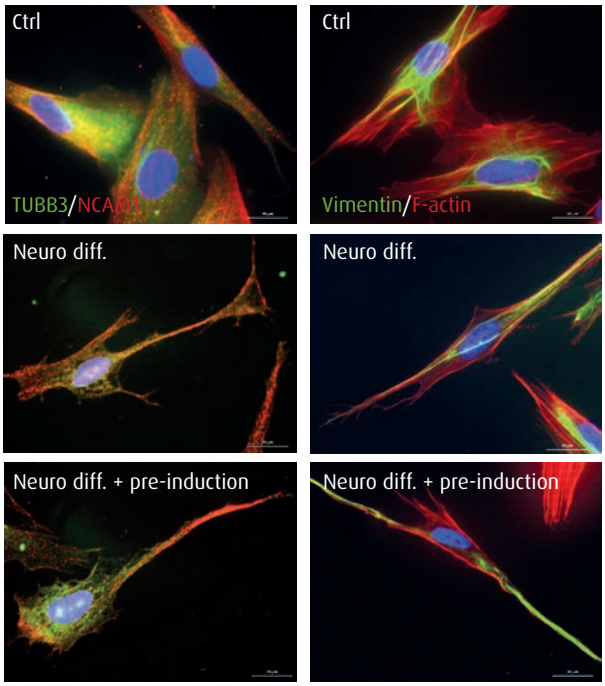
Small Molecule Treatments Improve Differentiation Potential of Human Amniotic Fluid Stem Cells

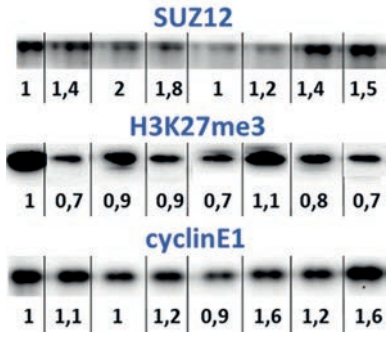
Human amniotic fluid stem cells (AFSC) share some characteristics with pluripotent stem cells, but they are still considered as multipotent. One possible approach to improve the plasticity and applicability of AFSCs could be the use of small molecules. Therefore, in this study we tested epigenetically active compounds, such as Trichostatin A (TSA), sodium butyrate, retinoic acid (RA), and vitamin C (vitC), and their treatments on AFSCs. Collected data revealed that combinations of these compounds triggered upregulation of genes involved in pluripotency, caused alterations in cell surface marker expression, and also stimulated AFSCs toward a more energetically active phenotype. AFSCs were induced to differentiate toward neurogenic lineage using several different protocols and compared to the same differentiation protocols with the addition of a pre-induction step consisting of a combination of small molecules (vitC, TSA and RA). The beneficial effect of small molecule treatment on differentiation potential was observed with significantly upregulated gene expression. This study demonstrates that short treatments with small molecule combinations could be used to improve stem cell characteristics and boost differentiation efficiency (Zentelyte et al. *Front Bioeng Biotechnol.* 2021, 9:623886).



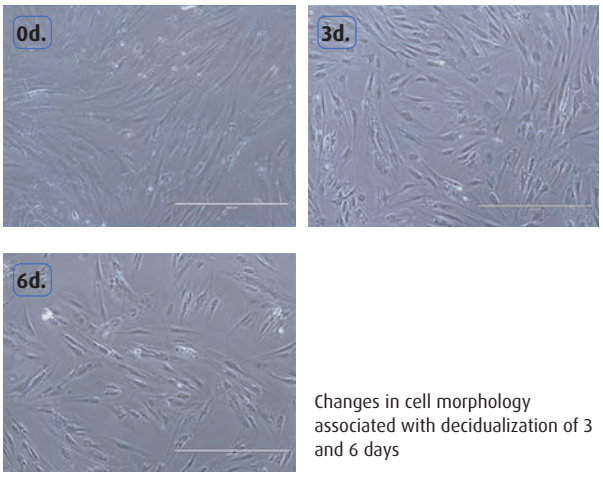
Immunofluorescence analysis of AFSCs differentiated towards neurogenic lineage with and without pre-induction step consisting of small molecule treatment

Decidualization Potency and Epigenetic Changes in Human Endometrial Origin Stem Cells during Propagation

Endometrial origin stem cells can be isolated from two sources: endometrium and menstrual blood and they share typical, with mesenchymal stem cells associated properties. It is thought that stem cells isolated from endometrium (EndSCs) and menstrual blood (MenSCs) could pose a significant advancement in the treatment of reproductive system disorders, especially unexplained infertility. In order to use these stem cells for clinical purposes in the future, it is important to underline (determine) the characteristics of these cells in a laboratory setting. Therefore, we investigated changes in gene and protein expression levels as well as decidualization potential in EndSCs and MenSCs during long-term cultivation. Our results demonstrated that the expression of typical mesenchymal stromal cell surface markers, stem cells gene markers, cell cycle control associated genes and genes associated with senescence remained at a similar level throughout long-term propagation. In addition, changes in protein levels associated to epigenetics and cell cycle control revealed slight fluctuation that is dependent on the patient, but overall these levels remained mainly consistent during passaging. Lastly, we demonstrated that in all induced hEndSCs the expression of decidualization markers Prolactin (PRL), IGFBP1 and WNT4 was upregulated. According to these findings, we suppose that endometrium-derived stem cells and menstrual blood-derived stem cells could have a potency not only for endometrium tissue regeneration, but could also become a successful therapy for reproductive system disorders, including infertility or recurrent pregnancy loss (Valatkaitė et al. *Front. Cell Dev. Biol.* 2021, 9: 765265).



Evaluation of protein level changes associated with epigenetics and cell cycle control in EndSCs and MenSCs using Western blot analysis



Changes in cell morphology associated with decidualization of 3 and 6 days

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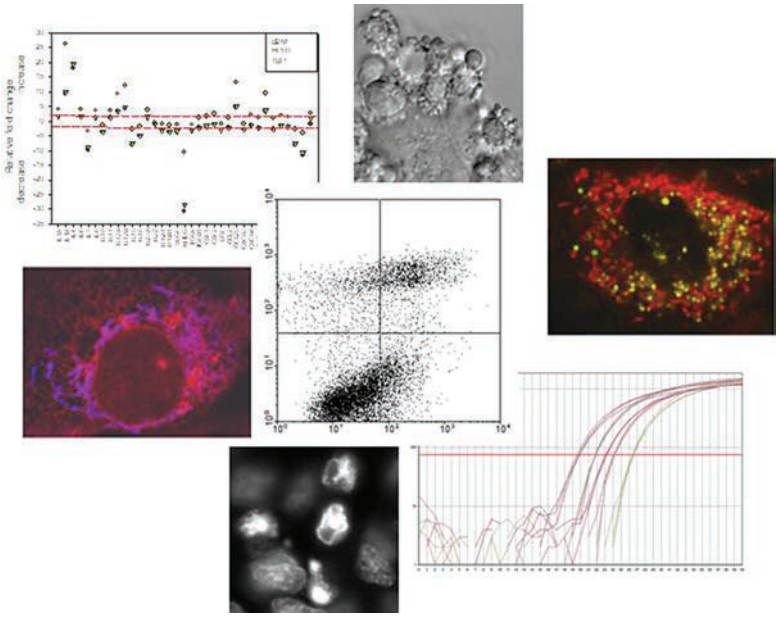

Molecular Mechanisms of Cell Death and Survival

Acquired chemoresistance is a major limitation of successful anti-cancer therapy. Resistance of cancer cells can emerge due to various factors: alterations in drug transport and metabolism, modification of drug targets, activation of DNA repair or changes in cell death induction. Deeper understanding of chemoresistant cell physiology, in particular cell death and survival signalling, suggests new possible targets to overcome cancer cell resistance. We focus our research on molecular mechanisms of cell death and survival pathways: Notch, Wnt, cytokine signalling, mechanisms of autophagy and necroptosis.

Inflammation and antitumor immunity are important determinants of colorectal cancer progression; it is mediated by cytokine signalling. We have determined that interleukin-8 (IL-8) and its receptor CXCR2 are upregulated in the chemoresistant colorectal cancer cells. However, chemoresistant cells remain sensitive to blockade of the CXCR2 pathway that reduces the cell number [1]. We found that cell treatment with exogenous interleukin-1 alpha (IL-1α) increased 5-fluorouracil (5-FU) cytotoxicity in both chemosensitive and chemoresistant colorectal cancer cell lines [2]. The combined exogenous IL-1α and 5-FU treatment changed the expression of cell adhesion molecules that may have an impact on adhesion-dependent chemoresistance and metastatic potential of cells.

Notch and Wnt signalling regulate differentiation of intestinal cells and alterations in these pathways may lead to carcinogenesis. We have determined that Notch and Wnt signalling is upregulated in chemoresistant colorectal cancer cells [3]. The roles of Notch and Wnt pathways for cell survival after 5-FU and oxaliplatin (OxaPt) treatment were different: in the case of 5-FU treatment, Wnt pathway was cytoprotective and supported chemoresistance, while inhibition of either Notch or Wnt pathways increased the cytotoxicity of OxaPt. Besides the studies of colorectal cancer, our group is interested in molecular signatures of endometrial cancer. We have summarized the current knowledge concerning the importance of Notch signalling in endometrial cancer in a review paper [4].

During 2021, the researchers of our group together with the scientists from the Institute of Biochemistry participated in two projects: 1) elucidation of the mechanisms of bacteriophage-derived nanotube entry to colorectal cancer cells (S-SEN 20-4), 2) evaluation of cytotoxicity of aromatic nitro compounds and N-oxides (DOTSUT-34/09.33-LMT-K712-01-0058). With researchers from the Institute of Biomedical Sciences of the Faculty of Medicine, we have performed functional analysis of primary cells of patients having rare genetic diseases. We aim to characterize functional changes of primary cells and correlate them with alterations in genome and transcriptome.

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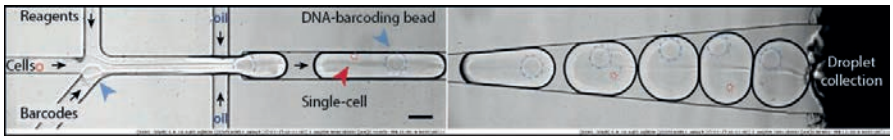

The principle of *inDrops* technique. Digital micrographs of cell encapsulation together with hydrogel beads and reagents. Scale bars, 100 µm. Cell loading into droplets with hydrogel beads and assay reagents occurs at the flow-focusing junction. Hydrogel bead ferries ssDNA primers attached to hydrogel polymer mesh via UV light-sensitive bond.

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Single-Cell Transcriptomics and Genomics

Recent advances in high-throughput single technologies and computational methods have opened new horizons for biological and biomedical sciences. Just over the last few years, we have witnessed significant efforts to develop various analytical techniques to isolate, amplify and sequence the genetic material of individual cells. As the applications of single-cell sequencing continue to expand to all branches of life sciences there is a growing need for technological solutions that can deliver increased molecular sensitivity and reaction throughput at a reduced cost. Droplet microfluidics, a technology that enables pico- and nano-litre volume reactions, plays a major role in this endeavour. Our group are experts in droplet microfluidics technology for single-cell and many biological applications. Our group is pursuing research in cancer and immune system biology, aiming at better understanding of the genetic programs that drive tumour heterogeneity, progression and immune response.

In collaboration with Harvard University, our group has pioneered the droplet microfluidics technique *inDrops* (*in*dexing *Drops*) for barcoding the transcriptome of individual cells (Klein, Cell, 2015). Since then, the technique has triggered immense attention among many scientists across different disciplines. We are applying *inDrops* and other techniques to better understand the gene expression programs that drive the development of complex diseases (e.g. tumours) and how the immune system responds. In collaboration with the Harvard Medical School (Prof. Allon Klein) and Memorial Sloan Kettering Cancer Center and Columbia University (Prof. Dana Pe'er), we have studied the immune system role in complex diseases [2], the gene-environment induced epigenetic changes [3], T-cell epigenetic landscape [4] and cancer cell plasticity [5].

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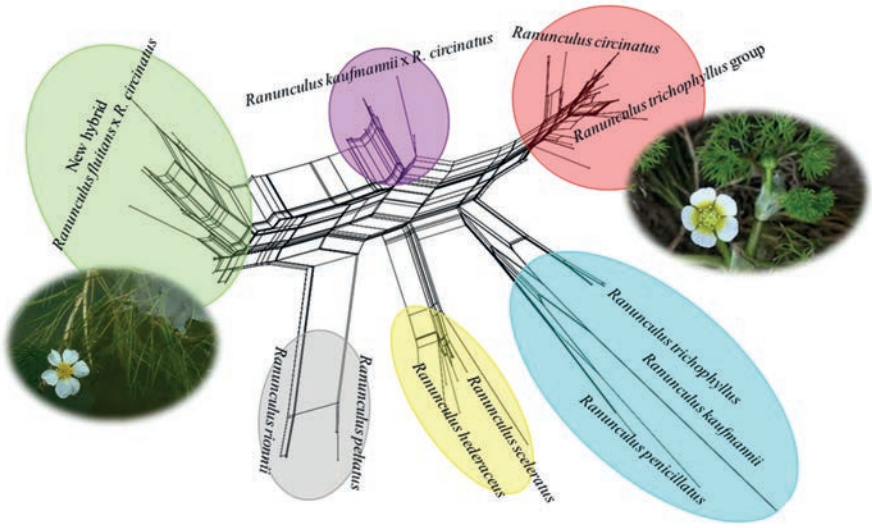

Plant Polymorphism, Genome Stability and Its Changing Factors

Plants are a source for sustainable food and other bioproducts, play essential roles in maintaining ecosystem and human well-being. However, little is known about the mechanisms that help plants survive and adapt to local and global environmental changes, and how these factors affect the plant's genome and gene expression. Many adaptation and developmental features have their chemical expressions related to the production of phytohormones, secondary metabolites and signalling molecules. However, chemical changes in the cell and whole organism are controlled by the structure and activity of the genome, its genes and epigenetic changes. Comprehensive studies of plant adaptation strategies should be carried out at the cell, individual and population level. DNA analysis reveals the relationship between the plant genome structure and its functioning as well as the survival and adaptation strategies of the plants. On the other hand, plants have unique developmental and reproductive features; they maintain a close relationship with the soil and its microflora. Therefore, they are often used as a test system to assess the ecological status of the environment, for phytoremediation and as producers of various metabolites.

We studied the natural and induced plant genome variability at the cell, organism and population levels using molecular, biochemical, statistical and bioinformatical methods. One of the traditional trends in our laboratory is studies of barley developmental mutants. We used *tw* mutants to study the impact of auxin pathway disturbances on plant immunity and the effects of plant immunity-related substances on the level of mouldy germinating grains (MGG). The study showed that auxin pathway disturbances specific for pleiotropic tw mutants are generally restricted to organogenesis but not to germination events [1]. Another aspect of our investigation concerns plant evolution and ecology with particular interest on the phenomena of hybridization and adaptivity. Our study confirmed the hybridogenic origin of distinctive *Batrachium* genotypes [2] and the role of the anthropogenic river modifications on the genetic diversity of *Phragmites australis* populations [3]. Some of our studies [2–5] were carried out in collaboration with colleagues from other Lithuanian research institutions and abroad. Our team has also a lot of experience in the field of genotoxicity studies on soil contamination by hazardous environmental pollutants using *Tradescantia* clone #4430 and other test-systems [5].

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Application of Barley *Tweaky Spike* Mutants for Study of Effects of Plant Immunity-Related Substances

Barley developmental mutants *tweaky spike* (*tw*) with disturbed auxin pathways possess a unique feature of an increased level of mouldy germinating grains (MGG), which serves as a convenient model to investigate the effects of plant immunity-related substances. The effects of the auxin 2,4-dichlorophenoxyacetic acid (2,4-D), auxin inhibitors, salicylic acid (SA), and transcinnamic acid (TCA) were studied using the tw-WT system in surface-sterilized and unsterilized germinating grains under high rates of natural infection. Significant differences among the allelic *tw* mutants were revealed at the natural MGG level and in response to 2,4-D, SA, and TCA. The most effective means against MGG were sterilization and TCA. 2,4-D inhibited root growth in *tw* and *tw2* mutants occurred only in unsterilized and not sterilized germinating grains, while the opposite was observed for TCA and SA. The *tw* mutations influenced variations in the seed-borne fungal spectra, decreasing the frequency of *Bipolaris sorokiniana* and increasing *Fusarium* spp. Hypochlorite-based surface sterilization methods should be used with caution in studies, where the action of exogenous 2,4-D will be analysed in germinating grains. Auxin pathway disturbances specific for pleiotropic *tw* mutants are generally restricted to organogenesis but not to germination events (Šiukšta et al. *Agronomy*. 2021, 11: 2180).

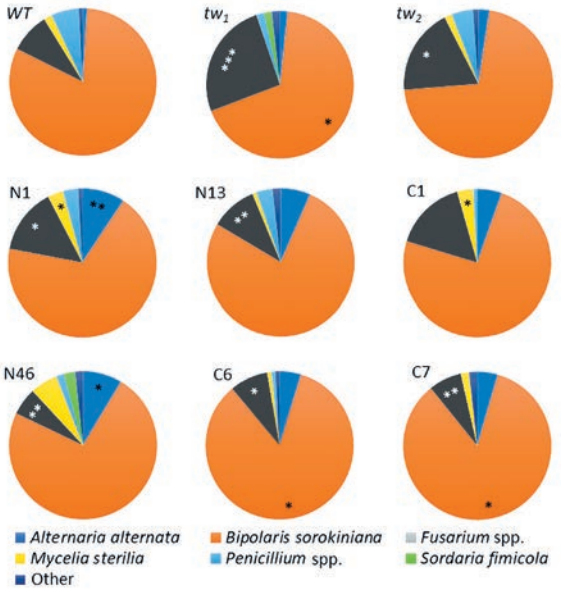


Fig. 1. Spectra of fungi (%) in the internal grain tissues of barley *tw* mutants and the *WT*. The middle row – revertants from tw_1; the lower row – revertants from tw_2. N – revertants with normal spike and floral structure, C – revertants with normal floral structure but compactoid spikes. The asterisks represent significant differences (* p<0.05; ** p<0.01; *** p<0.001) between the *tw*-type mutant and the *WT* (in the upper row) or between the revertant and the respective initial *tw* mutant (N1, N13 and C1 derived from tw_1, N46, C6 and C7 — from tw_2).

Genetic Diversity Patterns in *Phragmites australis* Populations in Straightened and in Natural River Sites in Lithuania

Like in many other parts of the world, *Phragmites australis* distribution and abundance are changing in Lithuania because of the impact of human activities on aquatic habitats. We studied *P. australis* genetic diversity patterns and the effect of hydrographic modifications, introduced in the mid-late twentieth century with great impact on the Lithuanian landscape. The genetic diversity was studied using chloroplast DNA sequences and nuclear microsatellite markers in natural and straightened river stretches for water regulation. We found haplotypes M and L and their variants in the studied populations. The analysis of microsatellites revealed high genetic diversity within populations and significant structure both at the population and river level. We did not find any differences in the distribution of genetic diversity between populations growing in natural and in straightened river stretches; however, at the local level, 5 populations in straightened river sites had higher genetic diversity values than populations in nearby natural sites within the same river, confirming seed establishment in disturbed habitats. Our results demonstrate that anthropogenic river modifications have had an impact on the genetic diversity of *P. australis* populations; however, disturbance is not the only factor that affects genetic diversity recruitment and dynamics in new *P. australis* stands (Naugžemys et al. *Hydrobiologia*. 2021, 848: 3317–3330).

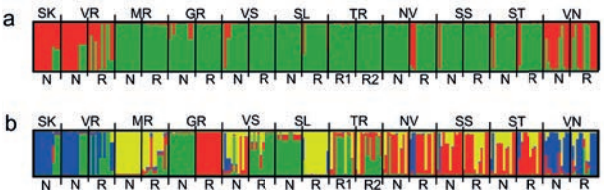


Fig. 2. Distruct graph of *Phragmites australis* STRUCTURE analysis results applying the Evanno Delta K method: a – genetic structure of 21 sampling sites (K=2); b – genetic structure of 21 sampling sites (K=4). Sites are separated by black lines; N – site in a natural river stretch, R – site in a straightened river stretch. Each individual is depicted by a thin line, and its colour depends on its partitioning into the K clusters.

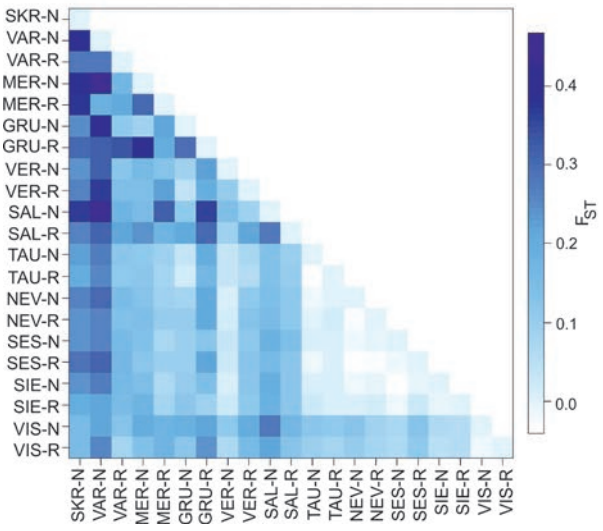
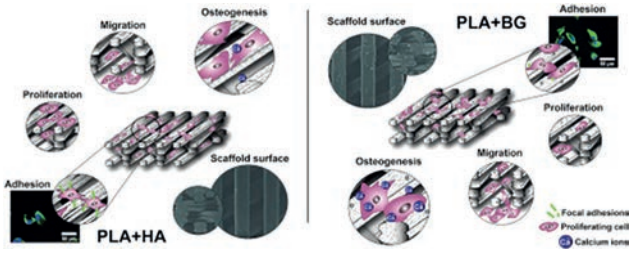


Fig. 3. Genetic differentiation between Lithuanian populations of *Phragmites australis* based on nuclear microsatellite data and revealed using Arlequin v3.5.2.2.



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Stem Cells for Tissue Engineering

Tissue engineering approaches, which combine cells, scaffolds, and biomolecules into functionally active constructs, are a promising strategy to regenerate diseased or damaged tissues that can yield satisfactory clinical outcomes. However, despite significant progress in this field, there are many scientific and technological challenges. Cells in the body experience a wide spectrum of different tissue-specific mechanical environments, e.g., the softest are adipose, neural, pulmonary tissues, intermediate are muscles, blood vessels, gingiva, and the hardest ones are cartilage and bone. Consequently, the tissue-specific environment plays a crucial role in maintaining tissue homeostasis. Infringement of these systems causes the development of various diseases, including idiopathic pulmonary fibrosis, cancer, cardiovascular diseases, and liver cirrhosis. The mechanical environment also plays an important role in cell differentiation and signalling processes. Therefore, it is apparent that the proper coordination of biochemical, structural, and mechanical signals is essential for the design of functionally engineered tissues. Current advances made in the field of mechanobiology have expanded our understanding of how mechanical cues influence cell behaviours ranging from cell motility, proliferation, metabolism, and differentiation to tissue organization, and function, but the molecular mechanisms involved in outside-in and inside-out mechanotransduction signalling pathways still have to be explored. Moreover, the response of cancer cells to various chemotherapeutic drugs is also dependent on the environment stiffness.

Previously, we have shown that biodecoration of the 3D scaffold made of polylactic acid (PLA)/ hydroxyapatite (HA) by a dental pulp stem cell (DPSC)-derived extracellular matrix can remarkably improve the osteoinductive properties of the surface *in vitro*. Current studies implemented in collaboration with the Institute of Odontology of Vilnius University (Prof. V. Rutkūnas) revealed that biodecorated scaffolds demonstrated better bone-forming properties *in vivo* [2]. In 2021, our objective was to elucidate the impact of the mechanical environment on healthy and cancerous cells and their response to oxidative stress and chemotherapeutic treatment [1]. We have also developed a method for the evaluation of cell number in 3D environment [3].

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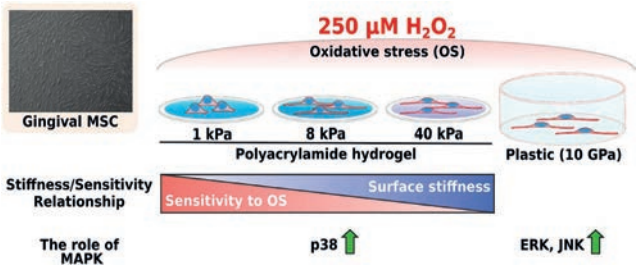
Impact of Extracellular Environment on Cell Fate

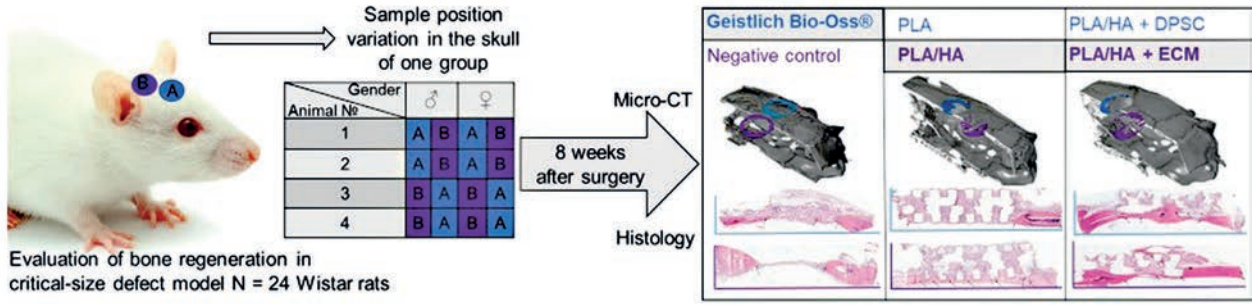
Oxidative stress (OS) induced by reactive oxygen and nitrogen species is involved in various processes in the organism, including wound healing, immune response, angiogenesis, and is responsible for the induction and progress of various disorders such as atherosclerosis, myocardial infarction, hypertension, diabetes, thrombosis, acute lung injury, and cancer. OS also plays a crucial role in stem cell death processes during cell therapy applications. We have shown that the sensitivity of gingival MSCs (GMSCs) to OS depends on the stiffness of the surface, on which the cells are grown. Furthermore, the activity and expression of mitogen activated protein kinases ERK, JNK, and p38 were surface stiffness-dependent. GMSCs isolated from intermediate/stiff gingiva tissue (~20 kPa) have shown better proliferative and survival properties than grown on the stiffest tissues mimicking polyacrylamide hydrogels (40 kPa). Therefore, the source of MSCs might determine their sensitivity to OS in different stiffness environments and should be considered when developing a treatment strategy.



Simoliunas *et al.*, *Free Radic. Biol. Med*. 2020, 169: 62–73.

Effect of Cell-Derived Extracellular Matrix on Bone Regeneration

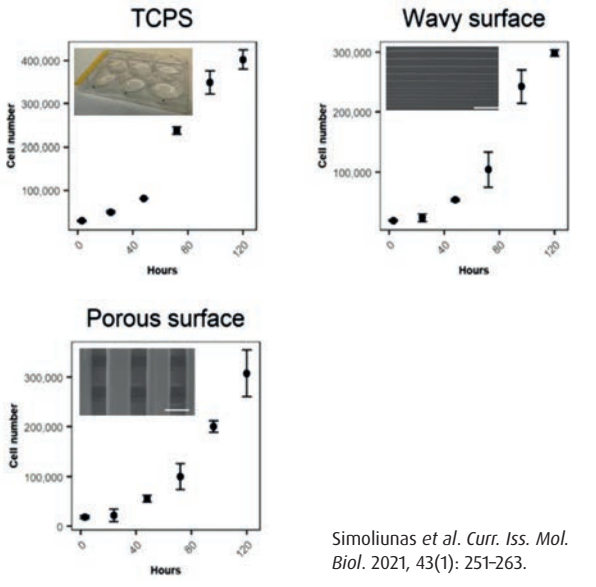
Bone substitute materials currently used in clinical practice are osteoconductive and have many limitations, so new materials and applications are being developed. The purpose of this study was to evaluate the new bone formation *in vivo* effect of 3D-printed PLA/HA scaffolds, enhanced by DPSC or the extracellular matrix (ECM) they produce. The decellularisation procedure used in the current study was sufficient, and an evenly dispersed ECM network remained on the PLA/HA ECM scaffolds. Interestingly, PLA/HA ECM group showed better bone regeneration results when compared to cellularised PLA/HA scaffolds. Moreover, gender-specific differences were observed in all experimental groups, and a statistically significant difference between cellularised PLA/HA and PLA/HA ECM groups in female rats was detected (License No G2-40, 2016-03-18).



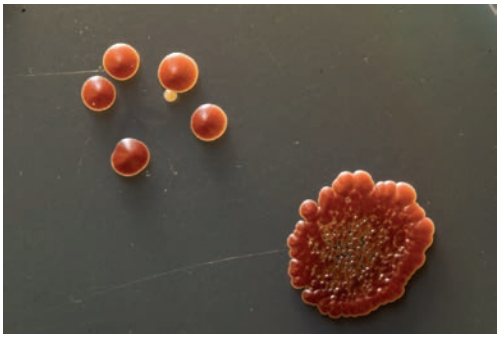
Gendviliene *et al*. *Eur. Cells Mater*. 2021, 41: 204–215.

DNA-DAPI Interaction-Based Method for Cell Counting in 3D

Effective cell number monitoring throughout the three-dimensional (3D) scaffold is a key factor in tissue engineering, however, the methods developed for cell number evaluation in 2D environments often encount limitations in 3D. For this purpose, we developed a method for cell quantification by measuring the DNA content. This method can be applied to cells grown in 2D, 2.5D, and 3D environments. It is applicable for the quantification of healthy, cancerous, and stem cell numbers. Since this DAPI-based method analyses cellular DNA content, its accuracy is not affected by changes in cell metabolism due to differentiation or other biological processes. Its quantification range depends on the cell type. However, we were able to perform accurate measurements of 250 to 100,000 cells with MCF-7, MH-22a, Swiss 3T3, and DPSC. This method uses simple buffers and a common DNA binding dye, DAPI, which can be combined with different assays where signal standardization to the cell number is required.



Simoliunas *et al*. *Curr. Iss. Mol. Biol*. 2021, 43(1): 251–263.



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Strategies in Antimicrobial Therapy and Protein Engineering

In biocontrol of the skin pathogens and analysis of their physiology, we are focusing on the application of pulsed electric field (PEF) in combination with the various chemical compounds to achieve the successful elimination of the skin pathogens, both bacteria and yeasts. The skin pathogens, *Candida* genera yeast, are capable of undergoing morphology switches and form pseudohyphae structures with highly increased resistance to the antifungal compounds. We discovered that, after growth in a rotary cell cultivation system (RCCS), a new, super-resistant and morphology-switching-unrelated phenotype of *Candida* is formed. RCCS is changing the pattern of the antibiotic resistance of *Pseudomonas aeruginosa* and *Staphylococcus aureus* as well. In our recent research we applied PEF on the cell containing weak or strong [PSI+] prions. Prions are misfolded, self-replicating, and transmissible proteins capable of causing different conditions that affect the brain and nervous system in humans and animals. We determined that prions significantly increase cell survivability and resistance to PEF treatment. The application of PEF to the purified Sup35NM fibrils showed that the electric field causes significant reductions in the length of fibrils, and the full disintegration of fibrils to Sup35 oligomers can be achieved in higher fields [1].

In 2020, we started a new project "The influence of intensive fish farming on aquatic microbiome and resistome", analysing and comparing the microbial communities in the fresh aquatic systems and fish farms. These studies provide information not only concerning the microbial communities present in the environmental samples, but also regarding the pathogens and antibiotic resistant strains that can be spread in the population [2, 3]. In this research, we are also focusing on the bacteria genotypes that could be related to the microplastic degradation, synthesis of the antimicrobial compounds, capability to grow on different carbon sources.

Protein engineering (directed evolution, rational design, enzyme fusion) is a powerful tool for developing new biocatalysts for different industrial fields. Lipolytic enzymes are extensively used in chemistry, food, pharmaceutical, detergent, cosmetics industry and biodiesel production. Our research team apply different protein engineering methods (random and site-specific mutagenesis, DNA shuffling, SHIPREC, epPCR, the design of new fused biocatalysts) to investigate structure-function relationships of microbial lipolytic enzymes and design more attractive polyesterases for industrial application [4–6].

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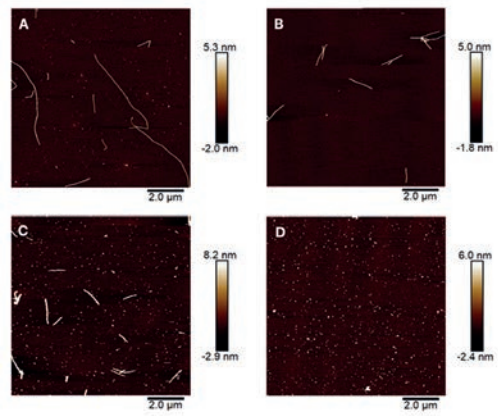
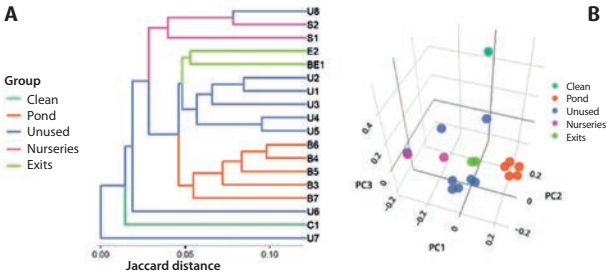
Prions are misfolded, self-replicating, and transmissible proteins that are related to the various neurodegenerative diseases in mammals. Yeasts are the perfect model to study prion formation, dissemination, and the structure of protein aggregates. The idea of applying PEF as a factor capable of disintegrating the amyloid aggregates arises from the fact that the amyloid aggregates form via noncovalent bonds and stabilize via electrostatic interactions. Electrostatic interactions, such as salt bridges (a combination of two non-covalent interactions: hydrogen and ionic bonding between the same amino acid residues) and self-energy, are the key factors in stabilizing the secondary and tertiary structure-forming elements in prions [1]. In our research, we showed that the application of PEF on prion fibrils with a longer pulse results not only in disintegration of fibrils but increases their diameter as well. The shift from 50 μs to 1000 μs resulted in the doubling of the fibril diameter.



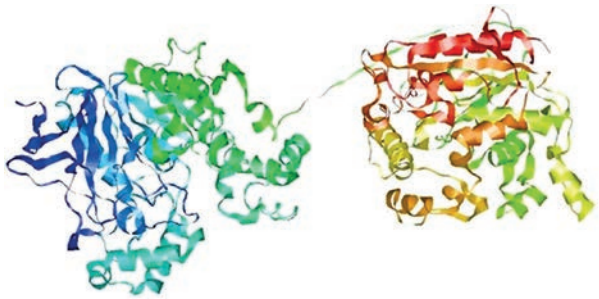
Atomic force microscopy images of Sup35NM amyloid fibrils before (A) and after exposure to a pulsed electric field (PEF) using different parameters; (B) 10 kV/cm, 0.05 ms pulse duration, 10 pulses; (C) 10 kV/cm, 1 ms pulse duration, 10 pulses; (D) 20 kV/cm, 1 ms pulse duration, 10 pulses. The adjacent colour scale indicates the height of the fibrils found in the samples

The aquaculture is one of the fastest growing food sectors all-over the world. The monoculturing in the fishery ponds can affect the local ecosystems as well as the ecosystems of the adjusting water bodies. In our research, we analyse 3 water ecosystems: fishery ponds, one lake downstream and one lake upstream fishery ponds. Analysis of the sediment microbiota as well as fish gut microbiota revealed that Lake Dusia (upstream from fishery ponds) sediment microbiota and fish gut microbiota composition differed significantly as compared to fishery ponds and Lake Simnas (downstream from fishery ponds). The distribution of different taxa in the sediments of fishery ponds and Lake Simnas was more similar, while the sediments of the Dusia had different frequencies of microorganisms. It can therefore be concluded that fishery ponds in direct contact with natural environmental water bodies have a significant impact on the sediment microorganism populations of these bodies.



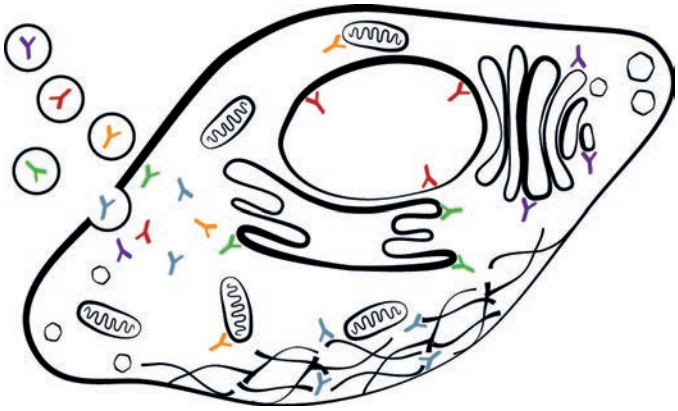
Microbial similarity (beta-diversity) of samples expressed as Jaccard distances and represented as a dendrogram (A) and principal coordinates plot (B)

Protein engineering, design of fused enzymes, immobilization and application of *Geobacillus* lipases and carboxylesterases is one of the major research fields of our group [4–5]. We study the functionality of individual domains in fused lipolytic enzymes, while using GDEst-lip, GDLip-lip and GDEst-est enzymes as a model system. Analysis of mutant GDEst-lip, GDLiplip and GDEst-est variants, where one domain is inactive, showed that both domains retained their activity. The experimental data proposed that the N-terminal domain mostly influenced the thermostability, while the C-terminal domain was responsible for thermal activity. GDEst-lip variants fused by using rigid (EAAELAAE) and flexible (GGSELSGG) linkers indicated that a unique restriction site or a rigid linker is the most preferable fusion strategy to develop new chimeric biocatalysts with domains of *Geobacillus* lipolytic enzymes.



The fused GDEst-lip enzyme used as an object in protein engineering and immobilization experiments [4–5]

Another object of our group is Microbially Induced Calcite Precipitation (MICP). MICP is an effective and eco-friendly technology that can be applied to solve soil problems, including soil erosion, pollution with heavy metals and radionuclides or for CO_2 sequestration. In geotechnical engineering, bioconsolidation is an effective technique to increase slope stability. The success of this process depends on ureases producing microorganisms. We have shown that using both 0.5–1 M concentration of urea with $CaCl_2$ and urease positive *Staphylococcus* sp. H6 cells successful MICP process can be carried out.



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Molecular Mechanisms of Intracellular Trafficking

Intracellular trafficking can be divided into several pathways: secretory mechanisms that transport cargo from the endoplasmic reticulum to the Golgi complex, and from there on – to the plasma membrane, lysosomes or cell outside. Endocytosis is responsible for cellular entry of various ligands, growth factors or viruses. A precise coordination of trafficking events at all levels, directions and pathways is inevitable to ensure cellular homeostasis.

Currently, we focus on two research directions in the field of membrane trafficking. Firstly, we analyse the endocytosis of integrins that are the major receptors of cell interaction to the extracellular matrix. Integrin endocytosis underlies cell migration, growth and stress response. Multiple pathways of integrin entry, recycling or degradation operate in one and the same cell providing with adaptable system rapidly responding to virtually any chemical and physical stimuli occurring in- or outside cells. We aim to understand how integrin endocytosis is regulated in response to cell size, shape and properties of the adhesive surface.

Secondly, in the application-driven project group, we investigate how the functional and morphological features of the endocytic machinery influence cargo internalization. The latter underlies intracellular delivery by chemical methods, such as lipofection. We harness the knowledge of endocytosis for more efficient and side-effects free transfection of cells with nucleic acids and proteins.

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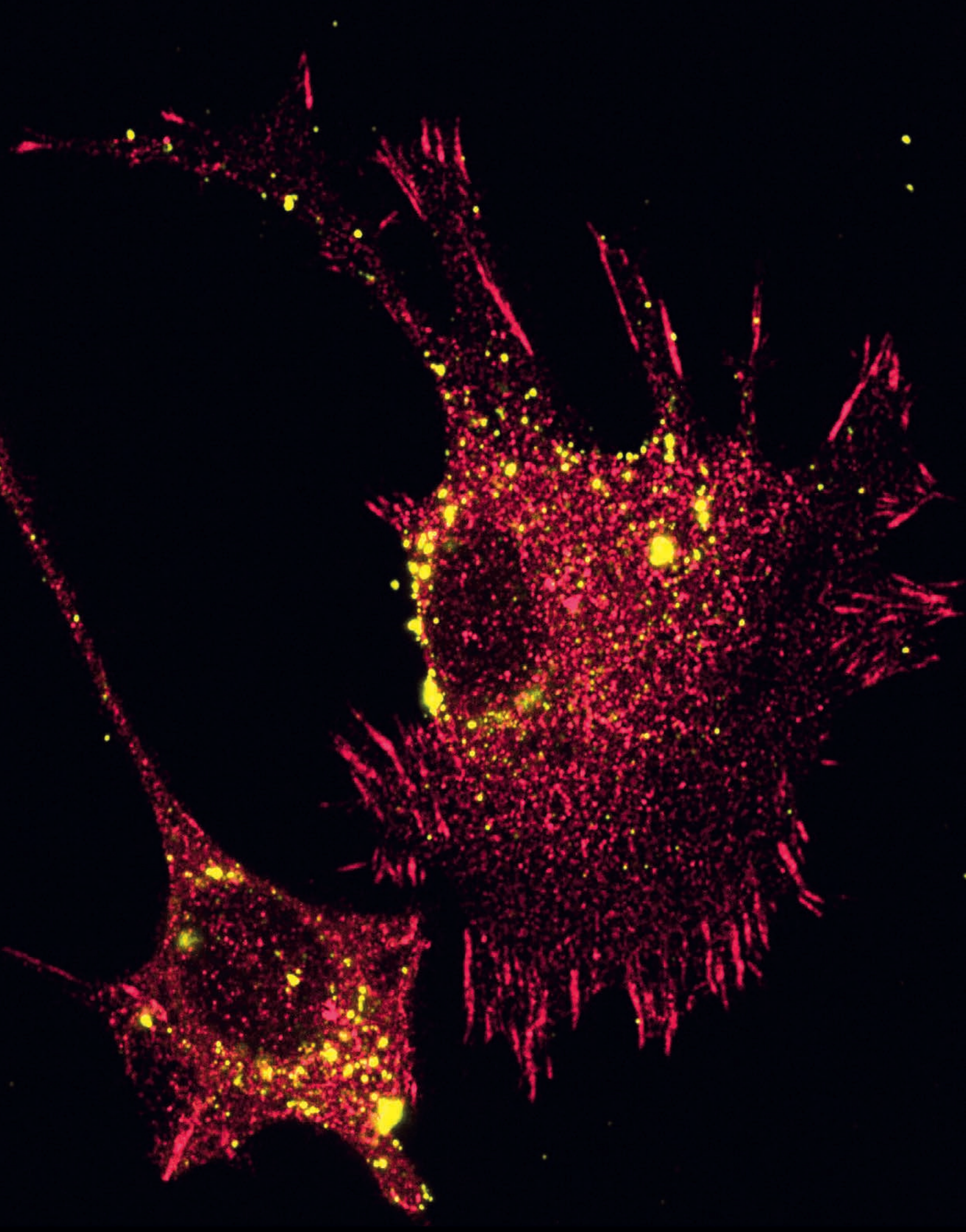

Red colour - cell focal adhesions labelled with paxillin and yellow colour - intracellularly delivered anti-Talin1 antibody. The transfected antibody induces loss-of-function of Talin1, one of the key component of focal adhesions. As a result, focal adhesions are destabilized, cell shape and migratory properties are altered

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Animal Biodiversity, Structure and Ecology of Populations

Animals are the most diverse group of organisms with enormous importance to ecosystems and humans. Hundreds of new species are described every year, however, many more are eradicated by human activity. Therefore, it is important to reveal the basic principles of their systematic and ecological evolution based on certain model animal groups. This includes research on Lithuanian fauna, with a particular concern about the ecology of rare and endangered, also alien and invasive species of animals and animals of medical or veterinary significance, changes in their abundance and distribution. The principal aims include: 1) the research on animal taxonomy and ecology based on the studies of particular animal groups; 2) the studying of the ecology of rare animal species, their abundance and distribution models in Lithuania and especially in the protected areas; 3) the carrying out of research on the biology and ecology of invasive organisms or animals with medical or veterinary significance.

The ongoing research of our team concerns insects (Diptera: Tipulomorpha; Bibionomorpha, Coleoptera, Lepidoptera, Hemiptera; Sternorrhycha: Aphidoidea and Adelgoidea; Hymenoptera: Apidae and Braconidae), spiders, slugs, snails and mussels (Mollusca: Gastropoda and Bivalvia), freshwater fishes, birds of prey and owls, black storks. Research topics include taxonomy and systematics, distribution, ecology, invasive species, medical and veterinary importance and protection as well as exploring of local faunas in the protected areas of Lithuania and elsewhere.

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Contribution of Insects to African Swine Fever Virus Dispersal: Data from Domestic Pig Farms in Lithuania

Outbreaks of African Swine Fever (ASF) in domestic pig farms in Lithuania typically begin in June and are detected through October, suggesting that insects might be involved in the transmission of the virus. Entomological collecting was performed to obtain two data sets: from farms with ASF outbreaks, and from farms without ASF outbreaks but in an ASF infected area. Target insects from the families Muscidae, Calliphoridae and Tabanidae were analysed for the presence of ASF Virus (ASFV) DNA. *Musca domestica*, Calliphoridae flies and *Stomoxys calcitrans* collected by entomological net during ASF outbreaks were confirmed to be ASFV positive. Viral DNA detected in insects collected by Nzi traps from farms with no ASFV outbreaks indicate that *Culex*, *Lucilia, M. domestica* and *S. calcitrans* are likely to play a role in spreading the ASFV mechanically. This finding could suggest contamination from outside of the farms: from infected wild boar or their carcasses. The role of *Stomoxys* flies as mechanical vectors could be accidental, because we did not find any significant correlation between the activity of *S. calcitrans* and the number of ASF outbreaks in pig farms, whereas temperatures positively correlated to the number of ASF outbreaks during 2018–2019.

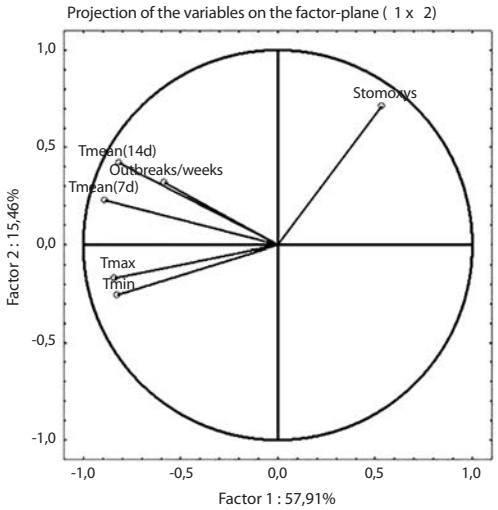

Fig. 1. Principal component analysis of two trapping seasons, from June to August 2018–2019. *Stomoxys* – total number of *S. calcitrans* trapped in 5 Nzi traps by weeks. Tmean(7days) and Tmean(14days) are the sums of mean air temperatures for 7 and 14 days before ASF outbreaks. Tmax and Tmin are means of maximum and minimum temperatures during a week of ASF outbreak. ASF – number of ASF outbreaks per week.

A New Genus of Limoniidae (Diptera: Tipuloidea) from the Mid-Cretaceous Burmese Amber

Limoniidae is a large family with more than 10,680 species distrib-uted across all zoogeographical regions. The oldest rep¬resentatives of Limoniidae are documented in fossil records as far back as the Upper Triassic. A new genus and species, *Orimarguloides* simplex gen. et sp. nov. described from Burmese amber. The type fossil speci-men studied herein was found in the mid-Cretaceous amber deposits (c. 98.79±0.62 Ma; lowermost Cenomanian) in Kachin (Hukawng Val-ley) of northern Myanmar. Based on venation, this new genus differs from other related genera by fused R3 and R4, by fused M3 and M4 which causes the absence of a discal medial cell, and by the absence of cell m1. These findings could give us a better understanding of the evolution of wing venation in family Limoniidae.

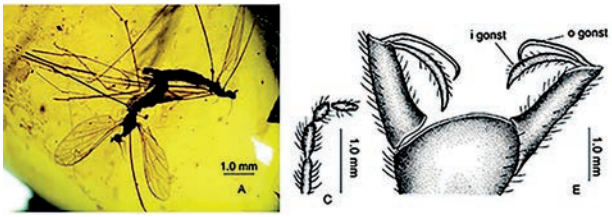

Fig. 2. *Orimarguloides simplex* gen. et. sp. n. Holotype No. AQNU-DIP-2020002. A. Habituses of male and female, dorsal view; C. Palpus of male; E. Male hypopygium, dorsal view. Abbreviation: i gonst – inner gonostylus; o gonst – outer gonostylus.

Review of East Palaearctic *Elliptera* (Diptera, Limoniidae) Immatures with Description of a New Species

The genus *Elliptera* Schiner 1863 is represented by ten species worldwide, but immatures of only the European species *E. omissa* Schiner has been described so far. Molecular methods were used to associate larvae and adults for two East Asian species from South Korea. *Elliptera jacoti* Alexander and *E. zipanguensis zipanguensis*. Alexander are common species in aquatic, hygropetric habitats in mountainous parts of the Korean peninsula. *Elliptera mongolica* Podeniene, Podenas & Gelhaus sp. nov. from Mongolia and China (Inner Mongolia) is described based on mitochondrial DNA COI gene barcodes sequences and morphological characters of larvae. This is the first new species described in superfamily Tipuloidea based on the larval stage alone.

Fig. 3. Bayesian phylogenetic tree constructed using mitochondrial CO I gene sequences of East Asian *Elliptera* Schiner, 1863 species with *Antocha nebulipennis* Alexander, 1931 as an outgroup. Posterior probabilities are indicated in the tree.

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Biodiversity and Ecology of Plants, Algae and Fungi

Plants, algae and fungi are among the most important organisms. They have huge influence on both natural and altered ecosystems, and on humankind ability to prosper. It is their diversity, abundance and vital roles that make them the object of biodiversity research and nature conservation programs, natural resource management and related activities.

The Botany, Algology and Mycology Research Group focuses on the diversity, biology, distribution and ecology of plants, algae, fungi and lichens. We integrate field and laboratory experimental methods to analyse plant and fungal biology and ecology questions. We also study the history of botany in Lithuania. The Herbarium of Vilnius University (WI), located at the Department of Botany and Genetics, has a large collection of dried plants, fungi, lichens, and algae for comparative and evolutionary studies.

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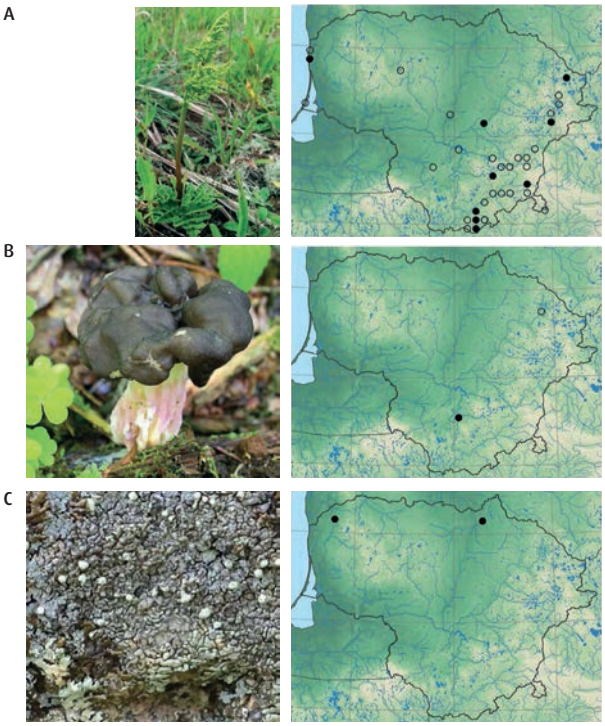
Research of Endangered Plant and Fungal Species in Lithuania

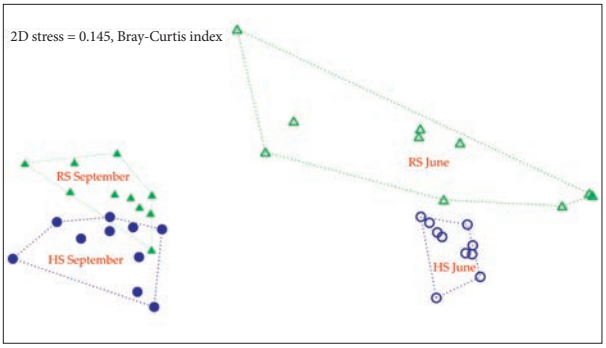
The evaluation of endangered species population size, distribution, decline, fluctuation, extent and quality of habitat, and identification of the main threats to survival of species represents an important field in modern conservation biology research. For the first time in the country's biodiversity conservation development the protected plant and fungal, including lichen, species have been evaluated against the criteria of the International Union for Conservation of Nature (IUCN), and classified into IUCN categories. Species, which were assigned to the threatened categories (Critically Endangered, Endangered, Vulnerable), were included in the current list of protected species of Lithuania. The research on biology, population size, ecology, distribution and threats of protected in Lithuania plant, fungal and lichen species was based on historical and recent observations. This study was initiated by The Ministry of Environment of the Republic of Lithuania, and was performed in collaboration with scientists from Nature Research Centre, Vilnius, and published in the *Red Data Book of Lithuania* (2021).



Distribution of endangered and protected species in Lithuania: *Botrychium multifidum* (**A**), *Gyromitra sphaerospora* (**B**), *Xanthoparmelia mougeotii* (**C**)

Effects of Hydroseeding and Regular Seeding on Native Plant Cover Formation, Species Richness and Abundance

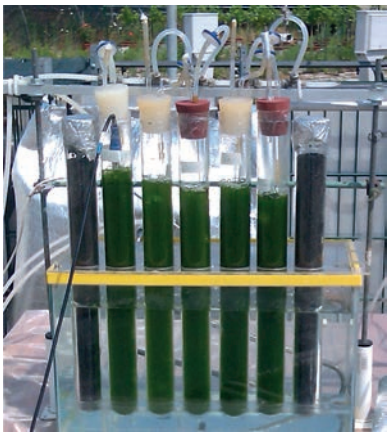
The use of native species is often desired in revegetation projects. However, there is a paucity of information about hydroseeding native species in Northern areas of Europe. Therefore, a total of 40 native plant species in Lithuania were sowed using hydroseeding and regular seeding. A comparison of species composition revealed significant differences between the sowing treatments that were more associated with species abundance than species diversity. Overall, our findings emphasize that legume species that display more competitive growth traits should be included in the seed mixture in lower proportions when hydroseeding is applied (Gudyniene et al. *Plants.* 2021).



NMDS diagram shows that more uniform species compositions were found in the hydroseeding (HS) compared to the regular seeding (RS) plots

Research of Medium Salinity Effect on Growth and Biochemical Composition of Green Microalga *Tetraselmis suecica*

Change in medium salinity due to evaporation or water addition is one of the significant factors affecting algal culture growth and biomass quality. This study evaluates the effect of different salinities on growth and lipid, protein, and carbohydrate content in the green alga *Tetraselmis suecica*. Decreased medium salinity had a positive effect on culture productivity as well as increased protein and carbohydrate content (Venckus et al. *Journal of Applied Phycology.* 2021).



Bubble column photobioreactors used in the outdoor experiments

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Environmental Assessment & Ecosystem Development

Our main research goal is the impact of various anthropogenic and natural stress factors on ecosystem state dynamics and environment assessments. During the last decades, the ecosystem development has been influenced by drastic changes in the socioeconomic and political systems. Anthropogenic and natural factors may adversely shape the present state and the perspectives of ecosystems in terms of their structure and material cycling. Restoration of disturbed ecosystems and its interferences with the anthropogenic pollution load have to be evaluated and understood. Wildlife-vehicle collisions (WVC) are of socioeconomic and ecological importance. We develop spatially explicit and other models how to predict and prevent WVC in anthropogenized landscape. Among natural factors, we focus on keystone species that are able to shape the ecosystem structure and function at different spatial scales. Assessment of the pollution of ecosystems requires reliable markers. We test the toxic impacts of the environmental pollutants on ecosystems using tests of luminescent microorganisms and biomarkers. The origin and migration of different pollutants through various environments may enable proper preventive means. Environmental uncertainty may provoke developmental plasticity reactions of some organisms which is very important for effective regulation of some target species (e.g., pests). Environment-friendly methods for pest control are within the scope of our team.

Our interdisciplinary team has contributed to different methods and different levels of ecosystem organization. Toxicity of various environmental samples from different contaminated sites (e.g. sediments of the fish farming ponds, phytoplankton biomass of eutrophicated water bodies, wastewater, lake sediments) using luminescent bacteria test (ISO 11348-3:2007) was evaluated [1]. The carbon-to-nitrogen ratio was found to influence chemical composition and developmental speed of model insects [2]. Ungulates (mainly *Cervidae* and wild boar *Sus scrofa*) are considered among the most problematic wildlife suffering on roads and causing the largest material losses due to high densities of ungulate populations, spatial and temporal movement patterns of these animals. Lunar light and wildlife-vehicle collision associations were found [3]. Comparative laboratory tests revealed novel properties of the fungus *L. aphanocladii*, its effectiveness as the leaf miner moth's entomopathogen and its suitability for field application trials while developing environment-friendly methods for horse-chestnut pest control [4].

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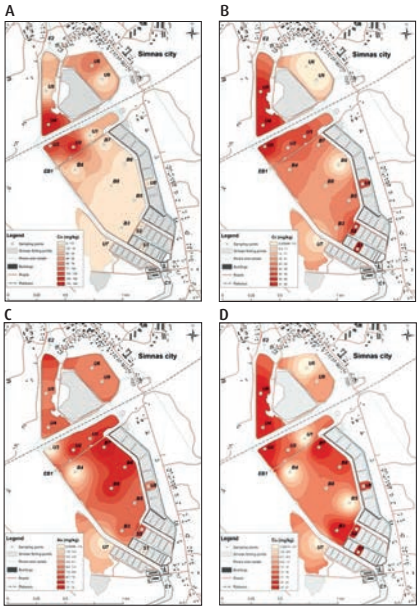
Impact of Intensive Fish Farming on Pond Sediment Microbiome and Antibiotic Resistance Gene Composition

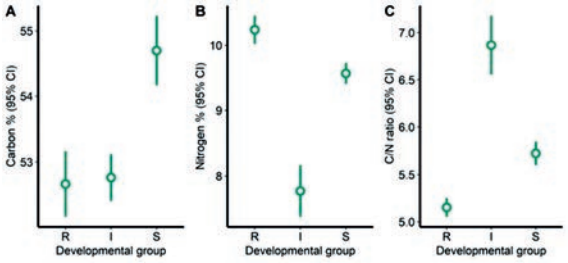
We evaluated the influence of intensive fish farming on the condition of water bodies used for the aquaculture and the environment, concentrating on the impact of the aquaculture on the surrounding water ecosystems as well as the possibility of transferring the pollutants and antibiotic resistance genes to both environment and the human hosts. All the tested sediment samples did not show significantly elevated heavy metal concentrations and no substantial veterinary antibiotic pollution. However, despite the lack of heavy metal and antibiotic pollution, the sediments showed toxicity, the cause of which should be explored more [1].



Distribution of heavy metals concentrations in the fish farming pond sediments: (**A**) Co, (**B**) Cr, (**C**) As, (**D**) Cu

Developmental Speed Affects Ecological Stoichiometry and Adult Fat Reserves in *Drosophila melanogaster*

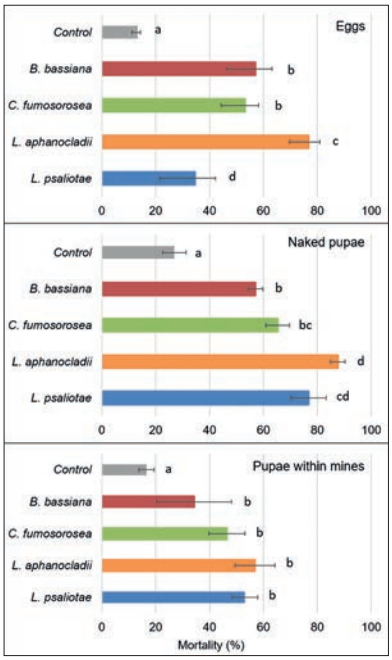
Associations between adaptive variations in developmental speed and elemental body composition are not well understood. We compared body mass, elemental body composition, food uptake and fat metabolism of *D. melanogaster* male fruit flies in relation to their larval development speed. Slowly developing flies had higher body carbon concentration than other groups of flies. Rapidly developing flies had the highest body nitrogen concentration, while slowly developing flies had higher body nitrogen levels than flies with intermediate speed of development. The carbon-to-nitrogen ratio was therefore lowest in rapidly developing flies. Overall, this study shows that a combination of bet-hedging, adaptive tracking and developmental plasticity enables fruit flies to respond adaptively to environmental uncertainty [2].



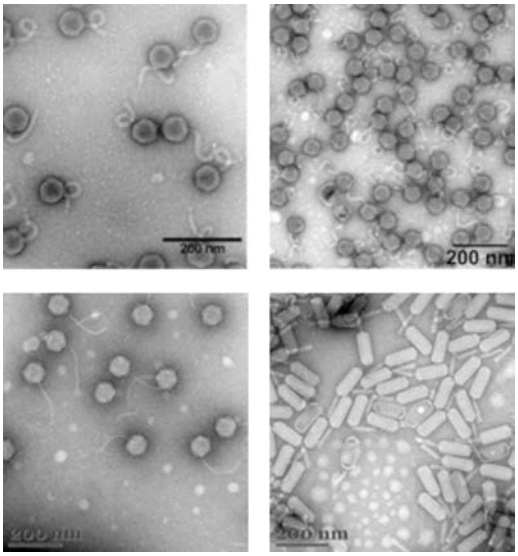
(**A**) Average (±95%CI) carbon, (**B**) nitrogen and (**C**) average carbon-to-nitrogen ratio (C/N ratio) in adult *D. melanogaster* of rapid (R), intermediate (I) and slow development (S) groups

Fungi Associated with Horse-Chestnut Leaf Miner Moth *Cameraria ohridella* Mortality

The total mortality of the leaf miner horse-chestnut pest, *Cameraria ohridella*, collected in nature, and the mortality associated with mycoses were assessed under laboratory conditions in stages: for eggs mortality rates of 9.78% and 61.97% were found, respectively; for caterpillars, 45.25% and 5.59%, respectively; and for pupae 21.22% and 100%, respectively. In the caterpillar and pupa stages, saprophytic fungi were most often recorded [4].



Mortality of *C. ohridella* eggs, naked pupae and pupae within the mines treated with *Lecanicillium aphanocladii, L. psalliotae, Beauveria bassiana* and *Cordyeps fumosorosea* (Tukey's test, $p<0.05$)



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Genetic and Structural Diversity of Bacteriophages

Bacteriophages (phages), the viruses that infect bacteria, are probably the most numerous biological entities on the planet, and they are also exceptionally diverse. Despite the fact that phages as model organisms have featured in many of the key studies of the last century and basically have helped transform biology into a modern science, they remain to be of great significance both in fundamental and applied research. For example, to combat the ever-growing antibiotic resistance in bacteria, a variety of promising phage-inspired antibacterial approaches as well as innovative techniques based on phage-borne enzymes (e.g., lysins) or structural proteins (e.g., tail spike/fibre) are being developed. The results obtained while studying unique phages isolated from different ecosystems by the scientists of the Department of Molecular Microbiology and Biotechnology show that the diversity of phages, in terms of virion structure, physiology and genetics, is enormous, and that we have not even begun to properly harvest it. In fact, every single phage studied not only provides novel insights into the nature of bacterial viruses, but can also be used as a source of novel building blocks for the construction of multifunctional nanomaterials or can be exploited in the detection/biocontrol of pathogenic bacteria.

The phage group of the Department of Molecular Microbiology and Biotechnology has long focused on the isolation and molecular analysis of novel phages with unique structure, host range or physiology. Over the last five years, researchers of the Department carried out a number of projects funded by Vilnius University (MSF-LMT-2) and the Research Council of Lithuania (P-MIP-19-259, 01.2.2-LMT-K-718-03-0099, 01.2.2-LMT-K-718-01-0019, 09.3.3-LMT-K-712-19-0102). Several projects were conducted in collaboration with the research groups of the Institute of Biotechnology (S-MIP-17-47), the Nature Research Centre (P-MIP-17-6, P-MIP-20-256) and the Institute of Biosciences (SIT-7/2015), as well as Kyoto University (S-LJB-17-1). The aims of the projects ranged from the investigation of gene expression profiles of novel bacteriophages, and elaboration of new systems for genome engineering of lytic bacteriophages to the investigation of molecular mechanisms of adaptation of viruses to the specific growth conditions. A number of unique *Achromobacter*, *Pantoea*, and *Escherichia coli* phages have been isolated, characterised and published in the process [1–5].

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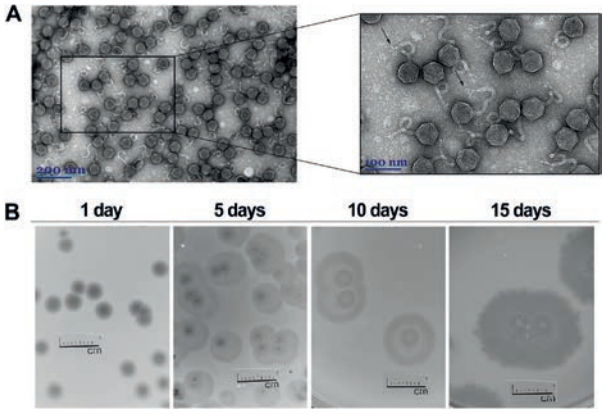
Pantoea Bacteriophage vB_PagS_MED16 - Siphovirus Containing 2´-deoxy-7-amido-7-deazaguanosine-Modified DNA

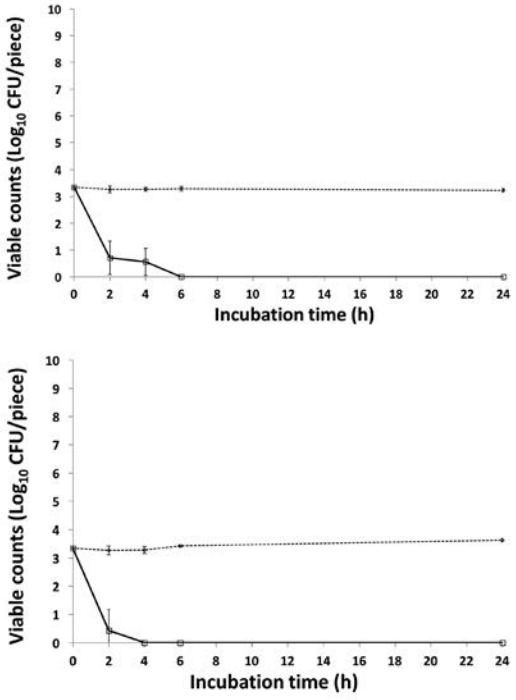
A novel siphovirus, vB_PagS_MED16 (MED16) was isolated in Lithuania using *Pantoea agglomerans* strain BSL for the phage propagation. The double-stranded DNA genome of MED16 (46,103 bp) contains 73 predicted open reading frames (ORFs) encoding proteins, but no tRNA. Comparative sequence analysis revealed that 26 of these ORFs code for unique proteins. Based on phylogenetic analysis, MED16 represents a new genus with siphovirus morphology. In total, 35 MED16 ORFs were given a putative functional annotation, including those coding for the proteins responsible for virion morphogenesis, phage–host interactions, and DNA metabolism. In addition, a gene encoding a preQ0 DNA deoxyribosyltransferase (DpdA) present in the genome of MED16 and the LC–MS/MS analysis indicates 2′-deoxy-7-amido-7-deazaguanosine (dADG)-modified phage DNA, which, to our knowledge, has never been experimentally validated in genomes of *Pantoea* phages. The data presented in this study provide new information on *Pantoea*-infecting viruses and offer novel insights into the diversity of DNA modifications in bacteriophages (Šimoliūnienė et al. *Int J Mol Sci.* 2021, 22(14): 7333).



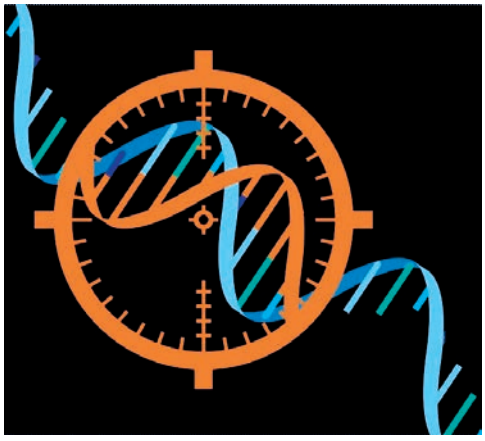
Electron micrographs of CsCl-purified MED16 particles (A) and morphology of plaques formed by phage MED16 on a lawn of *Pantoea agglomerans* strain BSL (B). (A) Black arrows indicate tail fibres; (B) plates were incubated at 22 °C, numbers above indicate days of incubation

Low-Temperature Virus vB_EcoM_VR26 Shows Potential in Biocontrol of STEC O26:H11

Shiga toxin-producing *Escherichia coli* (STEC) O26:H11 is an emerging foodborne pathogen of growing concern. Since current strategies to control microbial contamination in foodstuffs do not guarantee the elimination of O26:H11, novel approaches are needed. Bacteriophages present an alternative to traditional biocontrol methods used in the food industry. Here, a previously isolated bacteriophage vB_EcoM_VR26 (VR26), adapted to grow at common refrigeration temperatures (4 and 8 °C), has been evaluated for its potential as a biocontrol agent against O26:H11. After 2 h of treatment in broth, VR26 reduced O26:H11 numbers ($p<0.01$) by >2 log10 at 22 °C, and ~3 log10 at 4 °C. No bacterial regrowth was observed after 24 h of treatment at both temperatures. When VR26 was introduced to O26:H11-inoculated lettuce, ~2.0 log10 CFU/piece reduction was observed at 4, 8, and 22 °C. No survivors were detected after 4 and 6 h at 8 and 4 °C, respectively. Although at 22 °C, bacterial regrowth was observed after 6 h of treatment, O26:H11 counts on non-treated samples were >2 log10 CFU/piece higher than on phage-treated ones ($p<0.02$). This, and the ability of VR26 to survive over a pH range of 3–11, indicates that VR26 could be used to control STEC O26:H11 in the food industry (Zajančkauskaitė et al. *Foods*. 2021, 10:1500).



Effect of phage VR26 on numbers of STEC O26:H11 on fresh lettuce at 4 (a), and 8°C (b). Solid line, VR26-treated lettuce pieces; dashed line, control. Bars present standard deviation



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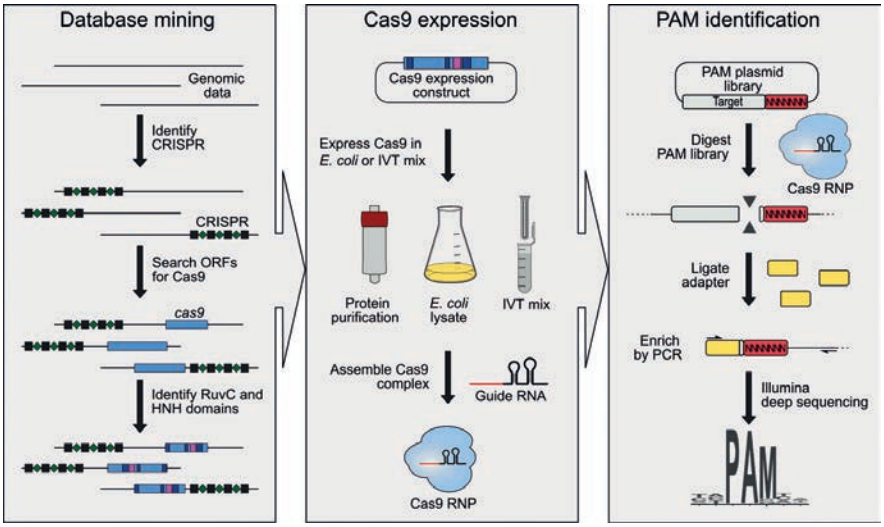
CRISPR-Cas Tools and Technologies

In recent years, Cas9 has revolutionized the genome-editing field and enabled a broad range of applications from basic biology to biotechnology and medicine. Cas9 can be guided to specific locations within complex genomes by a short RNA molecule [1]. Over the past several years, Cas9 and Cas12 endonucleases have been adopted as robust genome editing and transcriptome manipulation tools. Although both nucleases have been widely used, the size of Cas9 and Cas12 provides constraints on cellular delivery that may limit certain applications, including therapeutics that use the cargo-size-limited adeno-associated virus delivery vehicle. Recently, exploration of the natural diversity of the CRISPR-Cas systems identified more compact Cas systems [2, 3] that offer more therapeutic options and functionality. Moreover, we have shown recently that a small TnpB protein (400 aa) of *Deinococcus radiodurans* ISDra2 transposon functions as a programmable nuclease that is guided by non-CRISPR RNA and could be harnessed for genome editing applications [4]. This establishes TnpB as a prototype of a new system for genome editing.



To characterize DNA cleavage requirements of Cas9 nucleases we developed a phylogeny-guided bioinformatics approach that could be easily adapted for the characterization of other CRISPR-Cas nucleases that require PAM sequences and generate double-strand breaks following target recognition (Karvelis et al. *Methods Enzymol*. 2019, 616: 219–240)

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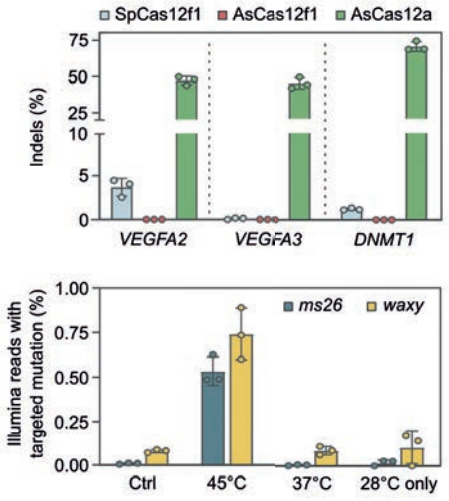
Miniature Type V-F CRISPR-Cas Nucleases Enable Targeted DNA Modification in Cells

Class 2 CRISPR systems are exceptionally diverse, nevertheless, all share a single effector protein that contains a conserved RuvC-like nuclease domain. Interestingly, the size of these CRISPR-associated (Cas) nucleases ranges from >1000 amino acids (aa) for Cas9/Cas12a to as small as 400–600 aa for Cas12f. For *in vivo* genome editing applications, compact RNA-guided nucleases are desirable and would streamline cellular delivery approaches. Although miniature Cas12f effectors have been shown to cleave double-stranded DNA, targeted DNA modification in eukaryotic cells has yet to be demonstrated. Here, we biochemically characterize two miniature type V-F Cas nucleases, SpCas12f1 (497 aa) and AsCas12f1 (422 aa), and show that SpCas12f1 functions in both plant and human cells to produce targeted modifications with outcomes in plants being enhanced with short heat pulses. Our findings pave the way for the development of miniature Cas12f1-based genome editing tools.



Genome modification by miniature nucleases in HEK293T (top panel) and plant cells (bottom panel)

Transposon-Associated TnpB – Novel Tool for Genome Editing

We have shown that TnpB protein of *Deinococcus radiodurans* ISDra2 transposon functions as a programmable nuclease that is guided by an RNA, derived from the right-end element of a transposon, to cleave DNA next to the 5′-TTGAT transposon-associated motif (TAM). We further demonstrated that TnpB can be adopted for genome editing of human cells (HEK293T). Plasmids encoding the TnpB protein fused with a nuclear localization sequence and reRNA constructs targeting five 20-nt sites next to the 5′-TTGAT TAM sequence in human genomic DNA (gDNA) were transiently transfected into HEK293T cells, and genomic DNA was analysed by sequencing for the presence of insertions and deletions (indels) at the targeted cleavage sites, indicating DSB repair events and genome editing. At the two tested sites

RNA-guided genome editor	Cas9	Cas12	TnpB
System	CRISPR-Cas	CRISPR-Cas	IS200/IS605 and IS607
Protein	1000-1500 aa	500-1500 aa	400 aa
gRNA	crRNA and tracrRNA	crRNA or crRNA and tracrRNA	reRNA
Effector complex (protein:gRNA)	1:1	1:1 or 2:1 (Cas12f)	1:1
Nuclease active site	HNH and RuvC	RuvC	RuvC
dsDNA target	Target and 3' PAM	5' PAM and target	5' TAM and target

(*AGBL1-2* and *EMX1-1*), TnpB introduced mutations at frequencies of 10–20%, similar to the levels observed for CRISPR–Cas9 and Cas12-based editing. These results indicate that compact RNA-guided TnpB nucleases can cleave eukaryotic gDNA and may be adopted as tools for genome editing.

Workpackage 4: Alternative Genome Editing Tools for the Retina

Over the past years, Cas9 and Cas12a nucleases have been adopted as robust genome editing and transcriptome manipulation tools. To recognize and cleave a dsDNA target, both Cas9 and Cas12 require a short sequence, termed the protospacer adjacent motif (PAM), in the vicinity of a DNA sequence targeted by the gRNA. The PAM requirement imposes a serious bottleneck in therapeutic editing, where often a precise targeting close to the mutation site is required. If there is no PAM near the target site, Cas9 and Cas12a proteins cannot access it. Next, there are difficulties with the delivery of Cas9 and Cas12a into tissues or cells to achieve therapeutic effects. AAVs often would be a preferable choice, however, these vectors have their own cargo packaging limit, thus small size Cas nuclease variants are highly desirable. Here we aim to assess the therapeutic potential of novel Cas9 orthologues and variants that cut close to the desired

EUROPEAN JOINT PROGRAMME RARE DISEASES

Project partners: NL, IT, SW, BE, DE, FR

Genetic therapy for *EYS*- and *USH2A*-associated retinal dystrophy

GET-READY

mutation sites in *EYS* and *USH2A*, and explore the gene editing potential of the miniature Cas12f nucleases in HEK293 cells. The most promising Cas nucleases will be selected and further optimized in either wild type, or patient derived retinal organoids, in collaboration with partners within the consortium.

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Advanced Microfluidics Technology for Biological and Biomedical Applications

Over the last few years, microfluidics have been established as an enabling technology in biological and biomedical sciences. Using droplet microfluidics technology highly monodisperse, aqueous droplets are generated in an inert carrier oil, and each droplet functions as an independent micro-scale reactor. In other words, each droplet is the equivalent of a well (or tube), yet the volume of a droplet is roughly a thousand to a million times smaller. Obviously, such significant reduction in reaction volume provides huge savings in reagent costs, when performing large numbers of reactions in a massively-parallel fashion. Furthermore, unlike the conventional microtiter plates or valve-based microfluidics, droplets are intrinsically scalable: the number of reaction "wells" is not limited by the physical dimensions of the chip but scales linearly with the emulsion volume. Different microfluidic modules can be employed to manipulate droplets in a sophisticated, yet highly controllable manner, therefore opening new opportunities for biology-related research. Our lab members are working at the interface of biology and biochemistry, physics and chemistry, engineering and computational biology and together we are developing novel microfluidic tools and molecular biology assays to address fundamental questions in cell biology and biomedicine. In 2021, our group members have collaborated with Harvard University, ETH Zurich and MSKCC to advance single-cell biology research in cancer, immunology and beyond.

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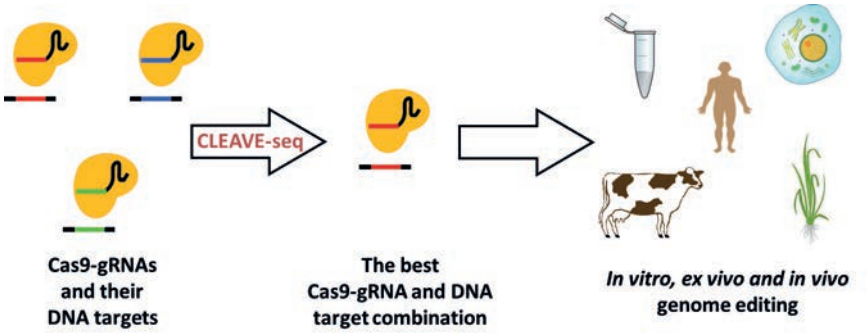

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Evaluation of the Specificity of Genome Editing Tools

The recent development of CRISPR-Cas9 technology revolutionized genome editing field. Due to versatility to target almost any DNA sequence in the genome, CRISPR-Cas9 technology has a potential to be adopted for human genome editing therapy. Variety of studies demonstrated the possibility to modify human genome; however, one of the limits that the technology is facing to be adopted in clinic is its safety. It was demonstrated that various Cas9 nucleases are prone to cleave the DNA sites that are similar to the target sequences (off-target cleavage), resulting in the chromosome rearrangements or mutations causing cell death or even their transformation to cancer cells. Therefore, in order to make genome editing technology safer it is crucial to utilize a sensitive and reliable method for the detection of double-strand breaks (DSB) to evaluate the specificity of a selected DNA endonuclease in every particular case. The successful development of the technology is critical not only for fundamental research to detect and quantify DSBs induced using DNA endonucleases (CRISPR-Cas9 nucleases, zinc finger nucleases, TALEN nucleases, restriction endonucleases etc.), but also for institutions and companies like, to make the application of nuclease-based (including Cas9) technology safer for personalized genome editing and engineering in the clinic. Until now, there were various off-target detection methods published; however, they lack sensitivity, are experimentally complicated and require large coverage in sequencing what make them expensive. Furthermore, the lack of user-friendly protocols limits their applicability.

Our multidisciplinary team is working to develop a sensitive, cost-efficient, high-throughput and user-friendly DSB detection method that will allow determination of DNA endonuclease specificity for broad interest laboratories. The method will be adopted for Illumina and Oxford Nanopore Technologies sequencing platforms, making it available not only for the institutions containing high-throughput sequencing capabilities, but also for small laboratories reluctant to invest vast amount of finances for sequencing equipment and infrastructure. Developing the method we collaborated with Corteva Agricience, DuPont, and our method prototype CLEAVE-seq was successfully used in maize genome editing, resulting in a publication and a patent application [1]. The DSB detection method is currently under further development (CPMA grant No. 01.2.2-CPVA-K-703-02-0010).

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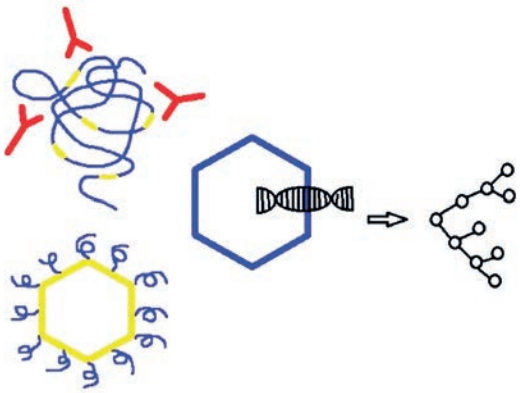

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Biosynthesis of Chimeric and Native Proteins

We investigate aspects related to the production of recombinant proteins in yeast expression systems and the development and optimization of expression systems dedicated to the production of recombinant proteins as virus-like particles (VLPs). VLPs generated in a yeast expression system of viral capsid and envelope proteins have an intrinsic capability of self-assembling into highly organized particles, often without the need for additional viral components. VLPs can induce a strong humoral immune response because of the correct folding of the monomeric proteins, the resulting formation of conformational antigenic determinants and the multimeric structure of identical subunits. Our aim is to understand and compensate the processes in yeast that are triggered by the synthesis of recombinant proteins and to identify the relevant factors for the efficient expression of recombinant proteins. In an attempt to elucidate the requirement of factors for the biosynthesis of recombinant viral and human proteins, we use proteomics, yeast mutant and gene collection studies. Our team is also interested in the search and characterization of new viruses as well as protein engineering based on the construction of chimeric VLPs that harbour foreign epitopes. Yeast-expressed recombinant proteins are applied in the tests for the detection of virus-specific antibodies in human serum and oral fluid samples. A large collection of more than 40 different VLPs derived from various polyomavirus VP1 proteins and papillomavirus 6, 16, 18, 31, 33 L1 proteins were generated. The proteins of measles, mumps, rubella, parainfluenza viruses (1–4), hantaviruses, porcine parvovirus, human bocaviruses (1–4), human metapneumovirus, hepatitis E, and human chaperons (calreticulin and BiP) were produced in yeast cells. Commercially available Microimmune (UK) measles and mumps diagnostic tests are based on the proteins developed in the Department. Moreover, we are focusing on the analysis and research of recombinant biopharmaceutical proteins and recombinant allergen proteins. Our studies include the exploration of a plant expression system for the transient production of a recombinant protein in *N. benthamiana*. We also concentrate on the research of plant anthocyanin synthesis regulation. Prof. D. Balčiūnas from Temple University, Philadelphia, has joined our group, and new research in the studies of molecular mechanisms of heart regeneration was started.

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Analysis of SARS-COV-2 Genomes Isolated from Lithuanian Patients

Genetic mutations of Sars-CoV2 virus detection study in Lithuania was established by the Decision of the Minister of Health as of February 04 2021, no V-221 "Organizing, coordinating and investigating in-depth testing of Sars-CoV-2 in the process of detecting genetic mutations". The aim of this project is sequencing of SARS-COV-2 genomes isolated from Lithuanian patients during the pandemic time and analysing the changes of the virus. The data obtained are shared by submitting it to the public databases such as GISAID. Sequencing of SARS-COV-2 genomes can support 'genomic epidemiology'- characterizing the virus and helping public health authorities to understand the identity of the virus, whether it is changing and how it is being transmitted - all in conjunction with other epidemiological data. On the other hand, genetic sequencing will help monitor COVID-19 mutations circulating in Lithuania, which can potentially improve diagnostic testing in time and foresee vaccination effectiveness (Dudas et al. 2021). During 2021, we sequenced more than 1500 SARS-COV-2 genomes circulating in Lithuanian patients.

New Technologies for Development of Recombinant Allergens

The main goal of this project was to develop a new universal platform for expression and purification of recombinant protein allergens and to prepare open access collection of recombinant allergens including allergen expression plasmids, strains-producers and allergen protein samples. The biosynthesis of recombinant allergens in the most relevant hosts such as bacteria, yeast, plant and mammalian cell cultures are in the process of implementation. The obtained results show that the platform is well applicable for bacterial expression systems (more than 40 allergens tested). Yeast- and plant-derived recombinant allergens are more sensitive to the host cell-protein modifications different from natural allergens, but still could be applicable in the cases, when bacterial hosts show incorrect alterations in allergen structure. Demonstrating application opportunities of the majority of produced and purified allergens have provided the positive binding results (ELISA and Western blot data) with allergic human IgE antibodies responsible for the allergic diseases.

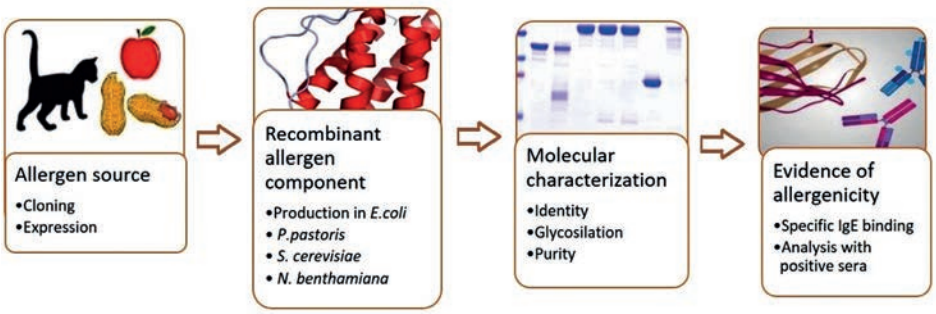


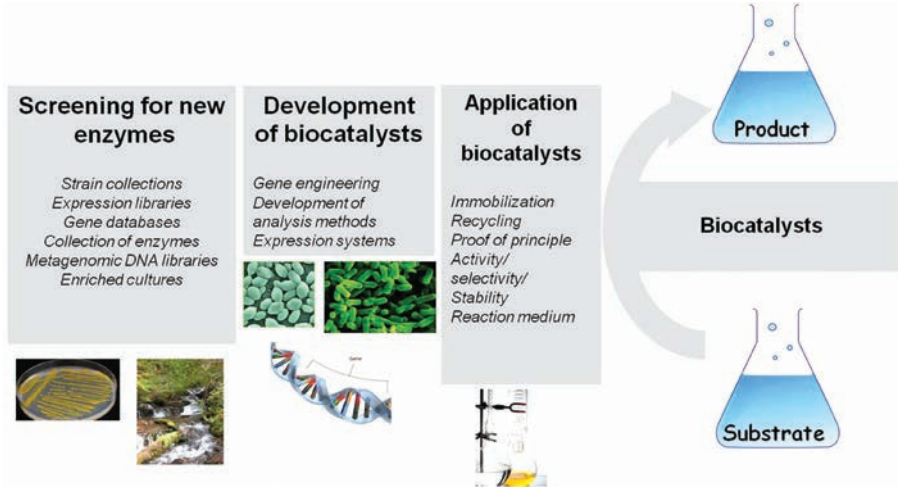
Fig. 1. Steps in production and characterization of allergen components.

New Methods of Screening for Treatment of Neurodegenerative Disorders

A range of recombinant human endoplasmic reticulum (ER) chaperones, which potentially could inhibit aggregation of proteins involved in progression of neurodegenerative disorders (ND), are tested using both *in vitro* and *in vivo* models for these diseases. Recombinant human ER chaperones in molecular interaction models as potential inhibitors of amyloid-like fibril formation are evaluated. Inhibition of aggregation was assessed using several model proteins related to different ND, such as Alzheimer's and Parkinson's diseases, amyotrophic lateral sclerosis, prion-related disorders and multiple sclerosis. Specific ND models in experimental animals are selected for further testing.

Molecular Mechanisms of Heart Regeneration. Genetic and Molecular Analysis of the Role of Tbx5a in Heart Regeneration

In order to improve regenerative capacity of the human heart, we must first thoroughly understand how this process occurs in an organism, which has innate regenerative ability. Zebrafish *Danio rerio* is an excellent model organism to study hurt regeneration. In our laboratory, we aim to identify transcriptional program required for cardiac regeneration to occur. We are focusing on two highly conserved transcription factors: *Tbx5a* in the myocardium and *Tcf21* in the epicardium. Using a conditional gene trap, we have demonstrated the *Tbx5a* essential for regeneration to occur (Grajevskaja et al. 2018, *PMID*: 29933372). In our studies, we are using CRISPR/Cas9 to engineer epitope-tagged alleles (Burg et al. 2016, *PMID*: 27892520) and conditional mutants (Burg et al. 2018, *PMID*: 30427827), and characterize these mutants using genomics, developmental biology and molecular biology methods.



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Development and Application of Biocatalytic Systems

Biocatalysis, which applies natural biological substances (microorganisms, enzymes, etc.) in various industrial processes, is one of the most popular alternatives to traditional technologies. The use of such biocatalysts fulfils the requirements that are needed for sustainable synthesis. They are very appealing as they exhibit high enantio- and region-selectivity toward targeted substrates and function under mild reaction conditions: a water/buffer medium, ambient reaction temperatures, no pressure is required. These advantages allow avoiding the burden of group-protecting procedures, saving time, materials (including the harsh, dangerous or toxic ones) and energy costs. Other advantages of biocatalysts are that they are easy to control and biodegradable. Thus, biocatalysis has proved, in many cases, to be a more superior pathway than the pathways of conventional chemical synthesis, not only in the simplicity of accomplishing the reactions but also from an economical and environmental point of view. Currently, enzymes are already used in many industries such as food, detergents, textiles, leather, wood and paper manufacturing, diagnostics and therapy, pharmaceuticals etc. Due to their wide application, the market of enzymes is growing very fast every year. Today, more than 2000 biocatalytic processes are implemented in industrial settings.

Our team focuses on the discovery and engineering of biocatalysts with properties for potential industrial application and development of efficient biocatalytic routs for producing the high-added value products from bio-based raw materials or industrial by-products. The sector's research is based on developing biocatalytic systems by screening for enzymes (environmental samples, enzyme and strain collections, metagenomic and expression libraries, the development of screening systems etc.); the development of biocatalysts (gene engineering, the development of analytical systems, protein purification, the development of expression systems etc.); the application of biocatalysts (immobilization, recycling, proof of principal activity/selectivity, stability, reaction media, an improved efficiency of bioconversions, the quality analysis of products obtained by biocatalysis etc.). We also strive to meet scientific challenges in the application of Green Chemistry principles in technologies and processes.

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Biodegradation of Polymers and Plastics

One of sector's topics of interest is biodegradation of various synthetic polymers and plastics. Synthetic polymers are used in various sectors; hence their recyclability is different and depends on their chemical structure. One of the hardiest synthetic polymers is polyurethanes (PU). Nonetheless, it is known to be biodegradable to some extent. The goals of our research are to explain this biodegradation phenomenon, and to identify enzymes and organism that are capable to degrade it. For this purpose this year various environmental samples and fungi collection of Mycotheca Universitatis Taurinensis (Italy) have been screened, identifying potential PU degrading organisms. Additionally, enzymatic PU degradation was being investigated using recombinant urethanase (ID: TBS 101 urethanase) produced in *Pichia pastoris* as expression host.

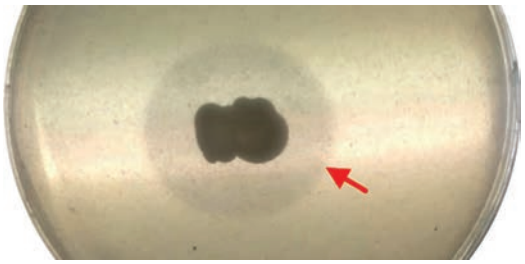


Fig. 1. Hydrolysis zone formed by *Kondoa* sp. on a selective growth media containing polyether PU. Yeast was isolated from microplastic waste found in Mediterranean Sea by The Mycotheca Universitatis Taurinensis.

Synthesis of Substrates for Functional Analysis

Laccase is an oxidoreductase used for various applications and shows the potential for even higher applicability and could substitute the most common commercial oxidizing agent, hydrogen peroxide. Nonetheless, current laccase research and utilization are limited by few factors. One of these factors is a lack of stable substrate variety for the functional analysis. In the current PhD project, we have developed a substrate named Ferbamine for laccase activity screening on agar plates. Our investigation has showed that this substrate is highly compatible with protein samples (Fig.2) and grown bacteria.

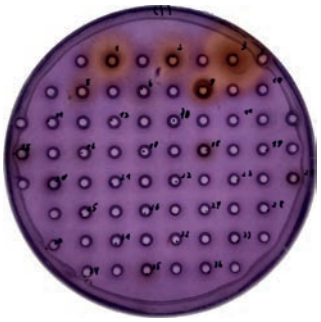


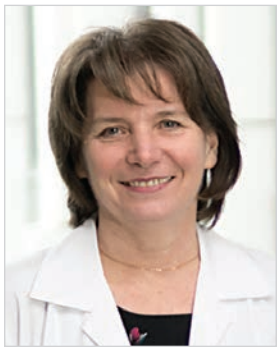
Fig. 2. Visual presentation of the developed agar-plate screening method.

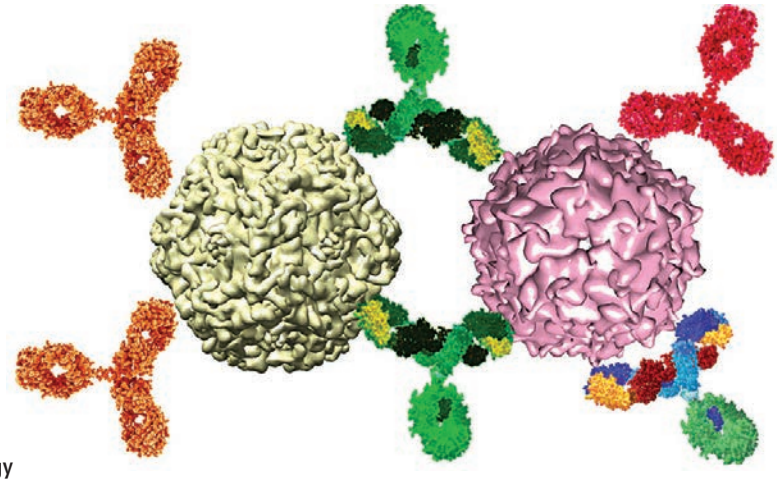
European Transdisciplinary Networking Platform for Marine Biotechnology

Marine organisms produce a vast diversity of various enzymes proteins, metabolites; up to now, over 35,000 natural products have been characterized from marine organisms, but many more are yet to be uncovered, as the vast diversity of biota in the marine systems remains largely unexplored. Since marine biotechnology is still in its infancy, there is a need to create effective, operational, transnational and transdisciplinary networks with a serious and ambitious commitment for knowledge transfer, training provision, dissemination of best practices and identification of the emerging technological trends through science communication activities. The Sector of Applied Biocatalysis took part in the establishment of such collaborative framework – Ocean4Biotech (www.ocean4biotech.eu) COST Action, which connects stakeholders with an interest in marine biotechnology in Europe and beyond. In 2020–2021, close scientific collaboration took place through visits, sample exchange, joint publications with the University of Torino (Italy), Jožef Stefan Institute (Slovenia) and Marine Research Institute (Klaipėda University).

Novel High-Performance Polymers from Lignocellulosic Feedstock

It is becoming increasingly clear that the society at large and the whole industry in particular need a transition from an unsustainable, fossil-based linear economy to a sustainable circular economy, based on the utilization of solar energy. Bio-based chemicals have great potential to reduce and replace the fossil feedstock used by chemical industry today. The project develops new sustainable platform for novel bio-derived polymers used in applications with strict demands for properties (e.g. automotive industry, coatings, packaging, etc). As a raw material, a stream of wood sugars, which very recently became available and are derived from low quality wood residues, is used. Importantly, the environmental benefits and possible adverse effects are fully analysed throughout the project, enabling the optimization from the sustainability point of view. The environmental sustainability profile of the bio-based polymers studied will be benchmarked against the conventional fossil-derived plastics. The project under Baltic Research Programme is implemented by close collaboration of the Sector of Applied Biocatalysis, University of Tartu, Norwegian University of Science and Technology and the Estonian company Graanul Invest.





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Generation and Analysis of New Antibodies

Monoclonal and recombinant antibodies are widely used in biotechnology, medicine and biomedical science. Monoclonal antibodies produced using traditional hybridoma-based technologies are valuable research tools and clinical diagnostic reagents. Recombinant antibodies generated by gene engineering approaches are increasingly being used as therapeutic agents for the treatment of cancer, autoimmune and infectious diseases. Therefore, there is a strong need for novel, well-characterized antibodies with the desired specificities and other characteristics.

Our team has extensive expertise in the development and characterization of monoclonal and recombinant antibodies. We have generated more than 500 monoclonal antibodies against different targets: viral antigens, bacterial virulence factors, cellular proteins [1], cytokines, hormones, allergens. The largest antibody collection is generated against viral antigens, including measles, mumps, human parainfluenza viruses, henipaviruses, hantaviruses, parvoviruses, human bocaviruses, hepatitis B virus, hepatitis E virus and others. These antibodies are valuable tools for investigating the antigenic structure of viruses, the development of diagnostic assays and the prevalence studies of viral infections. Virus research is carried out in collaboration with Prof. Dr. R. Ulrich (Friedrich-Loeffler-Institute, Greifswald-Insel Riems, Germany), Prof. Dr. D. Glebe (Justus Liebig University Giessen, Germany), J. O. Koskinen (ArcDia International Oy Ltd., Turku, Finland) and other partners. Together with our colleagues from the Department of Eukaryote Gene Engineering, we have developed a new technology for the use of virus-like particles as a carrier for target epitopes to increase their immunogenicity. This approach provides possibilities to generate antibodies against short and non-immunogenic protein sequences. For the construction of recombinant antibodies, gene sequences encoding the variable parts of immunoglobulin heavy and light chains are cloned from hybridoma cells producing well-characterized monoclonal antibodies against the target of interest. Recombinant antibodies are developed in different formats – as single chain antibodies (scFv) and Fc-engineered antibodies, where the scFv derived from hybridoma cells are joined to the human IgG Fc fragment [1]. In addition, we have exploited recombinant virus-like particles as carriers for antibody molecules, both scFv and Fc-engineered scFv. This innovative approach allows the generation of recombinant multimeric antibodies displayed on virus-like particles.

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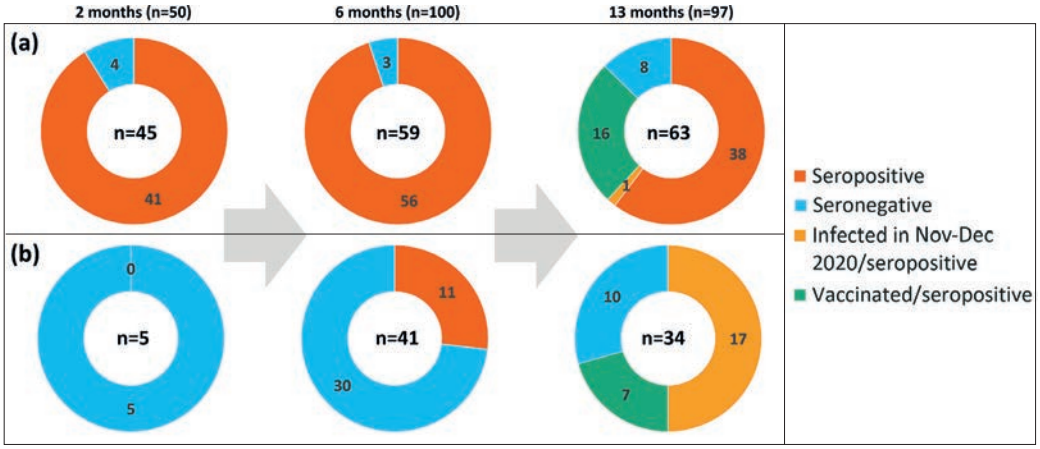
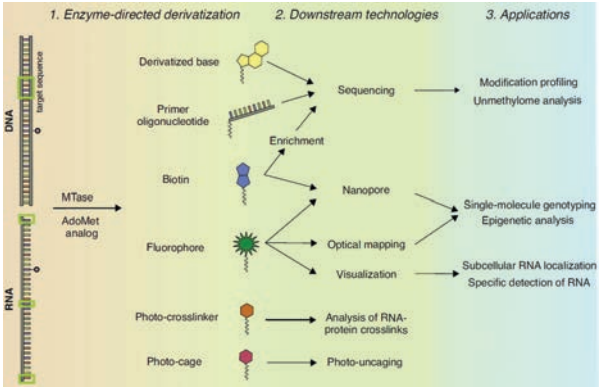
Our team is a member of the EuroMabNet, the European network of laboratories specialised in the production and use of monoclonal antibodies: https://www.euromabnet.com/.

We also participate in the activities of the European Federation of Immunological societies (EFIS), in particular in the EFIS Vaccination task force that provides evidence-based information on COVID-19 vaccines [2].

During COVID-19 pandemic, our team contributed to developing serologic assays for SARS-CoV-2 infection in collaboration with Lithuanian biotech companies *UAB Baltymas* and *UAB Imunodiagnostika*. These serologic tests have been used to investigate the duration of the humoral immune response after SARS-CoV-2 infection [3]. Our study demonstrated the persistence of virus-specific antibodies for 13 months after infection and the correlation of the seropositivity with protection (see Figure below). We have also generated antibodies against SARS-CoV-2 spike (S) and nucleocapsid (N) proteins that were evaluated in sensor-based immunoassays for SARS-CoV-2 detection [4]. The research on SARS-CoV-2 will be continued within the ongoing projects – SMART Specialization project (granted by the Research Council of Lithuania) and the Technological development project (granted by the Agency for Science, Innovation and Technology).



Seropositivity pattern in the groups of study participants tested SARS-CoV-2-specific RT-PCR positive (a) and RT-PCR negative (b) during the outbreak in April, 2020. On the top of each section, time after the outbreak and the number of study participants subjected to serologic testing are indicated (Kučinskaitė-Kodzė et al. *Viruses*. 2021)



Application of mTAG labelling in DNA and RNA

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Molecular Tools for Covalent Labelling and Profiling of Epigenome

Molecular Tools for Targeted Covalent Derivatization of DNA and RNA. Nucleic acids are linear biopolymers comprised of four major types of building blocks encoding the genetic blueprint of life. Analysis of such largely uniform biomolecules can be facilitated by targeted installation of suitable reporter tags. AdoMet-dependent methyltransferases (MTases) uniquely combine two features required for targeted labelling: recognition of a specific target and its covalent modification. We seek to repurpose these enzymes by using as targeting vehicles for the transfer of pre-derivatized (extended) versions of the methyl group from synthetic analogs of the AdoMet cofactor [1] leading to the mTAG technology (methyltransferase-directed Transfer of Activated Groups) for targeted covalent derivatization and labelling of DNA and RNA [4]. Beside their ability to transfer covalent labels on cytosine base in DNA, MTases have been devised to perform atypical C-C bond cleavage reactions, leading to removal of the oxidized one-carbon moieties (hydroxymethyl and carboxyl) and formation of unmodified cytosine in DNA [2]. By combining these MTase-promoted reactions, we develop novel tools for the analysis of epigenetic DNA modifications genome-wide, which could open new avenues in genomic research, diagnostics, and bionanotechnology.

Advanced Methods for Epigenome Analysis. Epigenetic regulation in vertebrates involves chemical variation of one-carbon groups of cytosine residues in CpG dinucleotides by enzymatic production of 5-methylcytosine followed by its oxidized forms 5-hydroxymethylcytosine, 5-formylcytosine, and 5-carboxylcytosine. Genomic distribution of these modified cytosines varies in different cell types, environmental conditions and disease states and is associated with many biological processes such as embryogenesis, establishment of cell identity, and development of pathological conditions, including cancer. However, research into the epigenetic regulation is hampered by limitations of available analytical techniques. Recently, we proposed a high-resolution economical technique named TOP-seq, which exploits non-homologous priming of the DNA polymerase at covalently tagged CpG sites [3]. Using mTAG and other covalent DNA labelling technologies, we develop new experimental approaches for profiling cytosine modifications genome-wide for epigenome studies and improved diagnostics. Our novel technological approaches targeting different DNA and RNA modifications allow sensitive and cost-efficient multiomic analysis of epigenetic changes in various eukaryotic organisms and disease contexts [5].

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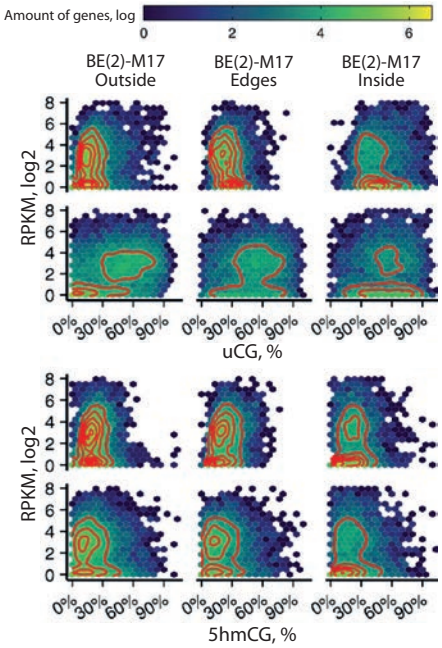
Related Patents: EP2414528 (B1), US8822146 (B2), US8889352 (B2), US9505797 (B2), EP2414527 (B1), LT5706 (B), US9988673 (B2).

Multi-omics Analysis of Neuroblastoma Cells Reveals Diversity of Malignant Transformations

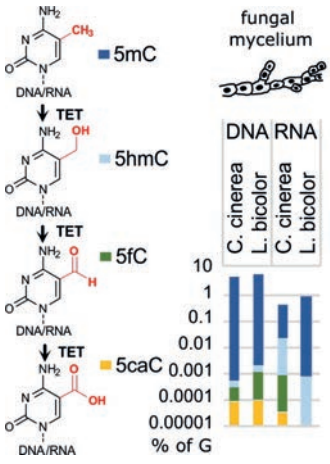
Neuroblastoma (NB) is a paediatric cancer of the developing sympathetic nervous system that exhibits significant variation in the stage of differentiation and cell composition of tumours. We used our recently developed single-base resolution approaches, hmTOP-seq and uTOP-seq, for the construction of 5hmC maps and identification of large partially methylated domains (PMDs) in different NB cell subpopulations. The 5hmC profiles revealed distinct signatures characteristic to different cell lineages and stages of malignant transformation of NB cells in a conventional and oxygen-depleted environment. The analysis of the cell-type-specific PMD distribution highlighted differences in global genome organization among NB cells that were ascribed to the same lineage identity by transcriptomic networks. Collectively, we demonstrated a high informativeness of the integrative epigenomic and transcriptomic research and large-scale genome structure in investigating the mechanisms that regulate cell identities and developmental stages of NB cells (Narmontė et al. *Front. Cell Dev. Biol.* 2021, 9: 727353).



Heatmaps and two-dimensional density estimates for uCG/5hmCGs and expression data distributions for genes localized outside of PMDs, within PMDs and crossing PMD boundaries

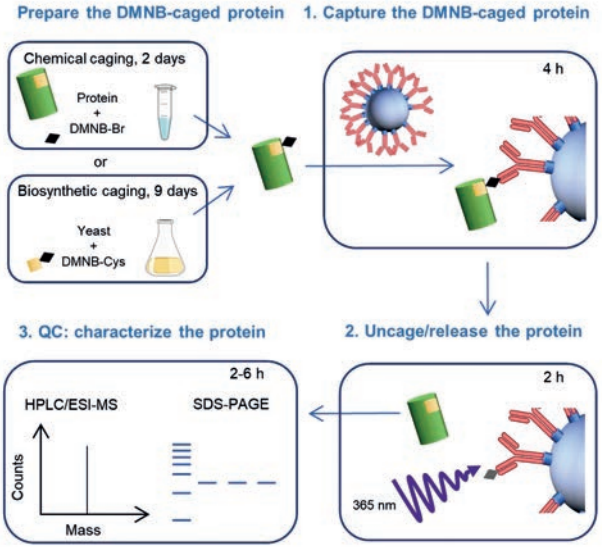
Distribution and Regulatory Roles of Oxidized 5-Methylcytosines in *Basidiomycete* Fungi

The formation of three oxidative DNA 5-methylcytosine (5mC) modifications (oxi-mCs), 5-hydroxymethylcytosine (5hmC), 5-formylcytosine (5fC), and 5-carboxylcytosine (5caC), by the TET/JBP family of dioxygenases prompted intensive studies of their functional roles in mammalian cells. However, the functional interplay of these less abundant modified nucleotides in other eukaryotic lineages remains poorly understood. We carried out a systematic study of the content and distribution of oxi-mCs in DNA and RNA of *Basidiomycetes* fungi, which are established models to study DNA methylation, developmental and symbiotic processes (Ličytė et al. *Open Biology*, in press. DOI: 10.1098/rsob.20160049).



Selective Immunocapture and Light-Controlled Traceless Release of Transiently Caged Proteins

The 4,5-dimethoxy-2-nitrobenzyl (DMNB) photocaging group imbedded into small biomolecules, peptides, oligonucleotides, and proteins is commonly exploited for spatiotemporal control of chemical and biological processes. Here, we demonstrate the use of our developed DMNB-selective monoclonal antibody for non-covalent capture of chemically or biosynthetically produced proteins containing surface-exposed DMNB caging groups followed by light-controlled traceless decaging and release of the bound proteins into solution. Such manipulations are suited for a variety of downstream applications including laboratory production of designer (seleno)proteins and development of miniature light-controlled systems involving microfluidic devices, microchips, and nanoparticles (Rakauskaitė et al. *STAR Protoc.* 2021, 2(2): 100455; EP21187460).



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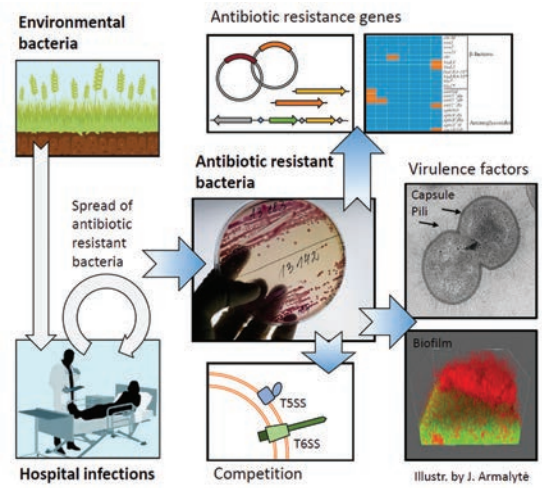

Antibiotic Resistance and Pathogenesis

We focus our research towards understanding the molecular basis underlying the bacterial antibiotic resistance in clinic and in the environment with the emphasis on novel resistance mechanisms and on the bacterial cell features contributing to pathogenesis. In addition to pathogenic microorganisms, environment and commensal microbiota can also serve as pool of genetic features that can be transferred among microorganism giving rise to novel variants. Infections caused by the group of gram-negative bacteria that are resistant to nearly all currently available antibiotics is a serious concern in clinical settings worldwide. Bacteria, previously considered as non-pathogenic, due to their ability to acquire multidrug-resistance and virulence traits, are currently becoming the ones of the most important hospital infection agents. The clinically important opportunistic pathogens cause a variety of difficult-to-treat nosocomial infections to critically ill patients. Their characteristic traits are the ability to withstand prolonged periods of dryness, form biofilms on various surfaces including medical equipment, upregulate intrinsic resistance mechanisms and acquire new resistance genes through plasmids, as well as the ability to adhere and colonise the host cells. All the listed features are crucial in the life of pathogens, and understanding their molecular basis might bring novel insights into pathogenicity and development of novel antibacterial strategies.

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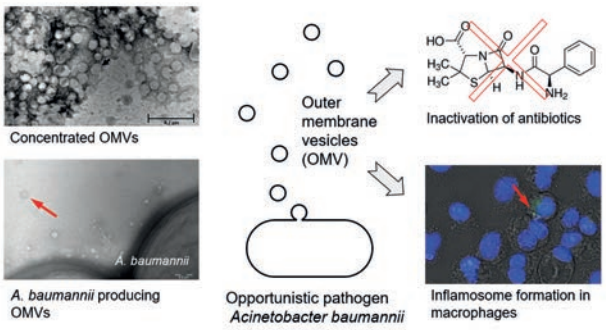
Acinetobacter baumannii Outer Membrane Vesicles (OMVs) Reduce Antibiotic Level and Transfer Virulence Factors into Host Cells

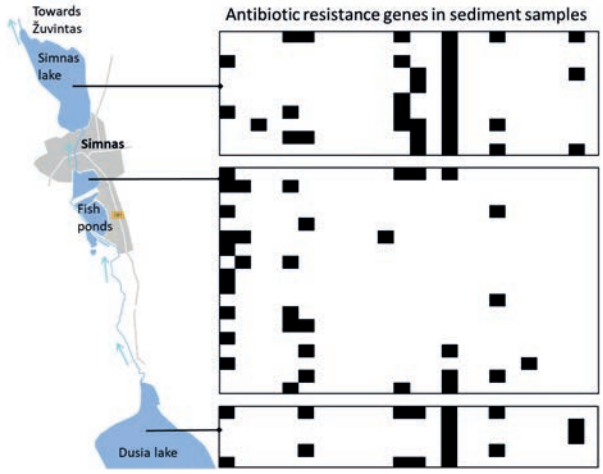
Opportunistic pathogen *A. baumannii* generates OMVs, which pack β-lactamases capable of neutralizing ampicillin. Using this mechanism bacteria can inactivate antibiotics before they enter the cell. In addition, *A. baumannii* packs multiple proteins into OMVs including virulence-related ones such as outer membrane protein A (OmpA). OmpA secreted by OMVs can modulate immune response in macrophages and regulate inflammasome-related cytokine expression. This implies that OmpA protein in bacterial OMVs could trigger a more intense proinflammatory response without the contact with the bacterium itself.



Skerniškytė et al. *Pathogens*, 2021; Skerniškytė et al. Molecules, 2019.

Impact of Fish Farming on Spread of Antibiotic Resistance Genes

Aquaculture is a fast growing animal food sector. As fish are susceptible to infectious diseases, antibiotics are used in aquaculture production systems. The objective of the project is to evaluate the influence of fish farming on environment, concentrating on the spread of antimicrobial resistance genes and resistant bacteria.
Clinically relevant antibiotic resistance genes were analysed in the three water body system in Lithuania. The resistance patterns indicate that the genes are widespread and possibly related to bacterial innate resistance.
The project is carried out together with the Lithuanian University of Health Sciences.



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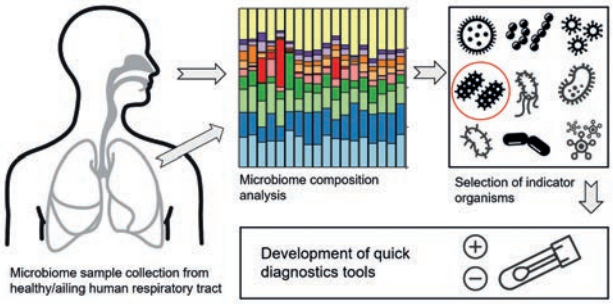
Individualized Analysis of Upper Respiratory Tract Microbiome

Human body, including its respiratory tract, is an ecosystem that includes a variety of microorganisms. As in any ecosystem its health could be predicted by analysing the composition of the inhabitants. In this project, we aim to analyse the human respiratory tract microbiome, and distinguish biomarkers (i.e. microorganism groups) that correlate with health conditions or disease exacerbations. The selected biomarkers can be used to create quick diagnostics tools, providing easily accessible information about a person's respiratory tract health and condition.
The project is carried out together with the Centre for Innovative Medicine and Vilnius University Hospital Santaros Klinikos.



Project *"Individualized Analysis of Upper Respiratory Tract Microbiome – a Novel Diagnostic and Healthcare Tool"* of the Programme for the European Union Funds Investments in Lithuania measure "Targeted Research in Smart Specialisation Areas"

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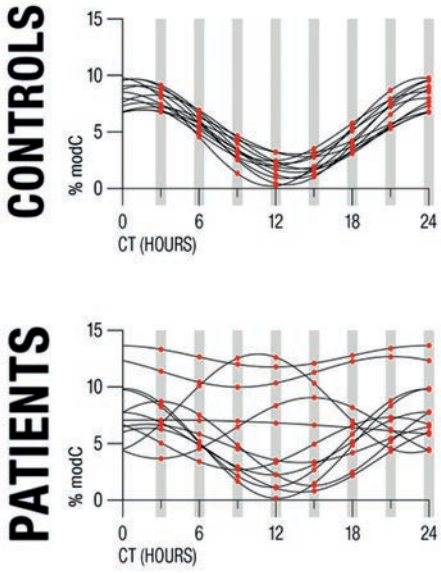

Epigenomic Studies of Human Disease

Twin, family, and adoption studies have surmounted numerous direct and circumstantial evidence supporting the gene-environment paradigm in human disease studies. In the last several decades, however, there is increasing realization that epigenetics, with its malleability and somatic heritability, can serve as a functional extension of genetic predispositions and mediator of environmental risk factors. In our laboratory, we have focussed on the less discussed epigenetic roles, i.e. phenotypic changes in the same genetic background such as disease dynamics and trajectories through the individual's lifespan. Within individuals born with genetic risk factors, how do diseases stay "silent" for a number of decades only to manifest at specific ages? Even more surprising is partial or complete recovery, which may occur spontaneously, without eliminating the causes or application of curable clinical intervention. Patients affected by major psychosis, asthma or attention deficit and hyperactivity syndrome can show significant improvement after years or even decades of suffering from delusions and hallucinations, periods of obstructed breathing, and inability to focus, respectively. The question of phenotypic transformations on the same genetic background - a blind spot of the traditional paradigm in disease studies - is of central importance in epigenetics of disease. In fact, it is simply a new formulation of the primary prerogative of epigenetics which, from the times of C. H. Waddington, has attempted to understand the basic mechanisms of development – changes of cellular phenotypes in genetically identical cells of the same organism.

In 2021, we finalized on building the key principles for chrono-epigenetic (*Greek* "chronos" – time) disease research program in human disease, which puts a strong emphasis on the temporal aspects of epigenetic studies. Chrono-epigenetics can change our perceptions of the key axes and dimensions of health and disease – stability vs dynamics, stochasticity vs determinism, and external vs internal risk factors. We also proposed that investigating temporality and individuality of the epigenome can advance our understanding of phenotypic transformations of health to disease and back to health. Based on this, we propose a new rationale, guidelines, and experimental approaches for chrono-epigenetic and -epigenomic studies of disease, marking the advent of the 3rd generation of epigenome-wide association studies.

We are collecting biological samples for chrono-epigenetic studies of colorectal cancer and psychiatric disease. We have performed a series of wet lab and computational experiments, which provide further support for the chrono-epigenetic theory and help to optimize large-scale chrono-epigenomic studies.

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Strategies in Fighting COVID-19 Pandemic

Since the first reported cases of coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) in China in December 2019, the pandemic has spread rapidly worldwide, resulting in a global slowdown with saturated healthcare systems and disrupted socioeconomic activities. Despite stringent control measures initially deployed by the governments (quarantine, reduction in contacts, work from home policies, etc.), the high transmission rate of SARS-CoV-2 has induced outbreaks in public gathering places as well as workplaces either due to their poor implementation or limited efficiency. In 2021, the emergence of novel virus variants, which were more contagious and could increase the risk of severe illness and consequent hospitalization, generated new waves of the ongoing pandemic. In the current context of insufficient vaccine uptake, development and characterization of methods for environmental monitoring remain important areas of research for identifying and preventing both local outbreaks and global spread. Our group focuses on developing new strategies for SARS-CoV-2 detection and improved diagnostic testing.

Environmental Surface Testing. The SARS-CoV-2 virus is primarily transmitted from person to person through inhalation of infected droplets or aerosols. Presymptomatic and asymptomatic SARS-CoV-2 carriers can be highly contagious, although they have mild or no symptoms and, therefore, are difficult to track. Such individuals can shed a high viral load in their workplace and expose co-workers to constant fomite spread. Based on the principle that detection of viral material on inanimate surfaces indicates at least one infected individual present in that area, we have performed screening-based testing at the Life Sciences Center, Vilnius University, and five other institutions (over 700 samples in total), followed by the validation stage at two institutions of pre-school and pre-primary education (145 samples in total). In over 98% of the cases, high levels of viral RNA in surface swab samples were traced back to one or more infected individuals. Most of the cases were detected prospectively (i.e. they were identified at a presymptomatic stage). The developed methodology was rapidly adopted for routine testing at pre-school and pre-primary education institutions as an attractive alternative to invasive nasopharyngeal or anterior nasal vestibular swabs.

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Genetic and Epigenetic Mechanisms of Cancer Development and Progression

During the last decade, the increased understanding of genetic profile of tumours has personalised cancer treatment through the usage of molecular diagnostic tools. Molecular tests have been developed in order to facilitate both the diagnosis of the disease and the selection of the most effective treatment scheme, as well as to avoid unnecessary interventional procedures for the patient. Using a variety of genome-wide and target-oriented methodologies, our group aims at the (epi)genetic characterization of various human tumours and the development of molecular biomarker systems for cancer detection, molecular classification, prognosis and early identification of resistance development [1–4]. The same tools of genomic analysis have been applied for timely identification of periodontitis-specific alterations [5].

Focusing on altered DNA methylation, epigenetic profile of renal cell carcinoma was recently characterized and the biomarker panel for timely and specific renal cancer detection using urine sediments was developed [2, 3]. Promoter DNA demethylation and the increase in gene *ABCB1* copy number was shown as a novel effective way leading to chemoresistance of breast cancer cell line exposed to doxorubicin and nongenotoxic ABCB1 inducer [4]. Using sensitive tools of genomic analysis, miRNA profile of periodontitis was analysed and circulating miRNA panel was developed for periodontitis diagnostics in various liquid biopsy samples (gingival crevicular fluid, saliva and plasma) [5].

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Clinical Significance of Novel DNA Methylation Biomarkers for Renal Clear Cell Carcinoma

Clear cell renal cell carcinoma (ccRCC) is the most common type of kidney tumour characterized by the highest mortality rate of the genitourinary cancers, and, therefore, new diagnostic and/or prognostic biomarkers are urgently needed. Considering this, the current study aimed to develop a gene-specific DNA methylation tool for non-invasive and early kidney cancer diagnosis and follow-up.

Genome-wide DNA methylation profiling utilizing microarrays allowed the identification of a set of novel presumable ccRCC-specific DNA methylation biomarkers, having moderate to high diagnostic and/or prognostic potential. Moreover, identified DNA methylation alterations at the regulatory regions of selected genes appeared to be amenable for non-invasive detection in the urine samples of ccRCC patients (Fig. 1), where they outperformed the diagnostic and prognostic value of previously described biomarkers and even some other parameters currently used in the clinical practice. Thus, the results showed the promising potential of the selected genes as candidates for further development of non-invasive tools for kidney cancer detection.

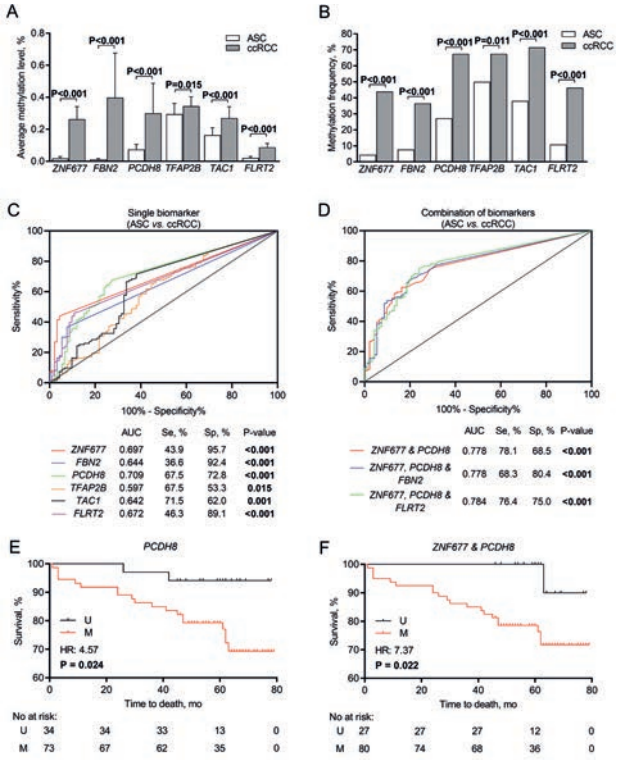

Fig. 1. DNA methylation analysis in urine samples of ccRCC patients and asymptomatic controls (ASC). A – Methylation levels and B – methylation frequencies of the selected genes; C–D – ROC curve analysis for single biomarkers and combination of two-three biomarkers in discriminating patients with ccRCC and ASC; E–F – Kaplan-Meier survival curves according to methylation status of selected genes.

Nongenotoxic ABCB1 Activator TPP⁺ Can Contribute to Doxorubicin Resistance in MX-1 Breast Cancer Cell Line

Hyperactivation of ABC transporter ABCB1 and induction of EMT are the most common mechanisms of acquired cancer chemoresistance. This study describes possible mechanisms that might contribute to upregulation of ABCB1 and synergistically boost the acquisition of doxorubicin (DOX) resistance in breast cancer MX-1 cell line. DOX resistance in MX-1 cell line was induced by a stepwise increase of drug concentration or by pretreatment of cells with an ABCB1 transporter activator TPP+ followed by DOX exposure. Transcriptome analysis (Fig. 2) revealed gradual activation of canonical EMT transcription factors with later activation of ABCB1 in DOX-only treated cells, while TPP⁺ exposure induced considerable activation of ABCB1 possibly through promoter demethylation and the increase in gene copy number. These cells were highly resistant to DOX and showed morphological and molecular features of EMT. The study suggests that nongenotoxic ABCB1 inducer can possibly accelerate development of DOX resistance.

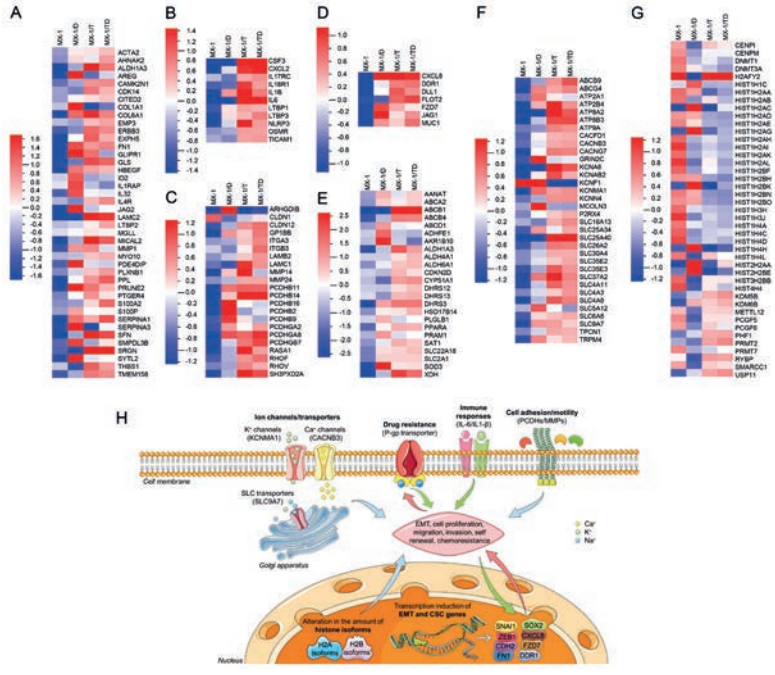

Fig. 2. Cellular responses/pathways induced in chemoresistant MX-1 cell sublines. Heatmaps of differentially expressed genes from gene expression microarrays of chemoresistant MX-1 cell sublines: A – EMT-related, B – immune response-related, C – cell adhesion and motility-related, D – stem cells-related, E – chemoresistance-related, F – ion channels and G – epigenetic regulators. H – the two hypothetical models of chemoresistance development made according the human gene expression microarrays and quantitative PCR results.

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Molecular Mechanisms of Cancer Cell Chemoresistance

Neoplastic diseases are one of the major causes of death worldwide. Chemotherapy, the main strategy for treating cancer, often fails due to the ability of the tumour cells to adjust to the therapy and to become even more malignant. A precise diagnosis of tumours and the development of new therapeutic tools as well as the ability to detect and destroy therapy-resistant cells are the essential areas for a successful tumour therapy. There are several avenues of research for overcoming the cancer treatment problems. First, one must understand the fundamental mechanisms of cancer genesis and target the crippled processes with specific agents. Second, to deal with the constantly rising drug resistance, it is necessary to elucidate the mechanism of drug resistance, to choose and individually apply the second-line therapy. We address these issues by pursuing the following long-term goals: I) to study the molecular mechanisms of cancer cell genesis, including cell signalling *in vitro*; II) by applying high throughput differential quantitative proteomic analysis, to identify signalling pathways and biological processes altered in drug resistant cells; III) to find second-line therapeutics that attacks primary drug-resistant cells.

We focus on the elucidation of novel as well as conventional pathways of cancer cell signalling. In collaboration with R. Prekeris Lab (UC, Denver, Colorado, USA) we investigated the novel role of midbody, cellular organelle previously associated only with cytokinesis, in cancer cell proliferation, survival and drug resistance [1]. The combined analysis of colorectal cancer cells proteome and miRNome enabled us to highlight partial endothelial–mesenchymal transition and altered mutant p53 signalling as the core processes in acquired resistance to standard FOLFOX chemotherapy [3]. In collaboration with L. Graves Lab (UNC, Chapel Hill, NC, USA) we applied kinomic and phoshoproteomic analysis to show the enhanced SCF receptor c-KIT signalling in multidrug-resistant breast cancer cells. The c-KIT inhibitor was shown to be a promising second-line therapy in the treatment of acquired chemotherapy resistance [2].

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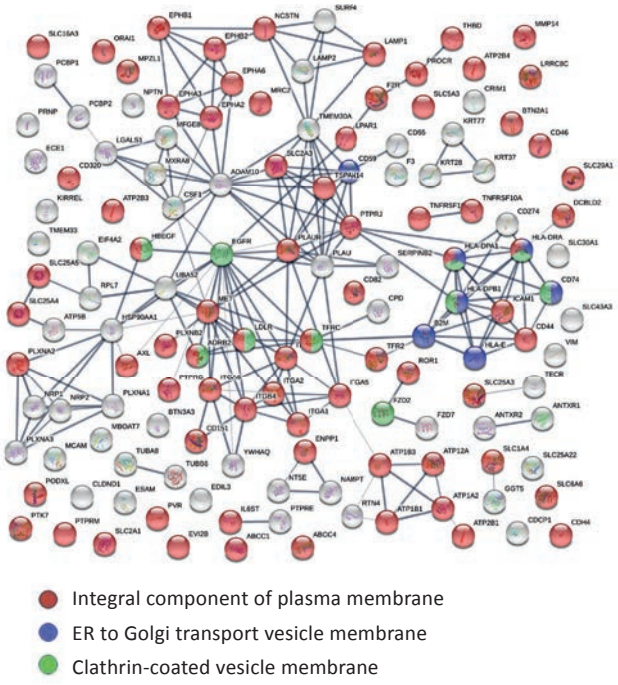
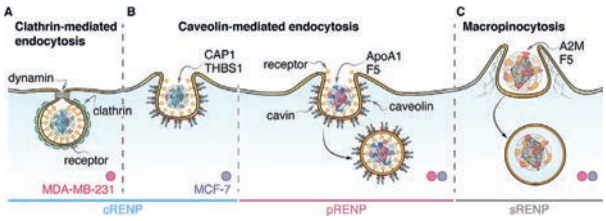
Differences in Protein Corona Underlie Varying Cellular Uptake of Upconverting Nanoparticles

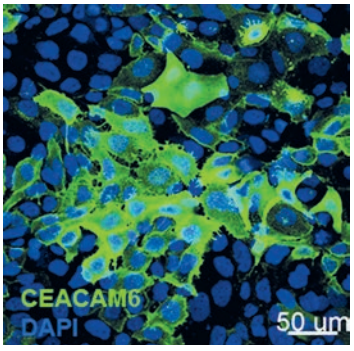
In biological media nanoparticles are exposed to proteins and other biomolecules, which form the so called "protein corona" around the nanoparticles and influence their targeted delivery and accumulation in cells and tissues. In the current study we investigated the role of protein corona in the cellular uptake by the breast cancers cells of LiYF4:Yb3+, Tm3+ upconverting rare-earth doped nanoparticles (RENPs) with various coatings, including citrate, phospholipid, and SiO2. RENPs with different coatings varied in their cell internalization efficiency and pathways. We hypothesized that protein corona around the nanoparticles might contribute to variations of RENPs intracellular uptake. Thus, we performed differential proteomic analysis to identify and compare the composition of protein corona that forms around the RENPs with different coating in cell culture media. This analysis highlighted several proteins unique for the protein corona of each RENPs type as the most likely candidates responsible for difference in cellular uptake. On the other hand, proteomic analysis of the cell surface proteome of breast cancers cells MCF-7 and MDA-MB-231 showed the differences of plasma membrane protein composition. Bioinformatic analysis predicted plasma membrane protein interaction with some of the nanoparticle corona proteins. Finally, by combining both differential proteomic analyses we showed that thrombospondin 1 (THBS-1) protein in the protein corona of citrate-coated RENPs and THBS1-interacting integrins in the MDA-MB-231 cell surface proteome may explain the high uptake of citrate-coated RENPs by MDA-MB-231 cells. Data show that RENPs surface coating defines variations in the corona protein composition that subsequently associates with cell plasma membrane proteins and defines efficiency of nanoparticle intracellular uptake (Voronovič et al. ACS *Appl. Mater. Interfaces.* 2021, 13(33): 39076–39087).

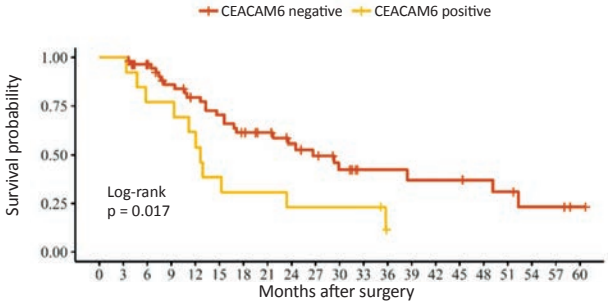


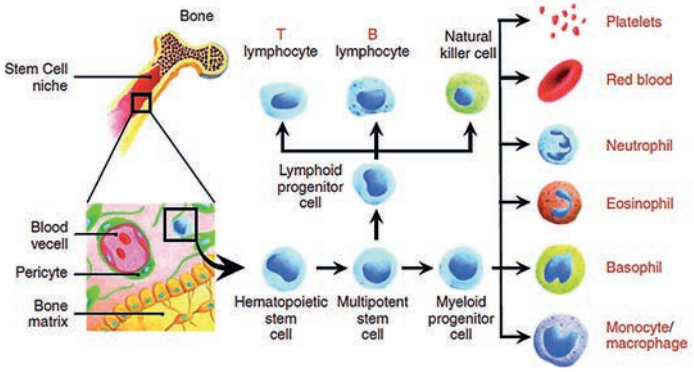


CEACAM6 is Novel Prognostic Biomarker for Pancreatic Cancer

Pancreatic ductal adenocarcinoma (PDAC) is one of the most fatal cancers due to the lack of efficient diagnostic and prognostic tools. Carcinoembryonic antigen-related cell adhesion molecule 6 (CEACAM6) was discovered as a possible promising pancreatic cancer biomarker during in-depth differential proteomic analysis of PDAC tumour tissues. The predominance of CEACAM6 to pancreatic cancer was validated in tissue and primary PDAC cell samples by Western blot and immunofluorescence methods. The analysis of CEACAM6 concentration in the serum of PDAC patients (n=142) demonstrated that increased CEACAM6 blood serum concentration significantly correlates with poorer overall survival (17.0 vs. 12.6 months, p=0.017) in PDAC patients after the radical treatment and adjuvant chemotherapy. Thus, CEACAM6 is a promising new biomarker with significant prognostic value for the prediction of chemoresistance properties, enabling the improvement of individualized approaches to the patients with pancreatic cancer (Kurlinkus et al. *Life*. 2021, 11(6): 542).







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Molecular Mechanisms of Cancer Cell Treatment

Cancer is the disease caused by alterations in genes coding cell proliferation, apoptosis and differentiation. At present, cancer therapy has shifted from the use of conventional cytotoxic drugs to molecular agents that target these specific regulatory molecules responsible for oncogenic transformation. However, therapeutic resistance, intrinsic or acquired, remains a major obstacle in the treatment of cancer. It is now increasingly recognized that not only genetic alterations but also non-genetic mechanisms are involved in drug resistance, as well as tumour progression. Cancer cells can acquire resistant phenotypes through epigenetic modifications, deregulation of signalling networks and other non-genetic mechanisms, dependent on both intracellular and extracellular factors associated with the tumour microenvironment.

Epigenetic Targeted Therapy Strategy for Leukaemia

Acute myeloid leukaemia (AML) is an aggressive group of cancers with high mortality rates with only ~20% of patients expected to survive 5 years after diagnosis. Despite significant advances in the pathophysiology of AML, only a few new treatments for AML have moved to the clinic due to a subset of AML cases; therefore, relapse of AML remains a significant problem that adversely affects the survival of patients with AML [DOI: 10.1158/1078-0432.CCR-05-0468, DOI: 10.1056/NEJMoa0901409].

Cytarabine (AraC) and daunorubicin (DNR) / Idarubicin (IDA) are conventional chemotherapy drugs widely used over the past three decades for induction therapy, which aims to eliminate the bulk of AML blasts by targeting rapidly proliferating cancer cells. Many AML patients achieve initial remission and will receive consolidation therapy, such as high-dose AraC in order to target the remaining AML blasts. Unfortunately, these therapeutic regimens are extremely intensive and toxic, making them unfeasible for debilitated elderly patients. Some patients could be diagnosed as “refractory AML” when after two cycles of induction chemotherapy patients still have residual leukemic cells in their bone marrow. Some patients after reached remission could have leukaemia cell re-turn in the bone marrow (“relapsed leukaemia”). In these cases, new treatment strategies must be find for relapsed/refractory AML.

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OxPhos Inhibitors and Epigenetic Regulators as Treatment Strategy to Improve Conventional Leukaemia Therapy

Today, cancer is understood as an epigenetic as well as a genetic disease. DNA methylation and histone modifications are the main epigenetic hallmarks of the cancer cells. The main goal of using different epigenetic modifiers would be restoration of gene expression of those tumour-suppressor genes that have been transcriptionally silenced by promoter-associated histone modifications. Recently, studies on the fundamental roles of cellular metabolism reprogramming in tumour development and progression are given more emphasis. Increasing research studies have emphasized that, in addition to glycolysis, some tumours like brain cancer and AML depend more on enhanced mitochondria-specific oxidative phosphorylation (OXPHOS) for bioenergetic and biosynthetic processes. In particular, residual chemotherapy-resistant leukaemia cells are demonstrated to show increased mitochondrial mass, retain active polarized mitochondria, and rely more on mitochondrial OXPHOS for survival [doi: 10.1158/2159-8290.CD-16-0441].

Our research is focused on the following topics:

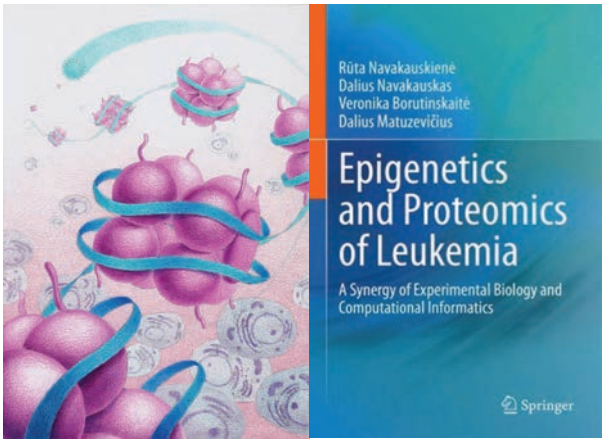
- on the pharmacological manipulation of chromatin remodelling that might develop into a potent and specific strategy for the treatment of leukaemia;
- on the study of mitochondrial-specific oxidative and bioenergetics features in AML;
- on the establishment of novel, potentially prognostic biomarkers useful for the diagnostics of leukaemia or disease outcome predictions.

Signalling in Lung Cancer Cell Fate Regulation

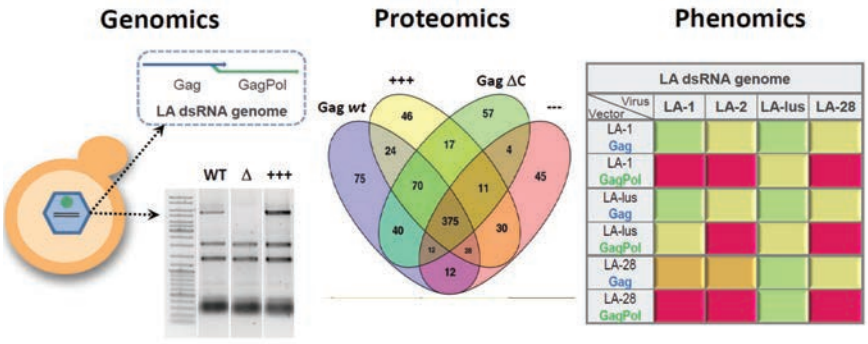
During the implementation of the SMART project "Designing of the patient-specific, heterogeneous lung cell ex vivo model system for drug efficiency prediction in personalized oncotherapy" in 2021 (project leader Dr A. Kalvelytė) the cross-talk and feedback mechanisms between of PI3K / Akt and MEK / ERK signalling pathways during therapeutic treatment using a panel of phenotypically and genotypically different lung cancer cell lines were investigated. Studies have highlighted the potential role of cell state. In addition, modelling studies of different cell states – adherent cells, single-suspension cells, and cell aggregates - showed an opposite dependence of MAP kinases JNK and p38 basal phosphorylation on substrate loss compared to Akt kinase. However, abnormal activation of ERK1/2 in lung cancer A549 cells, in contrast to decrease in ERK1/2 phosphorylation in other cell lines studied, was observed in anchorage-independent state. The opposite dependence of members of the same signalling pathway, transcription factor cJun and kinase JNK, on cell-cell contacts was also shown.

Epigenetics and Proteomics in Leukaemia – Synergy of Experimental Biology and Computational Informatics

A scientific achievement of more than 20 years of active and productive research in the field of cancer treatment in collaboration with VILNIUS TECH University researchers resulted in the publishing of the monograph "Epigenetics and Proteomics in Leukemia – A Synergy of Experimental Biology and Computational Informatics" by Prof. Dr Rūta Navakauskienė, Prof. Dr Dalius Navakauskas, Dr Veronika Borutinskaitė, Dr Dalius Matuzevičius.



Aging of the hematopoietic system is associated with a variety of changes at the molecular, cellular, and physiological levels. The changes of the expression and function of different molecular factors during aging process are the main factors in the development of blood cancer, leukaemia. Genetic and epigenetic changes are very individual among leukaemia patients. Therefore, the treatment of leukaemia should be individualized according to the patient's cytogenetic and epigenetic changes, biological age, drug tolerance, stage of the disease, and so on. The monograph focuses on the mechanisms of action of various epigenetic modifiers, their influence on the growth and death of cancer cells and their possible use in the treatment of leukaemia.



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Molecular Virology: Mechanisms, Evolution, Antivirals

The *Totiviridae* family dsRNA viruses found in *Saccharomycetaceae* family yeast are ubiquitous yet poorly understood benign inhabitants of the host. The impact of the dsRNA viruses is uncovered by genomic, transcriptomic, proteomic and phenomic analysis in our lab. We aim to create a paradigm framework for establishing of the universal mechanisms for virus-host interactions. The understanding of intra- and extracellular relations of yeast dsRNA viruses is vital to elucidate the evolutionary pathways of these viruses and reveal the ultimate principles of distribution within an ecosystem [1,2].

Nucleoside and nucleotide-based antivirals constitute the essence of modern high-efficacy antiretroviral HIV treatment, and proved already to be efficient for the constrain of SARS-CoV-2. Once a revolutionary approach, nowadays it suffers from an emerging resistance and multiple side effects. Recently, innovative and more advanced measures against genuine retroviral replication enzymes have been proposed and substantiated. The aim of our research is to develop compounds active at the level of a catalytic cycle of retroviral replication enzymes, linking an exclusive specificity and efficacy into a binding approach.

Our team focuses on systems biology approaches to address the interactions of yeast double-stranded RNA *Totiviridae* viruses with the host cell. Basing on a virus genome cloning technique, developed in our lab [2,3], constituent genes of a virus genome were re-introduced into model hosts to manipulate the phenotype conferred by the virus [1]. We were able to either achieve a complete clearing of the target virus or boost the synthesis of the viral genome, making it the most prevalent form of an individual RNA molecule in the yeast cell. The developed techniques allowed us to perform a transcriptomic and proteomic analysis, aimed at understanding the molecular mechanisms behind the establishment of *Totiviridae* viruses in host cell [3].

To create novel universal antiviral compounds, we took advantage of the catalytic mechanisms of viral polymerases. In particular, the catalytic flexibility of reverse transcriptases from HIV and M.MuLV were exploited to prepare and investigate the conjugates of nucleotide and small molecule inhibitors. We demonstrated the feasibility of altering the action of a polymerase, forcing a shift from the processive to the distributive mode [4]. The conformational alterations of productive complexes were postulated to determine the impaired turnover of the target enzymes, ensuring the selectivity toward a variety of cellular polymerases.

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Specificity Determination in *Saccharomyces cerevisiae* Killer Virus Systems

Saccharomyces yeasts are widely distributed in the environment and microbiota of higher organisms. The killer phenotype of yeast, encoded by double-stranded RNA (dsRNA) virus systems, is a valuable trait for host survival. The mutual relationship between the different yet clearly defined LA and M virus pairs suggests complex fitting context. To define the basis of this compatibility, we established a system devoted to challenging inherent yeast viruses using viral proteins expressed *in trans*. Virus exclusion by abridged capsid proteins was found to be complete and nonspecific, indicating the presence of generic mechanisms of *Totiviridae* maintenance in yeast cells. Indications of specificity in both the exclusion of LA viruses and the maintenance of M viruses by viral capsid proteins expressed *in trans* were observed. This precise specificity was further established by demonstrating the importance of the satellite virus in the maintenance of LA virus, suggesting the selfish behaviour of M dsRNA.

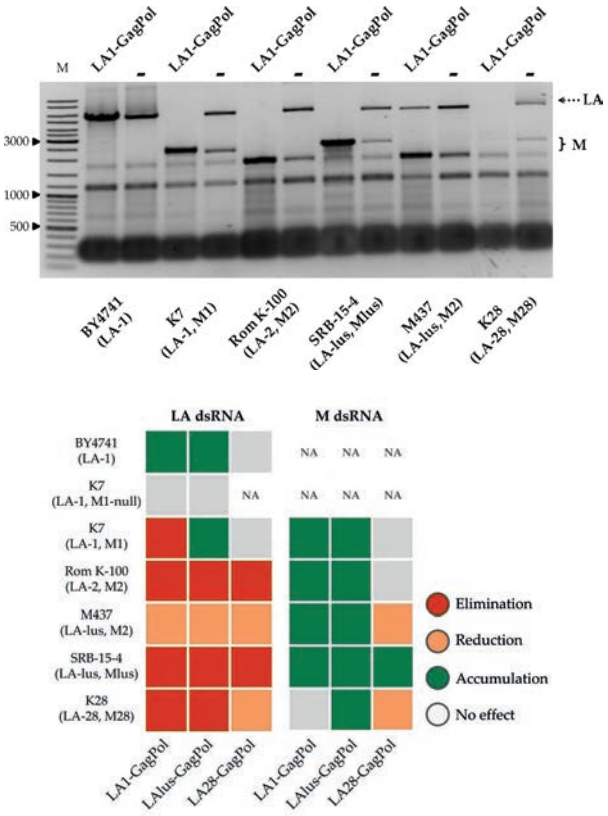
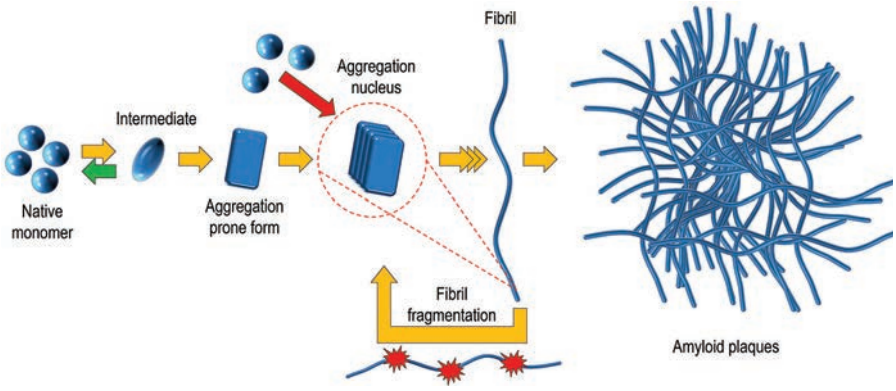


Fig. 1. The impact of overexpression of LA-1 capsid proteins on the maintenance of LA and M dsRNAs in the native hosts.



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Protein Misfolding and Aggregation

Protein misfolding and aggregation into amyloid structures is involved in many diseases, including such neurodegenerative disorders as Alzheimer's and Parkinson's, systemic amyloidoses and even some localized diseases such as type II diabetes or cataract. There is increasing evidence of the amyloid nature of proteinaceous infectious particles – prions. One of the possible ways of prion spreading is a self-replication of amyloid-like fibrils; thus, there is a chance of all amyloid-associated diseases to be potentially infective.

The ability of the same protein to adopt distinct pathogenic conformations was first reported in studies of infectious prions, and such conformations were referred to as strains. Strain-like polymorphism was reported for several other amyloid proteins. It highly increases the complexity of disease mechanisms and may be one of the reasons for the slow progress in drug research.

Our team studies the effects of environmental factors such as temperature, pressure, intensity and type of agitation, pH, ions, macromolecular crowding and the presence of different organic solvents, ligands and biomolecules on aggregation kinetics, thermodynamic stability and the structural properties of amyloid-like fibrils. We believe that only comprehensive knowledge of all factors may provide a genuine understanding of the mechanisms of amyloid self-replication, complexity of fibril polymorphism and lead towards curing amyloid-related diseases.

We are interested in comparing the aggregation profiles of different proteins and testing possibilities of their co-aggregation. The group has experience in the expression and purification of recombinant amyloid beta, alpha-synuclein, different isoforms of full-length Tau proteins, a variety of mammalian prion proteins (derived from different species and with different mutations), S100A9 protein, superoxide dismutase and sup35. The main methods used to follow amyloid formation include UV, visible and fluorescence spectrometry, Fourier transform infrared spectrometry and atomic force microscopy.

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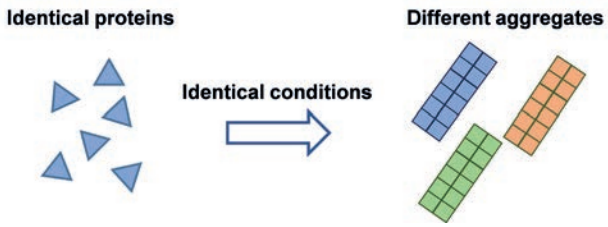
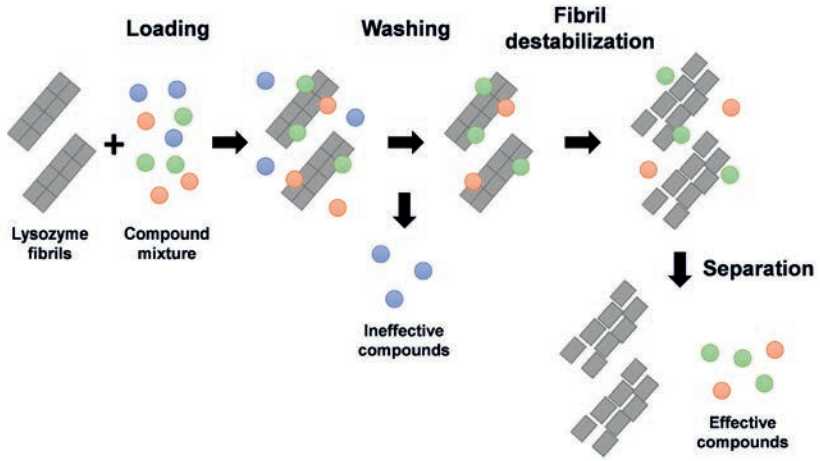
Polymorphism of Amyloid Fibrils

Amyloid proteins are capable of forming fibrils with various distinct secondary structures and morphologies under different environmental conditions. We have demonstrated that in certain cases, such as alpha-synuclein (Ziaunys et. al. *International Journal of Molecular Sciences.* 2021a) and prion protein (Ziaunys et. al. *International Journal of Molecular Sciences*. 2021b), even identical experimental conditions can lead to the formation of distinct structure aggregates. For alpha-synuclein, the aggregates varied in length, width and periodicity patterns, while prion protein conformations had specific self-association tendencies and amyloid dye binding properties.



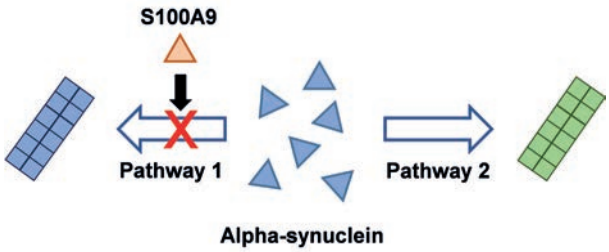
Inhibition of Amyloid Formation

The widespread prevalence of amyloid-related disorders is still a major issue, as very few effective treatment modalities have been developed. It has been shown that certain natural, plant-derived compounds, such as flavones or flavonoids, have strong anti-amyloid properties. We have shown that autoxidation is an important factor in enhancing the aggregation-inhibiting potential of flavone derivatives (Sakalauskas et. al. *Antioxidants.* 2021). We have also devised a novel method for such compound separation from complex mixtures by using their affinity towards the amyloid structure of lysozyme fibrils (Ziaunys et. al. *Biotechnology Journal.* 2021).



Cross-Interactions in Amyloid Aggregation

Amyloid protein cross-interactions have been implicated as one of the multiple possible causes of disease onset and progression. Certain fibril-forming protein/peptide pairs were observed in infected tissue samples, suggesting that they alter each other's aggregation rates or pathways. We have demonstrated that the inflammation-related S100A9 protein is capable of altering the pathway of alpha-synuclein aggregation, leading to the formation of a specific fibril conformation (Toleikis et. al. *International Journal of Molecular Sciences*. 2021). We have also shown that superoxide dismutase-1 is capable of increasing the lag-phase of prion protein aggregation, as well as the resulting fibril secondary structure (Ziaunys et. al. *Archives of Biochemistry and Biophysics.* 2021).



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Electrophysiological Brain States: Modulating Factors and Clinical Applications

Electrophysiological brain responses assessed non-invasively with electroencephalogram (EEG) stand as a widely used cost-effective tool to estimate brain functioning in norm and pathology. Depending on the recording conditions, information about the resting state or brain responses to particular stimuli or tasks can be evaluated. However, the factors affecting electrical brain responses in pre-clinical and clinical settings are not fully understood, especially those related to the state of the study participant or a patient. The knowledge on the potential modulators of electrical brain states is important for correct objective interpretation of the observed patterns of brain activity and for further optimization and practical application of the method.

Employing electroencephalography as the main tool, also behavioural measures and subjective evaluation, the Brain States Research Group is investigating the origin and the outcome of the observable electric brain states from the viewpoint of everyday functioning and from diagnostic/clinical perspective. We evaluate how subjects' traits (like sex, personality, general ability to sense one's own body) and states (like level of arousal, attention, hormonal background), the task they perform and stimulation we provide affect brain activity. We use various stimulation approaches (i.e. classical P300, P50, Go/NoGo, MMN) with a special focus on the brain ability to entrain with periodic events as measured by steady-state responses (SSRs) to stimuli of various modalities. In close collaboration with partners from the USA, Switzerland, Poland, New Zealand, Chile, Czech Republic we have evaluated the promise of electrophysiological resting state activity and auditory evoked brain responses to evaluate the state of the nervous system in various normal conditions (i.e. dependence on subject's sex and the subjective experiences during the experiment [1]) and pathological states (i.e. prolonged disorders of consciousness, schizophrenia and association to clinical symptoms, behavioural addictions [2, 3, 5]), and as tool for modulation of brain functioning (brain-computer interface, neurofeedback [4]).

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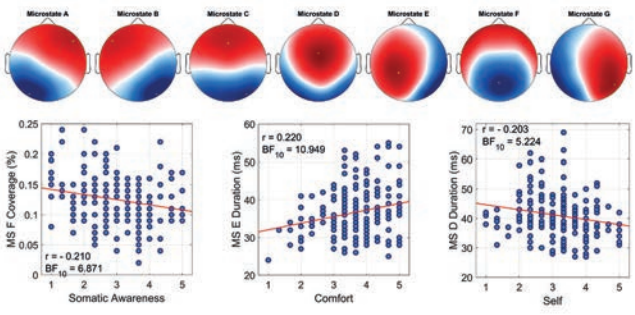
Resting-State Experience and EEG

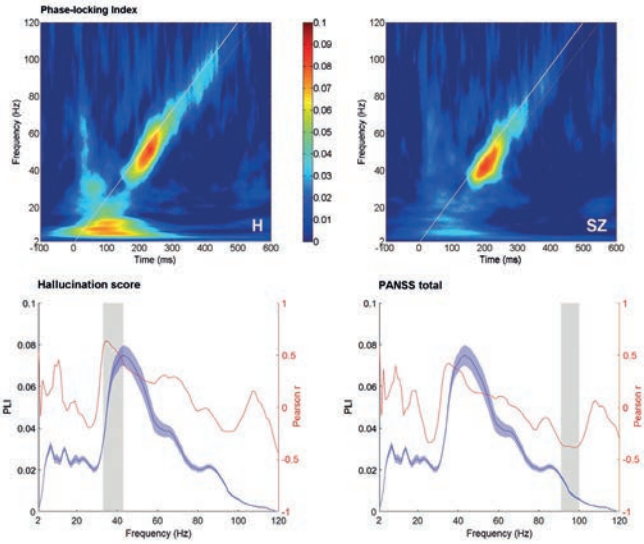
The resting-state paradigm is frequently applied in electroencephalography (EEG) research; however, it is associated with the inability to control participants' thoughts. To quantify subjects' subjective experiences at rest, the Amsterdam Resting-State Questionnaire was introduced covering ten dimensions of mind wandering. 5 min. resting-state EEG data of 197 subjects was used to evaluate temporal properties of seven microstate classes. Several associations between Comfort, Self and Somatic Awareness domains and temporal properties of neuroelectric microstates were revealed. This indicates the relevance of assessments of spontaneous thought occurring during the resting-state for the understanding of the intrinsic brain activity reflected in microstates. (Tarailis et al. *Journal of Personalized Medicine*. 2021, 11(11)).



Seven resting-state EEG microstates extracted from recordings taken during mind-wandering in 197 subjects. The parameters of microstates were related to subjectively experienced levels of Somatic Awareness, Comfort and Self

Responses at Multiple Frequencies: Changes in Schizophrenia and Relation to Clinical Symptoms

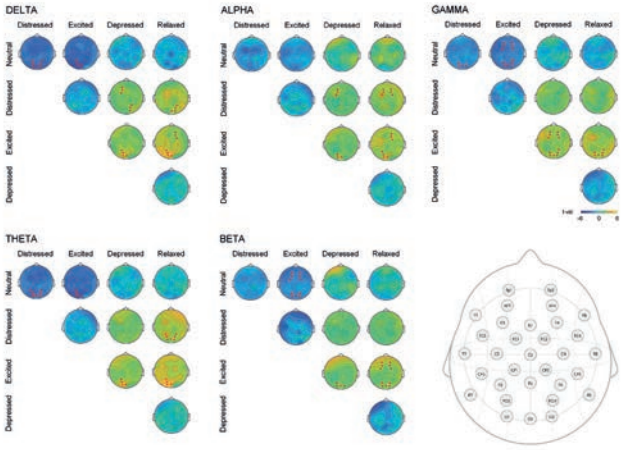
In order to obtain the full picture of potential EEG impairment in schizophrenia, it is important to test responses at different frequencies. The EEG-derived envelope following responses were obtained in a group of male patients with schizophrenia (N=18) and matched controls (N=18). Brain networks showed impaired capabilities to generate EFRs at different frequencies in schizophrenia; moreover, even when responses of patients did not significantly differ from the controls on the group level, they still showed potentially clinically relevant variability. (Griskova-Bulanova et al. *Brain Sciences*. 2021, 11(1)).



Phase-locking index in response to increasing modulation rates of chirp stimulations shows impairment in theta-beta and high gamma ranges in schizophrenia (SZ) as compared to healthy subjects (H). Response at around 40Hz is positively related to the prevalence of hallucinations and response at around 100Hz negatively related to the general level of psychopathology

Active Emotional Musical Performance: Spectral Characteristics of EEG

The research on neural correlates of intentional emotion communication by the music performer is still limited. In this study, we attempted to evaluate EEG patterns recorded from musicians who were instructed to perform a simple piano score while manipulating their manner of play to express specific contrasting emotions and self-rate the emotion they reflected on the scales of arousal and valence. The spectral analysis of the signal was applied as an initial step to be able to connect findings to the wider field of music-emotion research. The experimental contrast of emotional playing vs neutral playing was employed to probe brain activity patterns differentially involved in distinct emotional states. The EEG activity differences were observed between distressed/excited and neutral/depressed/relaxed playing. (Pousson et al. *Sensors* 2021, 21(22)).



Topographical plots of T values for different emotional conditions (neutral, distressed, excited, depressed and relaxed) and for EEG bands (delta (1–4 Hz), theta (4–8 Hz), alpha (8–12 Hz), beta (12–30 Hz), and gamma (30–45 Hz) reveal differences between emotional playing conditions. Red dots represent electrode clusters where differences between conditions were significant

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Neuroscience and Cellular Biophysics

The understanding of the functioning of the most complicated structure – the nervous system – in norm and pathology is one of the most challenging questions of modern science. We investigate mechanisms within the nervous system at different levels – starting with the electrophysiological properties of single neurons and excitable plant cells up to an investigation of the different brain states, the modulatory effects of sex steroids, the pathological mechanisms of depression, schizophrenia and various addictions. We employ various methods: EEG, eye tracking, *in vivo* and *in vitro* electrophysiology as well as video patch clamping and behavioural methods.

The vast experience in the evaluation of normal and pathological traits and states at the level of electrical activity along with close collaboration with scientists from the US, Switzerland, France, Sweden, Australia, Japan emerged into several successful international projects and an introduction of certain developed approaches into clinical settings both in Lithuania and abroad. Collaboration with neuroscientists, mathematicians and biophysicists from Denmark, Poland, Japan and Lithuania in electrophysiological data analyses' approaches resulted in an investigation of the response properties of single cells (plant cells and motoneurons), cell communication (bone marrow mesenchymal stem cells and chondrocytes, *Nitellopsis obtusa* cells) and signalling pathways involved in learning and memory in animal models. In cooperation with our colleagues from Switzerland, the cognitive functions and their dependence on individual hormonal concentrations are performed at the behavioural and electrophysiological levels.

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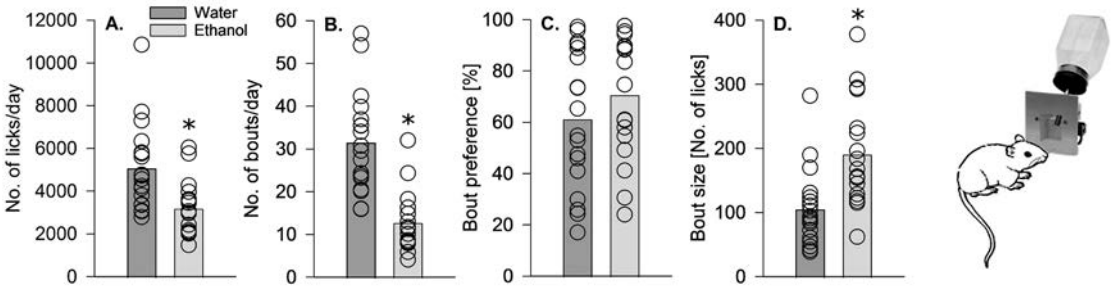
Anticonvulsant Lamotrigine Reduces Bout-Like Alcohol Drinking in Rats

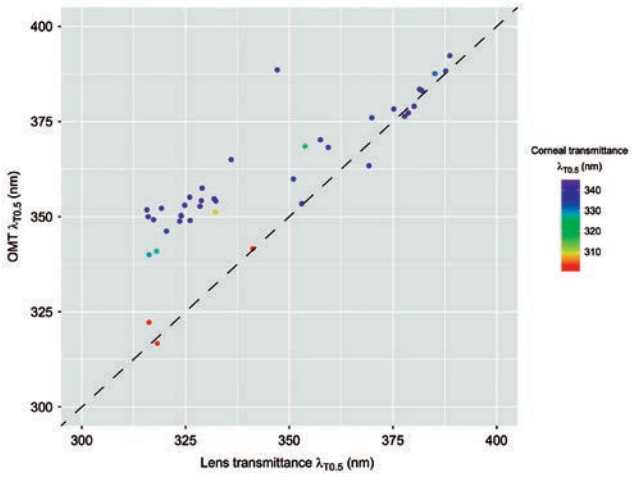
In this study we used an optical lickometer system to study drinking microstructure and effect of lamotrigine in voluntary alcohol drinking rats. We showed that, similar to humans, animals differ by their drinking microstructure where some consume alcohol exclusively in a bout-like patterns. The study suggests that anticonvulsants, such as lamotrigine, may be one treatment strategy specifically affecting this type of drinking.

Water and 10% alcohol drinking characteristics shown as (A) number of licks and (B) number of bouts during 24 h, (C) bout preference (calculated as (number of licks within bouts/total number of licks)*100, %) and (D) bout size (number of licks during one bout). A bout was defined as 20 or more licks separated by less than 60 s pauses between successive licks.



Lens and Cornea Limit UV Vision of Birds - Phylogenetic Perspective

Most vertebrates have UV-sensitive vision, but the UV-sensitivity of their eyes is limited by the transmittance of the ocular media. We described the transmittance of all ocular media (OMT), as well as that of lenses and corneas separately for 66 species of birds belonging to 18 orders. The wavelength at which 50% of light is transmitted through the ocular media to the retina ($\lambda_{T0.5}$) ranges from 310 to 398 nm. Corneal $\lambda_{T0.5}$ varies only between 300 and 345 nm, whereas lens $\lambda_{T0.5}$ values are more variable (between 315 and 400 nm) and tend to be the limiting factor, determining OMT in the majority of species. Corneal and lens transmittances do not differ between birds with UV- and violet-sensitive SWS1 opsin when controlling for eye size and phylogeny. Phylogenetic relatedness is a strong predictor of OMT, and ancestral state reconstructions suggest that from ancestral intermediate OMT, highly UV-transparent ocular media (low $\lambda_{T0.5}$) evolved at least five times in our sample of birds. Some birds have evolved in the opposite direction towards a more UV-opaque lens, possibly due to pigmentation, likely to mitigate UV-damage or reduce chromatic aberration.



Relationship between total ocular media transmittance (OMT) and the lens and corneal transmittances

Impact of Mammalian Two-Pore Channel Inhibitors on Long-Distance Electrical Signals in Characean Macroalga *Nitellopsis obtusa* and Early Terrestrial Liverwort *Marchantia polymorpha*

Inhibitors of human two-pore channels (TPC1 and TPC2), i.e. verapamil, tetrandrine, and NED-19, are promising medicines used in treatment of serious diseases. The impact of these substances on action potentials (APs) and vacuolar channel activity was examined in the aquatic characean algae *Nitellopsis obtusa*. Verapamil (300 µM) caused reduction of AP amplitudes and depolarization of AP excitation threshold, indicating impaired Ca^{2+} transport. Tetrandrine (100 µM) evoked a pleiotropic effect: reduction of resting potential and AP amplitudes and prolongation of AP repolarization phases. NED-19 (75 µM) caused similar specific and unspecific effects on *N. obtusa* APs. The results indicate an inhibiting effect on Ca^{2+} – permeable channels governing plant excitation. Research by our collaborators from Poland pointed a similar action of selected substances on electrical signalling in liverwort *Marchantia polymorpha*. Overall, we showed that verapamil, tetrandrine and NED-19 affect different ion channels in plant cells, which opens a question of their specificity as pharmaceuticals due to possible side effects.

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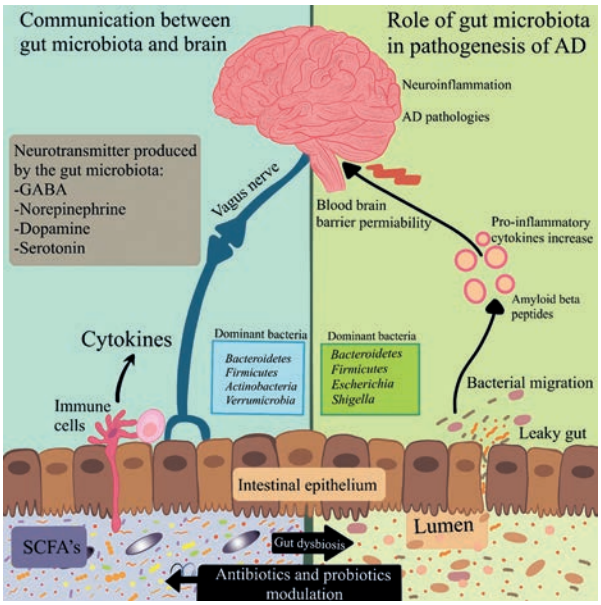

Role of Microbiota-Gut-Brain Axis in Neuropsychiatric Disorders

It is recognized that the microbes resident in the gastrointestinal tract can influence brain physiology and behaviour. Recent research has shown that the gastrointestinal microbiota can signal to the brain via a diverse set of pathways, including immune activation, production of microbial metabolites and peptides, activation of the vagus nerve, and production of various neurotransmitters and neuromodulators in the gut itself. The bidirectional signalling between the gastrointestinal tract and the brain is vital for maintaining homeostasis and is regulated at the neural (both central and enteric nervous systems), hormonal and immunological levels. Collectively, this bidirectional pathway is known as the microbiota-gut-brain axis and it is involved in a variety of psychological processes and neuropsychiatric disorders. These include mood and anxiety disorders, neurodevelopmental disorders such as autism spectrum disorder and schizophrenia, and even neurodegenerative disorders such as Alzheimer's and Parkinson's diseases. Thus, we aim to better understand the role of the microbiota in brain health, age-associated cognitive decline, Alzheimer's disease and autism spectrum disorder. Our research is supported by grants (No. 01.2.2-LMT-K-718-02-0014, No. S-SEN-20-9, and No. 01.2.2-LMT-K-718-03-0099) from the Research Council of Lithuania.

To study the role of the microbiota-gut-brain axis in neuropsychiatric disorders and mental health, we are using animal models for Alzheimer's disease, aging, and autism spectrum disorder. The impact of diet and microbiota interactions on brain health and cognitive functions is studied by combining animal behavioural experiments, ELISAs, inflammatory and metabolic states of microglia, microbiome and virome analysis. We intend to identify key microorganisms and their metabolites that play a crucial role in the microbiota-gut-brain axis and to develop a method to detect a specific microbiota profile that will serve as a biomarker for early diagnosis of Alzheimer's disease. The project related to autism aims to investigate the faecal microbiota transfer therapy in children with autism spectrum disorder and to identify possible biomarkers of the gut microbiota in this pathology to improve the therapy.

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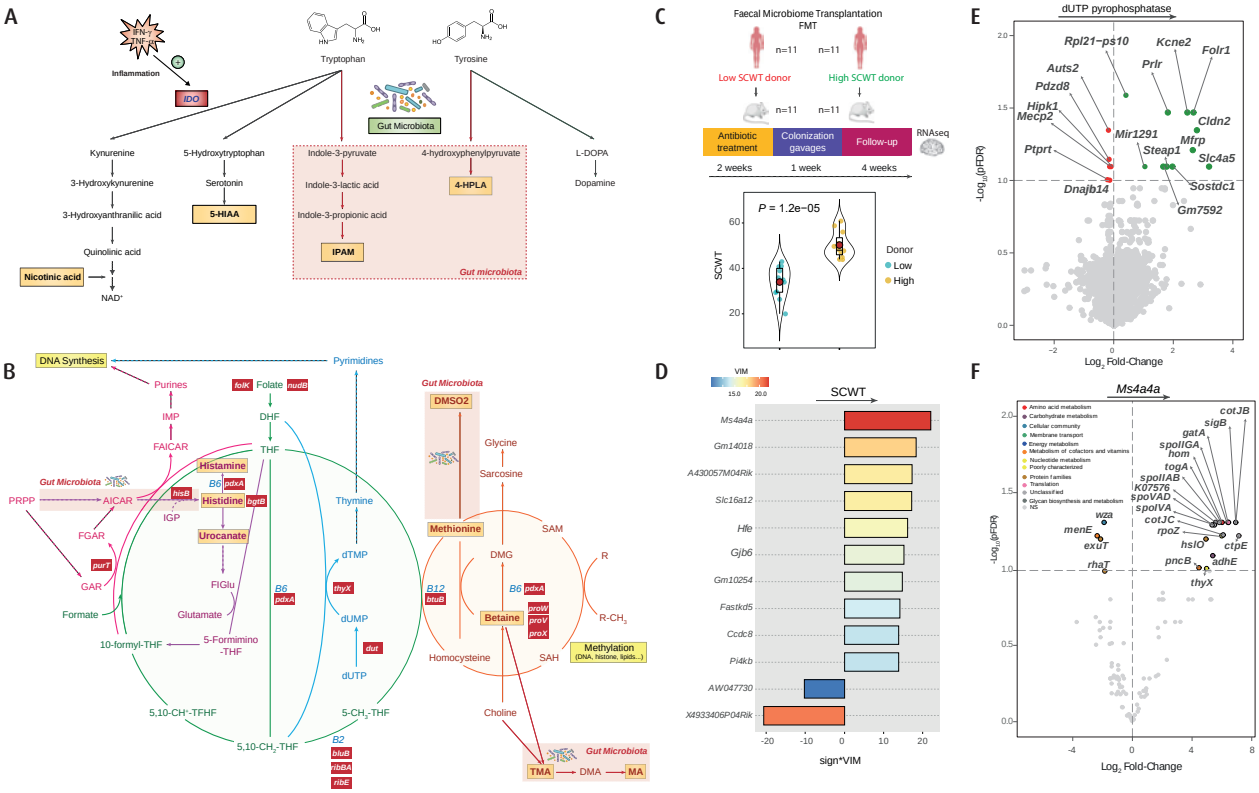
Modulation of Microbiota–Gut–Brain Axis by Probiotics, Prebiotics, Antibiotics

The communication between the gut microbiota and the brain includes neuronal, immune-mediated, and metabolite-mediated pathways. Gut dysbiosis leads to activation of the immune response and alters the production of neurotransmitters as well as bacterial metabolites. Bacterial metabolites such as SCFAs are considered the key mediators for microbiota–microglia interaction. Moreover, different bacterial metabolites may have a contribution to abnormal signalling through the vagus nerve. Reduction in the integrity of the gastrointestinal barrier causes bacterial migration and inflammation. Pro-inflammatory cytokines induce disruption of the blood–brain barrier permeability. Antibiotics can hinder the growth of certain bacteria, and probiotics or/and prebiotics have the potential to normalize the gut microbiota in microbiota–gut–brain processes (Megur et al. *Nutrients.* 2021).

Targeting Obesity-Related Impulsive Behaviour through Induction of Gut Microbiota Shifts

Inhibitory control (IC), a fundamental component of executive function, overrules automatic intentions to directly respond to stimuli without thought and is critical to keep long-term goals in everyday life. Bidirectional relationships between IC deficits and obesity are behind unhealthy eating and physical exercise habits. An interplay among IC, brain structure (in humans) and medial prefrontal cortex transcriptomics (in mice), plasma/faecal metabolomics and the gut metagenome was found. We with our partners in Spain have revealed the obesity-dependent alterations in one-carbon metabolism, tryptophan, and histidine pathways were associated with IC in the two independent cohorts. Bacterial functions linked to one-carbon metabolism and the anterior cingulate cortex volume were associated with IC, cross-sectionally and longitudinally. The microbiota transplantation from individuals with obesity led to alterations in mice reversal learning. In an independent microbiota transplantation experiment, human donor's bacterial functions related to IC deficits were associated with the medial prefrontal cortex expression of one-carbon metabolism-related genes of recipient's mice (Arnoriaga-Rodríguez et al. *Gut*. 2021).



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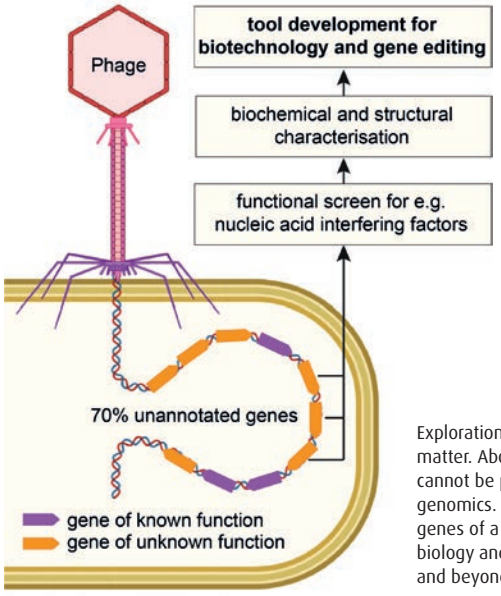

Exploration of phage genomic dark matter. About 70% of gene functions cannot be predicted by comparative genomics. We are characterizing all genes of a given phage to discover new biology and new factors for gene editing and beyond.

Advancing Genome Editing Tools

Viruses and prokaryotes are a biotechnological treasure trove of evolution. Billions of years of prokaryote-virus conflict and co-evolution have generated a fascinating and enormous diversity of nucleic acid interacting and modifying enzymes, which can be used to manipulate nucleic acids both *in vitro* and *in vivo*. A recent example is the prokaryotic anti-phage system CRISPR-Cas, which is widely used as a programmable gene editing system for diverse biotechnological applications. Notably, even phages carry CRISPR systems that are useful for gene editing (Pausch et al. 2020, 2021a,b).

The Pausch Lab is working on the discovery of new, and characterization of known, nucleic acid modifying enzymes – to understand their biological function and to develop tools for biotechnological, diagnostic and therapeutic applications.

Our approaches:

- For the discovery of novel enzymes, we are developing new high-throughput technologies to experimentally explore the genomic dark matter of microbial mobile genetic elements, such as phages (see Figure above). The methods will overcome the limitation of traditional approaches that rely on *a priori* knowledge, such as comparative genomics. Our approach will identify gene products that interfere with specific cellular pathways and will uncover enzymes that cut, process, or modify DNA and RNA.
- For the development of new biotechnological and gene editing tools, we are biochemically and structurally characterizing a range of diverse bacteria-phage conflict systems, such as novel CRISPR-Cas variants. The acquired mechanistic and atomistic knowledge will then be used to engineer and improve tools for next-generation gene editing in complex living systems.

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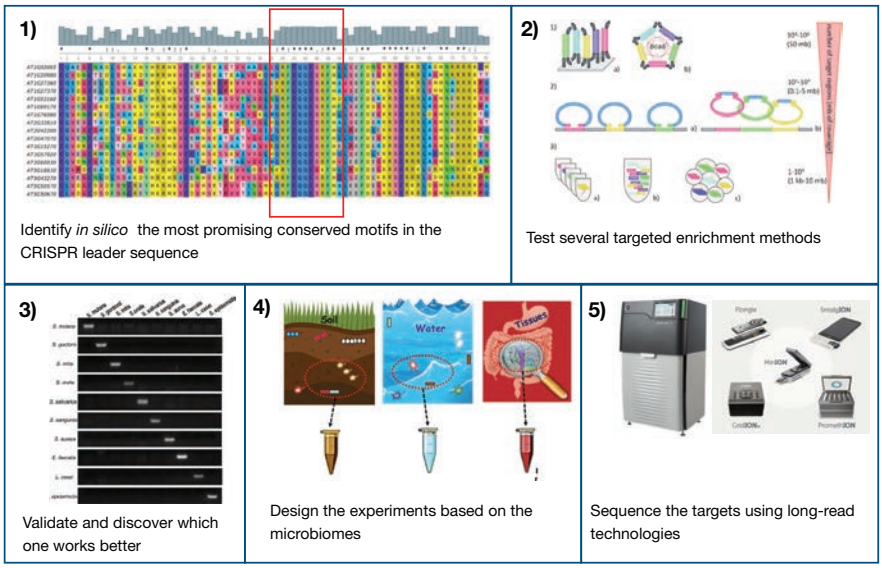

Applications of Long-Read Sequencing Technologies to Advance Knowledge of CRISPR-Cas Systems

CRISPR-Cas is a formidable example of acquired immune response in prokaryotes. In the context of genome sequencing, research efforts in this area have been based on short read-data and, to date, the potential of long-read sequencing technologies for CRISPR-Cas applications has not been assessed.

The Russo Lab intends to fill such a gap in the CRISPR-Cas field, leveraging our expertise in single molecule, long-read sequencing technologies and systematically deploy them as a tool in the CRISPR world.

The research in our group follows two main lines. On the one hand, we want to emphasize the capability of long-read sequencing technologies to obtain full-length sequences of the CRISPR arrays. Such a feature, paired with the development of a suitable protocol for targeted sequencing of CRISPR-Cas systems in microbial ecosystems, would open an avenue for full-length, multiplexed, high-throughput CRISPR metagenomics and give access to an unprecedented pool of CRISPR systems in uncultured bacteria. Envisioned applications include a better understanding of bacteria-phage co-evolution in natural ecosystems, with potential ramifications in the deployment of phage therapy, improvement in the classification of CRISPR types, expansion of the toolbox of CRISPR-based genome editing.

On the other hand, since those technologies sequence native DNA, one can retrieve unbiased, genome-wide information on several DNA modifications. It is known that restriction-modification enzymes and CRISPR-Cas systems can act in concert and they preferentially co-exist, however, there is no systematic map of the co-development of methylation patterns and CRISPR-Cas loci. Since methylation and CRISPR-Cas both represent bacterial defence mechanisms, elucidating the interaction between them would broaden our knowledge of the immune response. Additionally, monitoring the metabolic activities of the bacteria during those interactions could shed light on the fitness cost associated with fighting phages. Finally, new mechanisms behind phage-driven inactivation of antibiotic resistance genes could be unravelled.

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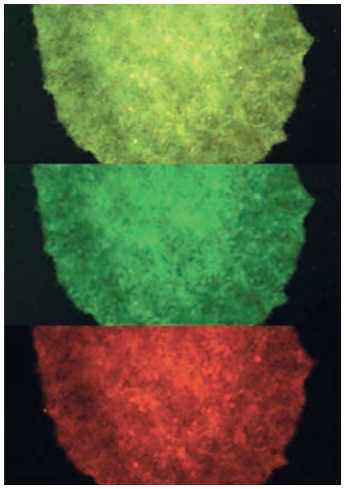

Colony of iPS cell engineered with CRISPR-Cas9 to express a synthetic single nucleotide polymorphism relevant in neurodegeneration - SNCA p.A30P [2]. Patent WO2017129811A1

Cell-Based Therapeutics Enabled by CRISPR Nucleases

Regenerative medicine is prone to yield a wide range of therapeutic solutions for currently incurable diseases over the upcoming decade. Its potential is made tangible through the technology of (1) induced pluripotent stem (iPS) cells and (2) genome engineering, mainly nucleases of the CRISPR family. Thus, iPS cells can be prepared to carry therapeutic functions and used as the source to manufacture human cells with clinical applications. Remarkably, we can now create most of the cell types present in adult human tissues, such as bone marrow, heart, pancreas or brain cells. These cells can be used in clinical practice to replace damaged or aged tissue, and also to carry synthetic properties, as those required for the treatment of cancer, metabolic or immune disorders.

The Arias Lab from the EMBL Partnership Institute pursues the creation of the next generation cell-based therapeutics. We combine the technologies of iPS cells and genome engineering to develop cells with translational potential to the service of human health. Our group, founded in 2022, is composed of experts in regenerative medicine, developmental biology and genome engineering. We pursue science with both innovative potential and positive impact to our society. Our research projects pursue the creation of immune cells, to better address pressing clinical challenges in oncology, hereditary, metabolic and immune disorders.

In collaboration with Karolinska Institutet (Dr. Inzunza and Dr. Nalvarte) and the private sector (Dr. Yu and Dr. Varshney), our group has succeeded in the manufacturing of iPS cell-derived immune cells of the hematopoietic stem cell (HSC) type, immune effector cells of the natural killer (NK) and cytotoxic/helper T-lymphocyte type [1].

As there are now new nucleases with improved properties capable of editing therapeutic cells, we integrate into our R&D pipelines the latest technologies on genome engineering. We introduce synthetic genes into iPS cells in a robust manner, preserving their genome stability, and with fine control of the genetic dose of these novel functions.

Traditionally, the differentiation of mature cell types from iPS cells is conducted following developmental biology principles. In our group, we integrate machine learning and other types of artificial intelligence approaches to identify and guide the differentiation of iPS cells towards cells with clinical potential. We accelerate our discoveries through the technologies of automation, high-throughput and high-content analysis [2], as well as single cell RNA-sequencing.

As iPS cells are a core component of regenerative medicine, our group has experience for the manufacturing of various adult cells types with translational potential, including cardiac, vascular, pancreatic, immune and neuronal [3] linages.

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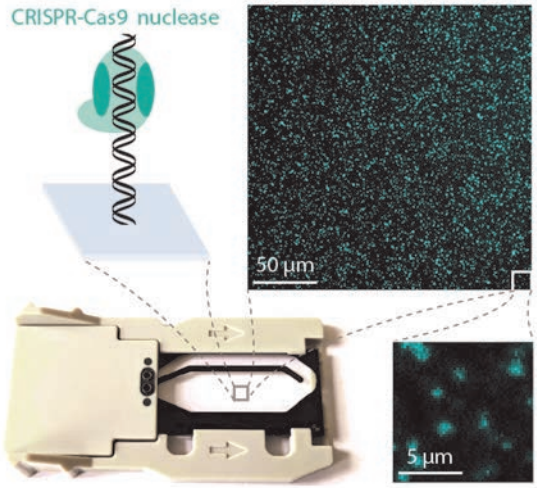

Fluorescently labelled CRISPR-Cas9 nuclease (deactivated) binds to DNAs on the surface of a high-throughput sequencing flow cell

High-Throughput Strategies for Evaluating CRISPR Nucleases and Their Targets

As the 2020 Nobel prize in Chemistry attests, CRISPR nucleases are transforming our approaches to disease, research, diagnostics and biotechnology. They provide cures for cancers, blindness and anaemia. They underlie tools for screening genomes and illuminating human development. They make diagnostics cheap and field able. They allow us to engineer models for disease in weeks instead of months, with cost savings to match.

Yet these amazing gene editing tools have a safety flaw: when targeting a specific gene, CRISPR nucleases alter other genomic DNA with similar sequence, so called 'off-targets.' To understand and overcome this challenge, the Jones Lab engineers and employs innovative 'next-generation biochemistry' technologies that pair classical biochemistry with the output of high-throughput sequencing. With them, we reveal how nucleases discriminate DNA targets at nucleotide-resolution, inform their nucleolytic mechanisms, and build biophysical models that benchmark nucleases and predict their activity. This way, users can select the best tools for their gene editing applications.

Our team currently develops strategies to produce and evaluate diverse, novel CRISPR nucleases. We want to know how their targeting specificity evolves, and how we can leverage the unique features of different nucleases to make gene editing more predictable and safe.

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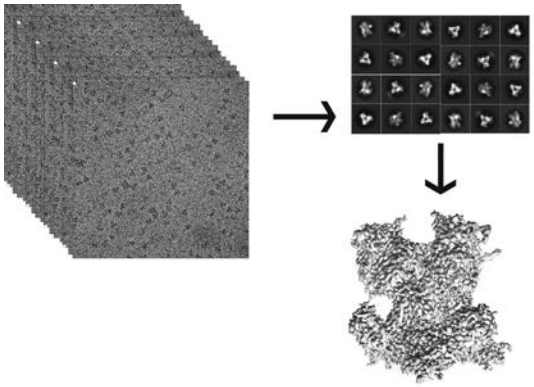

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Structure-Function Relationship of Procaryotic Defence Systems

Prokaryotes have evolved numerous defence strategies for preventing phage infection including innate immunity enabled by the restriction-modification system, and the adaptive immunity mediated by CRISPR-Cas (clustered regularly interspaced short palindromic repeats and its associated protein) system. In prokaryotes, genes encoding different anti-phage defence systems are often non-randomly clustered in specific genomic locations in prokaryotes, forming 'defence islands'. There is evidence to suggest that many additional phage resistance systems are present in the prokaryotes' genomes and are yet to be discovered. This field recently received a lot of attention due to an ingenious adaptation of the CRISPR-Cas system for precise gene editing and regulation.

The Malinaukaitė Lab employs X-ray crystallography and single particle cryo-electron microscopy (SP cryo-EM) to obtain 'snapshots' of macromolecules and their complexes in different stages of their action mechanism to understand their function and structural determinants of specificity. Furthermore, we can study binding kinetics using biophysical techniques, as well as capture and visualise different steps of reactions using SP cryo-EM, by freezing samples at specific time points, in this way locking functional states. In addition, due to extensive experience in membrane protein structural studies, our lab will focus on the mem-brane proteins associated with different defence systems, which have been studied to a lesser extent in the field. Therefore, functional and structural characterisation of these membrane proteins will lead to a better understanding of their role in the defence against phages.

Our research group aims to contribute to the structural and mechanistic characterisation of various prokaryotic phage defence systems, and a detailed mechanistic understanding will facilitate the development of novel tools for genetic engineering and biotechnological applications.

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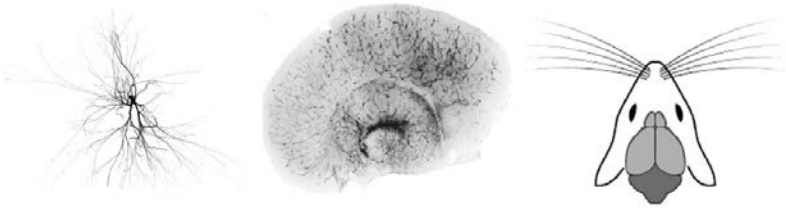

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Targeted CRISPR-Cas Delivery into Mammalian Nervous System

The discovery of CRISPR-Cas system and its application to specifically edit the genes *in vivo* provides new opportunities to treat neuropathologies at the genome level. To establish an effective approach of CRISPR-based gene editing in mammalian brain, a set of important aspects need to be addressed. Most effective CRISPR-Cas systems have to be screened, appropriate vectors for CRISPR-Cas delivery have to be selected, their efficiency has to be trialled in the living brain, and the outcome of selective gene editing needs to be thoroughly assessed. The Neniškytė Lab approaches this challenging task in a stepwise manner to develop CRISPR-Cas delivery system for selective gene editing of brain cells and to apply it in the models of nervous system disorders.

Using these new tools, we aim to define the molecular signalling pathways that drive this highly specific pruning of unnecessary synapses. For this, we use both *ex vivo* tissue cultures and genetically modified mouse lines. We are developing novel molecular tools for a rapid, selective and sensitive labelling of synaptic surface molecules. High-resolution fluorescent microscopy of developing circuits is supplemented with electrophysiology and animal behaviour experiments. We intend to define the synapses destined for elimination *in vitro*, and thereafter *in vivo*, and to elucidate their molecular signatures, giving first direct insights into the molecular cascades that are required for developmental synaptic pruning in the maturing circuits of the brain.

Projects implemented by our research group:

1) LIPSYNING: Lipid Scrambling as a Signal for Synaptic Pruning. European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement No. 705452 (2018–2021);
2) SINGLY: Glycobiology of Synaptic Pruning in Developing Brain. European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement No. 897958 (2020–2022);
3) Gliocobiology of glioblastoma multiforme. IBRO-PERC InEurope Grant in collaboration with the Albert Ludwig University of Freiburg (2020–2021);
4) Diet-microbiota-nervous system: the impact of maternal high fat diet for offspring neurodevelopment. Vilnius University Science Promotion Fund (2021–2022).

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OPEN ACCESS CORE FACILITIES

OPEN ACCESS CENTER

The Open Access Center (OAC) of the Life Sciences Center (LSC) was established in 2016 to enable researchers at VU LSC and other institutions to use modern equipment for research in biochemistry, biotechnology, molecular biology, genetics, neurobiology, molecular medicine, and other research areas.

Last year, EU-funded projects added new equipment to the OAC technical base, enabling both internal and external researchers to carry out a wide range of research using modern technology. Currently, there are eight advanced microscope systems from leading industrial companies enabling microstructure and other studies of small particles, allowing determination of the shape, dimensions and chemical structure of small objects.

Information about OAC as well as core facilities and instrumentation is available at: https://www.gmc.vu.lt/en/oac/

All open access facilities are listed in eight groups, short descriptions and main characteristics are provided for every item listed, contact scientist information is available. In addition, the full list of Core Facilities and Instrumentation is available for download at: https://www.gmc.vu.lt/en/oac/core-facilities-and-instrumentation

Animal Laboratory and *In Vivo* Testing

The laboratory animal facilities are designed to hold mice, rats and rabbits. The housing and handling of laboratory animals is controlled by the Animal Welfare Council. Our facilities have been approved by the Lithuanian State Food and Veterinary Service for animal breeding, supply and experimental work. Some of the facilities, including a fully equipped operating room and laboratory, are open access. The Ministry of Environment has approved the conditions as suitable for keeping genetically modified animals. Our staff has all the necessary certificates for animal research; they provide the technical assistance and housing of animals in accordance with the Directive 2010/63/EU on the protection of animals used for scientific purposes. The staff ensures that animal housing, handling and experimentation is in line with bioethical requirements.

The animal facilities at the Life Sciences Centre are specially designed to accommodate precisely controlled environments for the care and maintenance of experimental animals. They are kept either in high barrier SPF (specific-pathogen-free) or in low barrier (conventional) areas. The facilities are provided with key components: animal holding rooms, procedure rooms, a sterile operating room (equipped with all the necessary equipment: operating tables, surgical lighting, breathing apparatuses (Harvard 950), surgical blades (AARON 950), a pulse oximeter, a cardiograph (Custo Cardio 130), an ultrasound system (EUB-7000 HV, Hitachi), haematology analyser (Exigo EOS)) and all the other necessary animal laboratory areas.

Research in the facilities is focused on heart failure, stem cells and biocompatibility testing. Additionally, the following services are available: preclinical studies of novel drugs and chemical compounds, acute and repeated dose toxicity tests (oral, dermal, skin irritation, eye irritation, skin sensitization); immunization services and others. The facility provides qualified services to the scientific community of the Life Sciences Center and all external users. Regulatory and customized training courses on animal experimentation are regularly organized. The Laboratory Animal Science Training Program is certified by the Lithuanian State Food and Veterinary Service.

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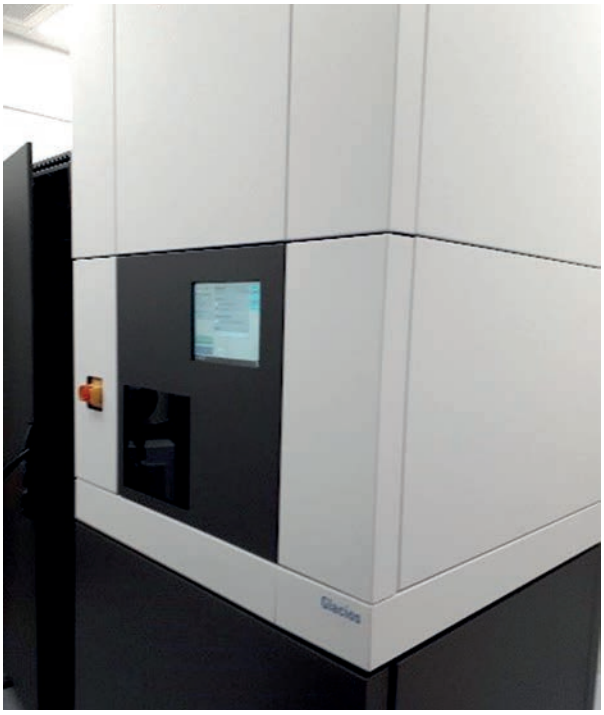
Cryo-Transmission Electron Microscopy

200 kV Cryo-Transmission Electron Microscope Glacios™ (Cryo-TEM) for Life Sciences with Falcon 3EC Direct Electron Detector and Volta Phase Plate (Thermo Fisher Scientific). The Glacios Cryo-TEM is dedicated for single particle analysis (SPA) workflow for pre-screening of sample quality before transferring to the 300 kV Krios Cryo-TEM for ultimate-resolution SPA data acquisition and for SPA data acquisition, and can be used for cryo-electron tomography. Sample preparation equipment available includes Vitrobot Mark IV (Thermo Fisher Scientific), which is used for automatic vitrification of samples for SPA or cryo-tomography and Gloqube Plus glow discharger (Quorum).



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High-Speed Atomic Force Microscopy

In 2020, a new high-speed AFM system SS-NEX (RIBM, Japan) became operational. This system is equipped with two scanners:

(i) a standard scanner for high-speed imaging such as enzyme reactions and structural changes of protein (scan speed – 50 ms/frame (20 frames/sec), maximum scan range – XY: 0.7 µm x 0.7 µm, Z: 0.4 µm);

(ii) a wide scanner for large samples with a high scanning rate (scan speed – 1 s/frame (1 frame/sec), maximum scan range – XY: 4 µm x 4 µm, Z: 0.7 µm).

This High-Speed AFM can observe real-time imaging as a movie; it allows a dynamic visualization of the nano-scale world.

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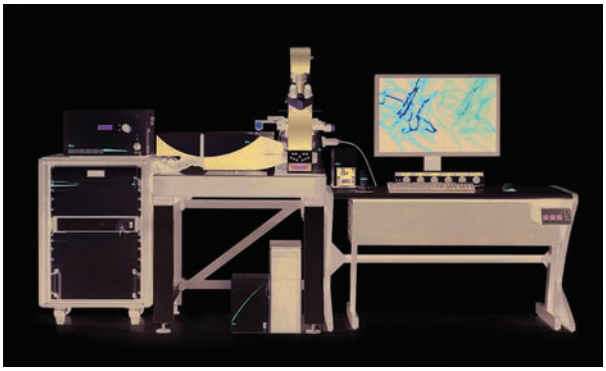
Super-Resolution Imaging Facility

Super-resolution imaging facility has recently acquired a Leica TCS SP8 confocal scanning microscope, equipped with motorized xy-stage 12 kHz tandem scanner, field of view scanner, gated HyD™ detection system, white light laser as well as LIGHTNING, FALCON and STED 3x modules. The white light laser source (470–670 nm) perfectly matches the excitation wavelength of any fluorophore and enables simultaneous use of up to eight excitation lines. By tuning both excitation and AOTF-based detection, complete two dimensional excitation-emission spectra can be acquired. Integrated LightGate technology removes unwanted fluorescence by adjusting the time gate for data collection. The SP8 LIGHTNING module combines the benefit of super-resolution (up to 120 nm) and simultaneous high-speed imaging for multiple fluorescent markers with low photo toxicity. SP8 FALCON module is a truly integrated solution for fluorescence lifetime imaging (FLIM) throughout the SP8 platform. It enables imaging fast molecular interactions via FLIM-FRET and the use of fluorescent biosensors with minimal training. The fully integrated stimulated emission depletion STED 3X system with 775 nm pulsed STED laser provides fast, intuitive and purely optical access to structural details beyond the light diffraction limit (up to 30 nm).



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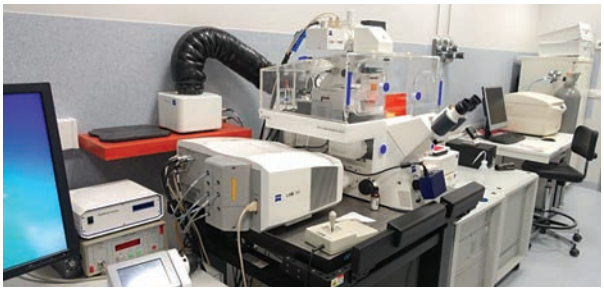
Proteomics and Imaging (Confocal Microscopy)

The Proteomics Center is designated to perform high throughput, differential, quantitative proteome analyses and analyse protein localization and functions in fixed or live cells. The Center is equipped with the Waters Synapt G2 higher definition mass spectrometer and the Sciex Qtrap4000 linear trap mass spectrometer, both directly coupled to nano-liquid chromatography systems and indirectly connected with a capillary range Dionex chromatography system. It offers the following services: 1) protein identification and quantitation in low and highly complex protein mixtures; 2) the implementation of a *de novo* sequencing of proteins from organisms with unknown or incomplete genomes; 3) discovery and quantitation of various covalent protein modifications; 4) performing a bioinformatic analysis to highlight the novel functions and molecular mechanisms of various biological systems. Center is involved in biomarker discoveries and validations including the search for biomarkers for the chemotherapeutic resistance of colon cancer chemotherapy and the early diagnostic markers of pancreatic cancer. It also performs a proteomic analysis of cell midbodies.



A confocal microscopy infrastructure offers unique possibilities by applying a Nicon C1 confocal microscope attached to a microinjection system as well as a Zeiss LSM710 confocal spectral microscope coupled with a fast, linear scanning microscope equipped with a live cell incubation unit to study proteins and other structures, including (1) protein co-localization and interaction, (2) protein movement in live cells, (3) cell movement, apoptosis and tissue-like structure formation, etc.

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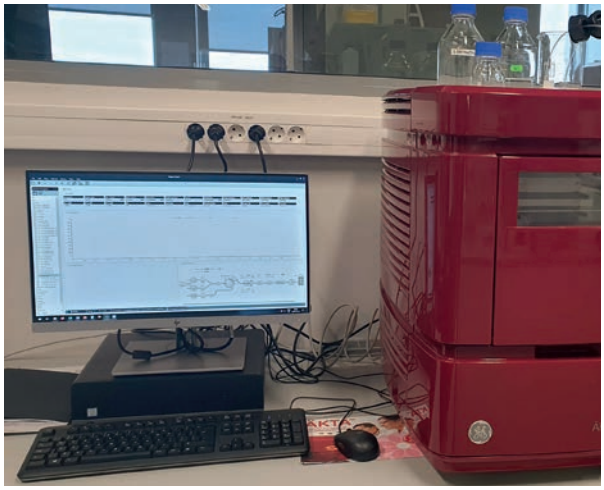
Automatic High-Protein Purification System with Refrigeration Function

ÄKTA avant 25 is a preparative chromatography system designed for fast and secure development of scalable methods and processes.

This system allows us to purify small or large amounts of biological macromolecules (proteins, nucleic acids or their complexes). The instrument has a modular design, with all valves, monitors, and columns mounted on the side of the system for easy access. The built-in fraction collector with cooling functionality protects purified samples. UNICORN 7-control software gives real-time control of the chromatography system, and provides an intuitive interface for method editing and evaluation of results.



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Crystallography Open Database

The Crystallography Open Database (COD, http://www.crystallography.net/cod/) is the largest to date open access collection of small molecule crystal structures, including organic non-polymer, inorganic and metal-organic compounds and minerals. All data are available in standard Crystallographic Interchange Framework (CIF) format. The COD presents facilities to browse and access individual entries, download the whole data collection at once and to keep a synchronized copy locally. A means to search the database by structural formulae is provided in addition to the interface to query bibliography and crystal parameters. Contributions from everyone are accepted in automated, Wikipedia-like fashion. All new entries are checked and fixed if necessary to ensure their compliance to the CIF format syntax as well as validation criteria established by the International Union for Crystallography. Changes made to each of the COD entries are preserved and made publicly available for the provenance. The development and the curation of its data collection is carried out at VU Life Sciences Center with the help of an international advisory board.

The COD is used by scientists world-wide. The two papers [1,2] were cited together over 1000 times by various media according to Google Scholar.

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X-Ray Diffractometer and Crystal Growth Equipment

Fig. 1 a. Robotic equipment for crystal growth and automatic crystal observations; a crystallization plate preparation robot (in the back).

Fig. 1 b. An X-ray diffractometer for small molecule and macromolecule crystal determination.

The X-ray crystallography core facility offers the possibility to crystallize biological macromolecules (proteins, protein nucleic acid complexes and their complexes with small chemical ligands) using crystal growth and solution preparation robotics (Fig. 1a) and to determine their three-dimensional structures by means of single crystal X-ray crystallography techniques. The current diffractometer (Fig. 1b) comprises the Rigaku MM-007HF rotating anode microfocus generator with a Cu anode, VariMax focusing mirrors and two detectors: the Raxis-IV++ Image Plate detector (for protein crystallography) and the Pilatus 200k direct-conversion detector with a kappa stage (suitable for both small molecule and protein crystals). The Cu K_α radiation used in experiments is suitable for most organic crystals with light elements, and it allows determining the absolute configuration of small chiral compounds. Measurements are possible at temperatures from 90K to 290K (room temperature) in a nitrogen gas stream or in sealed capillaries. Crystals the size of 50 µm to about 1 mm are suitable for investigation.

Protein crystals can be grown in high-throughput experiments from 100 nl–5 µl drops in standard polycarbonate or polystyrene crystallization plates. Robots are available for both crystallization solution preparations and for crystallization drop setups. For initial screenings of crystallization conditions, a range of commercial and in-house-made buffer collections is available. Help with data processing and structure solution is offered as well, if necessary.

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Chemical Synthesis of Organic Compounds for Industrial and Academic Purposes

Our mission lies in bridging the gap between the laboratory and the market via pilot-scale development. Our research is aimed at the cooperation with Lithuanian and foreign business entities interested in introducing the results of research into practice.

We offer services to fellow scientists and business representatives in the field of organic synthesis:

- development and optimization of technologies for the synthesis of chemical compounds;
- testing of the scalability of chemical technology designed by the interested developers;
- investigation of synthesis methods for organic compounds of different classes, development and design of multi-step synthesis schemes;
- custom synthesis of fine chemicals for research, commerce and industry.

We have experience in the synthesis of amino acids and their derivatives, the search of synthesis pathways and the development of technologies for macrocyclic and linear polyethers and the investigation of the synthesis, structural and other properties of various heterocycles. Our product portfolio contains over 200 compounds of various classes: O, N and S-heterocyclic compounds, thiols, thioethers and thioamides, stereoisomeric disubstituted cyclohexane derivatives, aromatic carboxylic acids, aminoacid derivatives, mono- and disubstituted cyclic polyethers, monodisperse derivatives of polyethylene glycols. These high-quality fine chemicals for scientific and commercial purposes are produced in quantities from grams to hundreds of

kilograms, depending on the compound structures and the requirements of the customers.

Our reactor equipment scale includes different volume glass (20–100 L), glass-lined (10–1600 L) and stainless-steel reactors (10–600 L) as well as autoclaves for catalytic hydrogenation (0.2–10 L) and different kinds of auxiliary equipment. Reactors of various types and volumes enable us to carry out a number of different projects simultaneously.

We have provided our services to Ramidus AB (Sweden), Synthon Chemicals GmbH (Germany), Polypure AS (Norway), Thermo Fisher Scientific Baltics, UAB (Lithuania), Elymus, UAB (Lithuania), Certumtech, UAB (Lithuania), Ekorama, UAB (Lithuania), Vilniaus Ventos Puslaidininkiai, UAB (Lithuania).

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DNA Sequencing Centre

The DNA Sequencing Centre (SC), part of the Institute of Biotechnology (IBT) at the Life Sciences Center of Vilnius University, has been successfully running since March 27, 2003. The SC was founded to help researchers, at both IBT as well as other institutions in Lithuania, to process DNA samples in an efficient and economical manner. The Centre is equipped with the Applied Biosystems 3130xl Genetic Analyser 16-capillary automated DNA sequencer that yields from 700 to 1000 bases per template. It performs cycle sequencing reactions using fluorescent dye terminators ABI Big Dye® Terminator v3.1 on any kind of DNA (plasmid, phage or PCR product) provided by the users. We also run reactions made by the users themselves. Usually, the turnaround time takes 2–3 days after receiving samples. The sequencing of the larger samples may take longer. The results of the DNA sequencing are provided to the customer with an e-mail as a text document (.seq) and with the chromatograms provided in ABI format (.abI).

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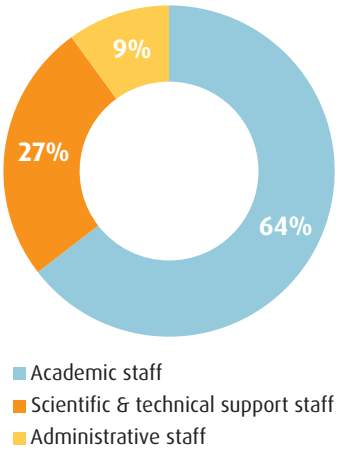
FACTS & FIGURES

Staff and Students

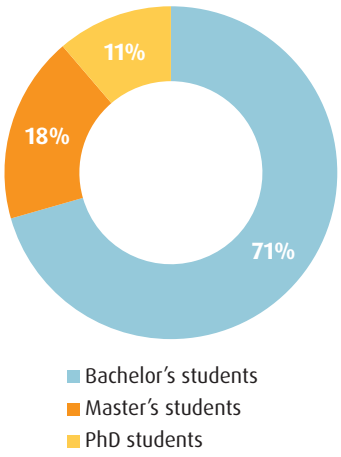
Staff

Academic staff	290
Scientific & technical support staff	120
Administrative staff	42
Total staff	**452**



Students

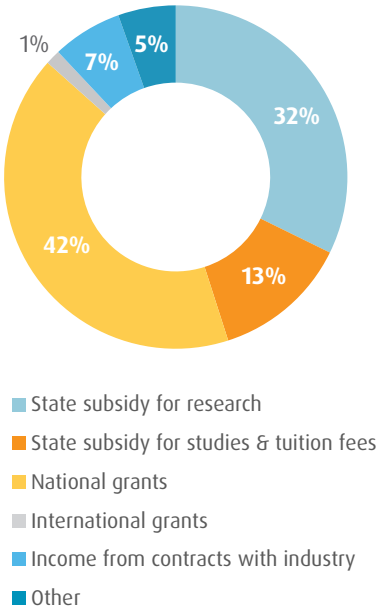
Bachelor's students	827
Master's students	213
PhD students	132
Total students	**1172**



Financing Sources

Total income – 19.5 m EUR
Income increase 36%

Source of funding	*2021*
State subsidy for research	6 286 507 €
State subsidy for studies & tuition fees	2 495 303 €
National grants	8 095 512 €
International grants	272 808 €
Income from contracts with industry	1 281 883 €
Other	1 058 441 €
Total	**19 490 454 €**



International Advisory Council

International Advisory Council of the Life Sciences Center was established at the end of 2017 seeking for the insight, high quality guidance and advice of the outstanding scientists, industrial leaders and administrative experts who could contribute to further development and growth of the Life Science Center into one of the leading research and education centres in Europe. It consists of seven members appointed for a five-year term. In 2021, VU LSC hosted the third meeting of the International Advisory Council.

HEINRICH LEONHARDT
Professor
Ludwig Maximilians University
Munich, Germany

CHRIS LOWE
Professor
Director
Institute of Biotechnology,
University of Cambridge, UK

BARBARA MAZUR
Strategic advisor
Pontifax Global Food and
Agriculture Technology Fund

ANDRES METSPALU
Professor
Estonian Genome Center,
Institute of Genomics,
Head of the Estonian Biobank,
University of Tartu, Estonia

TOMMY NYLANDER
Professor
Lund University, Sweden

TAINA PIHLAJANIEMI
Vice Rector for Research
Oulu University, Finland

SILKE SCHUMACHER
External Expert
Directorate-General for Research &
Innovation
European Commission

International Advisory Council meeting at the LSC on October 28, 2021. From the left: D. Lukšienė, K. Buivydaitė, M. Šimoliūnienė, D. Baltriukienė, I. Prigodina Lukošienė, E. Lastauskienė, B. Mazur, A. Metspalu, G. Valinčius, T. Pihlajaniemi, T. Nylander, S. Schumacher, K. Krikštopaitis, V. Šikšnys, V. Smirnovas

International Grants

Horizon 2020

Title	*Head of the project*	*Duration*
Single-cell temporal tracking of epigenetic DNA marks (EpiTrack) ERC-2016-ADG: 742654	S. Klimašauskas	2017–2022
High-throughput droplet-based single-cell small RNA sequencing technology; ID: 101030265	L. Mažutis S. Juzėnas	2021–2023
Eat me microglia: lipid scrambling as a signal for synaptic pruning MSCA-IF-2015-EF: 705452	U. Neniškytė A. Alaburda	2016–2021
Glycobiology of synaptic pruning in developing brain (SINGLY) Marie Skłodowska Curie Actions Individual Fellowship	A. Parimisetty U. Neniškytė	2020–2022

Title	*Lead scientist*	*Duration*
Directed EVOlution in DROPS (EVOdrops) MSCA-ITN-2018: 813786	L. Mažutis	2018–2022
Infrastructure for transnational access and discovery in structural biology (iNEXT-Discovery) 871037	G. Tamulaitienė	2020–2024

EMBO Installation Grant

Title	*Lead scientist*	*Duration*
Single-molecule force studies on Cas3 helicase motor involved in CRISP EMBO IG 4763-2020	A. Toleikis	2021–2024

Baltic Research Programme

Title	*Lead scientist*	*Duration*
Novel high-performance polymers from lignocellulosic feedstock (No. EMP426)	I. Matijošytė	2020–2023

European Joint Programme on Rare Diseases

Title	*Lead scientist*	*Duration*
Unveiling the role of glutamate in dopamine transporter deficiency syndrome (URGENT); S-EJPRD-20-1	J. Razumienė	2020–2023
Genetic therapy for EYS- and USH2A associated retinal dystrophy (GET-READY); EJPRD-21-1	V. Šikšnys	2021–2024

EuroNanoMed3 - European Innovative Research & Technological Development Projects in Nanomedicine

Title	*Lead scientist*	*Duration*
A liquid corneal glue-filler as an alternative to transplantation in high-risk patient (LIQD-CORNEA)	V. Bukelskienė	2019–2022

Lithuanian-French Programme *Gilibert*

Title	*Lead scientist*	*Duration*
Characterization of antiplasmodial agent plasmodione and its metabolites (S-LZ-19-4)	N. Čėnas	2019–2020
New methods for modelling protein-protein and protein-ligand interactions (S-LZ-19-5)	Č. Venclovas	2019–2020

Lithuania-Japan Research Programme

Title	*Lead scientist*	*Duration*
Individual gamma frequency-based neurofeedback: development and implementation study (S-LJB-20-1)	I. Griškova Bulanova	2020–2022

Lithuania-Latvia-Taiwan Cooperation Programme

Title	*Head of the project*	*Duration*
Brain-computer music interfacing for embodied musical interaction (S-LLT-19-3)	I. Griškova-Bulanova	2019–2021
Development of lead inhibitor of carbonic anhydrase IX as anticancer drug (S-LLT-20-2)	D. Matulis	2020–2022

Lithuania-Poland Cooperation Programme *Daina*

Title	*Lead scientist*	*Duration*
Long-distance electrical signalling systems in plants – adaptation to the change from water to terrestrial environment (S-LL-18-1)	V. Kisnierienė	2018–2021
Genomic insights into the mechanisms of drug resistance, virulence, and transmission of *Mycobacterium tuberculosis* strains from Lithuania and Poland (P-LL-18-4)	P. Stakėnas	2018–2021
CRISPR tools for the study of embryonic development in zebrafish (S-LL-18-7)	G. Tamulaitis	2018–2021

Other International Projects

Title	*Lead scientists*	*Duration*
Multicellular organoids: modelling, mechanisms and therapy development for C9ORF72-associatedd neurodegeneration	D. Baltriukienė	2020–2023
IBRO Return Home Fellowship	R. Guzulaitis	2020–2022
Antimicrobial photoinactivation approach based on natural agents for control of bacteria biofilms in spacecraft. European Space Agency (ESA)	L.Kalėdienė A.Gricajeva	2020–2021
IBRO-PERC InEurope Short Stay Grants	U. Kuliešiūtė	2020–2021
Cdk5 confers tumour resistance to adaptive immunity by PDL1 upregulation (St. Baldrick's Foundation Grant)	A. Petrošiūtė	2020–2021
IBRO-PERC InEurope Short Stay Grants	P. Tarailis	2021

COST

Title	*Management Committee members*	*Duration*
In vitro 3-D total cell guidance and fitness (CA16119)	D. Baltriukienė V. Bukelskienė	2016–2021
A sound proteome for a sound body: targeting proteolysis for proteome remodelling (CA20113)	V. Borutinskaitė I. Kelpšienė	2021–2025
European Research Network on Signal Transduction (ERNEST) (CA18133)	R. Budvytytė M. Jankunec	2020–2023
European Network for Problematic Usage of the Internet (CA16207)	I. Griškova-Bulanova	2017–2021
The neural architecture of consciousness (CA18106)	I. Griškova-Bulanova	2019–2023
International Nucleome Consortium (CA18127)	S. Klimašauskas G. Vilkaitis	2018–2023
European transdisciplinary networking platform for marine biotechnology (CA18238)	I. Matijošytė R. Šiekštelė	2019–2023
International Network for Translating Research on Perinatal Derivatives into Therapeutic Approaches (CA17116)	R. Navakauskienė J. Savickienė	2018–2022
Delivery of Antisense RNA Therapeutics (CA17103)	S. Serva A. Konovalovas	2018–2022
Personalized nutrition in aging society: redox control of major age-related diseases (CA16112)	V. Smirnovas L. Baranauskienė	2016–2021
New exploratory phase in research on East European cultures of dissent (CA16213)	V. Vaitkevičius I. Kelpšienė	2017–2021

National Projects

Individualized analysis of upper respiratory tract microbiome – a novel diagnostic and healthcare tool (YourAirwayMicrobiome) (No. 01.2.2-LMT-K-718-03-0079)	J. Armalytė	2020–2023
Development of virus-like particles-based vaccine against *Acinetobacter baumannii* (No. S-SEN-20-1)	J. Armalytė	2020–2021
Supramolecular recognition-based sensors for electro- detection of biomolecules (No. P-MIP-20-45)	G. Bagdžiūnas	2020–2022
Genetic and molecular analysis of the role of Tbx5a in heart regeneration (No. 09.3.3-LMT-K-712-17-0014)	D. Balčiūnas	2020–2023
Centre for genetic modelling of animals (No. 01.2.2-CPVA-K-703-03-0032)	D. Baltriukienė	2020–2023
Interactions of misfolded proteins and phospholipid membranes: possible key in neurodegeration (NeuroMisFolDe) (No. 09.3.3-LMT-K- 712-18-0003)	R. Budvytytė	2020–2022
Artificial urethra for the treatment of hypospadias and urethral strictures (No. 01.2.2-LMT-K-718-03-0087)	V. Bukelskienė	2020–2023
Targeting the microbiota-gut-brain axis in Alzheimer's disease: the role of the endocannabinoid system (No. 01.2.2-LMT-K-718-02-0014)	A. Burokas	2019–2023
Biomarkers of the gut microbiota in autistic spectrum disorders (No. 01.2.2-LMT-K-718-03-0099)	A. Burokas	2020–2023
A healthy microbiota for a healthy brain ageing (No. S-SEN-20-9)	A. Burokas	2020–2021
Impact of climate change on the sustainability of aquatic vegetation in rivers with Ranunculion communities (Habitat of European importance 3260) (No. S-SIT-20-1)	J. Butkuvienė	2020–2021
Self-assembling phage proteins for targeted nanomedicine (No. S-SEN-20-4)	V. Časaitė	2020–2021
Redox chemistry, biochemistry and cytotoxicity of aromatic nitrocompounds and N-oxides: new insights (No. 09.33-LMT-K-712-01-0058)	N. Čėnas	2018–2021
Screening for new methods for treatment of neurodegenerative disorders (No. 01.2.2-LMT-K-718-03-0021)	E. Čiplys	2020–2023
Biocatalytic systems for conversion of non-starch poli- and oligosaccharides (No. 01.2.2-LMT-K-718-01-0019)	M. Dagys	2018–2022
Development of biosensor research and engineering competence and technology transfer centre (BIOSENSE) (No. 01.2.2-CPVA-K-703-03-0010)	M. Dagys	2020–2023
Research on bat influenza virus (No. 09.3.3-LMT-K-712 -08-0002)	J. Juozapaitis	2018–2021
Determinants of quality of life in Lithuanian students: problematic usage of the Internet and neuropsychological profile (No. S-GEV-20-5)	I. Griškova Bulanova	2020–2021
Molecular mechanisms of adaptation of low-temperature phages to the mesophilic host (No. P-MIP-19-58)	L. Kalinienė	2019–2022
Designing of the patient-specific, heterogeneous lung cancer cell *ex vivo* model system for drug efficiency prediction in personalized oncotherapy (No. 01.2.2-LMT-K-718-01-0072)	A. Kalvelyte	2018–2022
Hypoxia as cell stress in mRNA diversity and aging (No. S-SEN-20-17)	A. Kanopka	2020–2021
Characterization of new Cas12 nucleases for targeted genome modification (No. S-MIP-21-8)	T. Karvelis	2021–2023

Expanding the CRISPR toolbox for rapid detection and genomic surveillance of SARS-CoV-2 variants of concern (No 13.1.1-LMT-K-718-05-0021)	D. Kazlauskas	2021–2023
Single molecule TOP-Seq – an innovative technological platform for early non-invasive diagnostics of cancer and other epigenetic disorders (No. 09.3.3-LMT-K-712-01-0041)	E. Kriukienė	2018–2022
DNA Modification and Chromatin Dynamics during Growth and Differentiation of Healthy and Malignant Cells. (No. S-MIP-S-21-1)	E. Kriukienė	2021–2024
The impact of viral antigens on immune cells in the context of inflammaging (No. S-SEN-20-11)	I. Kučinskaitė-Kodzė	2020–2021
Discovery of novel bioactive microbial compounds in the unique environment: an investigation of the diversity, prevalence and expression (No. MIP-17-21)	N. Kuisienė	2017–2022
The influence of intensive fish farming on aquatic microbiome and resistome (No. S-SIT-20-6).	E. Lastauskienė	2020–2021
Analysis of the *Geobacillus* sp. Synthetized Silver Nanoparticles Mechanisms of Action on the Biocontrol of Pathogenic Skin Microbiota (No. KD-19142).	E. Lastauskienė	2019–2023
A system of restful web services for protein remote homology search in real time and protein modelling (No. 01.2.2-LMT-K-718-01-0028)	M. Margelevičius	2018–2022
Development of visualization systems for tumour and metastases detection in cancer diagnostics and optically-guided surgery using CA IX biomarker (No. S-SEN-20-10)	J. Matulienė	2020–2021
Design of Technological Prototype to Determine Compound Efficiency as Inhibitors of SARS CoV-2 Recombinant Viral Enzymes (No. 01.2.2-LMT-K-718-05-0011)	J. Matulienė	2021–2023
Design of pharmaceutical compounds for the treatment of cancer and neurodegenerative diseases (No. 01.2.2.-CPVA-K-703-03-0006)	D. Matulis	2020–2023
Design of compounds inhibiting BACE1 enzymatic activity and Aβ peptide aggregation for the treatment of Alzheimer's disease (No. 01.2.2-LMT-K-718-03-0003)	D. Matulis	2020–2023
Microfluidic technologies for single-cell geno- and phenotyping research (No. 09.3.3-LMT-K-712-01-0056)	L. Mažutis	2018–2021
Establishment of single-cell transcriptomics/genomics research parallel-laboratory (No. 01.2.2-LMT-K-718-04-0002)	L. Mažutis	2020–2023
Chemical annotation in the Crystallography Open Database (COD) (No. S-MIP-20-21)	A. Merkys	2020–2022
Centre for engineering of the next-generation enzymes (TVIRTAS) (No. 01.2.2-CPVA-K-703-03-0023)	R. Meškys	2020–2023
Selective enzymatic system for prodrug activation (No. 01.2.2-LMT-K-718-03-0082)	R. Meškys	2020–2023
Development of innovative targeted therapies and prognostic tools for chemotherapy-resistant acute myeloid leukaemia (No. S-SEN-20-2)	R. Navakauskienė	2020–2021
The role of epigenetic oscillations in predicting biological age (No. S- MIP-19-66)	A. Petronis	2019–2021
Identifying chronoepigenetic markers in schizophrenia (No. 09.3.3-LMT-K-712-17-0008)	A. Petronis	2020–2023
Next generation epigenetic markers for accelerated ageing in colorectal cancer (No. S-SEN-20-19)	A. Petronis	2020–2021

Studies on the virulence potential of meningococcal isolates: implications for an improved molecular diagnostics of invasive meningococcal disease (No. 01.2.2-LMT-K-718-03-0036)	M. Plečkaitytė	2020–2023
Biosensor platform for fast, cheap and accurate quantification of amino acids in patients undergoing renal replacement therapy (No. 01.2.2-LMT-K-718-03-0005)	D. Ratautas	2020–2023
Development of non-invasive method platform for early diagnostics and prognosis of acute pancreatitis (No. 01.2.2-LMT-K-718-01-0025)	J. Razumienė	2018–2022
Adaptation mechanism in Class 2 CRISPR-Cas systems (No. S-MIP-19-32)	G. Sasnauskas	2019–2022
System for virus spread control and extreme situation (S-DNR-20-2)	S. Serva	2020–2021
Cross-interactions in amyloid fibril formation: from mechanisms to inhibition (No. S-SEN-20-3)	V. Smirnovas	2020–2021
Enzyme toolkit for the synthesis of fucosylated oligosaccharides (No. 01.2.2-LMT-K-718-03-0045)	J. Stankevičiūtė	2020–2023
Molecular mechanisms of new bacterial antiviral systems (No. 09.3.3-LMT-K-712-01-0126)	V. Šikšnys	2018–2022
Diversity and distribution of viruses infecting sulphur metabolising bacteria (No. P-MIP-20-38)	E. Šimoliūnas	2020–2022
Search of Anti-CRISPR proteins and research of their action (No. S-MIP-20-39)	T. Šinkūnas	2020–2022
Structural and Functional Studies of Thoeris Bacterial Antiphage Defence System (No. S-MIP-21-6)	G. Tamulaitienė	2021–2024
Studies of genome editing tools at the single-molecule level (No. S-MIP-20-55)	M. Tutkus	2020–2022
Development of novel proteomics-based drug selection approach for pancreatic cancer individualized therapy (No. S-SEN-20-16)	M. Valius	2020–2021
Computational study of evolutionary relationships, genomic distribution, structural and functional properties of DNA polymerases (No. 09.3.3-LMT-K-712-01-0080)	Č. Venclovas	2018–2022
Quantitative assessment of the phospholipid membrane damage exerted by the pore-forming toxins (S-MIP-19-33)	G. Valinčius	2019–2022
Analysis of 5`-capped RNAs and its modulating proteins in *E. coli* and probiotic lactic acid bacteria (No. S- MIP-19-31)	G. Vilkaitis	2019–2022
Sequencing centre of DNA double stranded breaks (No. 01.2.2-CPVA-K-703-02-0010).	M. Zaremba	2018–2021
Structural and functional studies of split prokaryotic Argonaute proteins (No. S-MIP-20-37)	M. Zaremba	2020–2022
Development of a Highly Sensitive Digital RT-PCR Method for Absolute Quantification of SARS-CoV-2 Virus (No. 01.2.2-LMT-K-718-05-0033)	R. Žilionis	2021–2023
Novel affinity binders for immunodetection of antimicrobial resistance (No. 01.2.2.-MITA-K-702-05-0003)	A. Žvirblienė	2020–2023
New technologies for development of recombinant allergens (No. 01.2.2-LMT-K-718-01-0008)	G. Žvirblis	2018–2022

Partnership Highlights

MINISTRY OF EDUCATION, SCIENCE AND SPORT OF THE REPUBLIC OF LITHUANIA

EMBL

The VU Life Sciences Center Partnership with the European Molecular Biology Laboratory (EMBL)

VU Life Sciences Center and EMBL Partnership has started on 8 September 2020 and is gaining momentum. In 2021, the VU Life Sciences Center – EMBL Partnership Institute consisting of six research groups was established. Ministry of Education, Science and Sport of the Republic of Lithuania has allocated EUR 6 million from the EU funds to implement the partnership project. Therefore, the selection of the principal researchers for the newly established Partnership Institute was carried out through an open international competition following the international standards and best practices applied by the EMBL. A 5-member committee was formed for the selection of the candidates. The committee included Director General of the EMBL Prof. Edith Heard, Director of the EMBL Scandinavian Partnership Institute Prof. Poul Niesen, VU Researcher Prof. Virginijus Šikšnys (Chairman), Prof. Saulius Klimašauskas and the VU LSC Director Prof. Gintaras Valinčius.

Six group leaders were selected and started building their research groups: Dr Jonathan Arias' group will focus on gene editing of stem and immune cells (see p. 88), Dr Stephen Knox Jones' group will focus of the mechanisms and specificity of the genome editing tools (see p. 89), Dr Lina Malinauskaitė's group will focus on structure-function relationships across gene editing systems (see p. 90), Dr Urtė Neniškytė's, group will focus on gene editing of neurons and cell systems (see p. 91), Dr Patrick Pausch's group will focus on discovery and structures of genome editing systems (see p. 86) and Dr Giancarlo Russo's group will focus on the gene origins and (epi)genomics (see p. 87).

On 18 November 2021, EMBL Director General Prof. E. Heard participated in the inauguration of the new EMBL and Vilnius University Life Sciences Center Partnership Institute. Speaking at the inauguration event, Prof. E. Heard said, "Understanding how life works through basic research will be critical for addressing the challenges facing humanity. By working together with the Life Sciences Center, we can help boost knowledge of biological processes and disease mechanisms, and enable deep understanding to bring maximum benefit across Europe. Lithuania's membership of EMBL is helping life science expertise and training to reach more researchers, and will offer fantastic opportunities in the coming years."

Speaking at the event, the VU LSC Director Prof. G. Valinčius said, "As institutional partners of EMBL, we have every opportunity to collaborate with EMBL scientists, to strengthen our scientific competencies and international academic reputation. Equally importantly – through the partnership – we are transferring the best practices of EMBL's research and academic organisation and activities, gaining new knowledge to operate in the global international research environment and talent acquisition markets. We hope that close cooperation will help to attract international funding for the VU LSC's research, and enable it to take advantage of competitive international funding opportunities."

Jurgita Šiugždinienė, Minister of Ministry of Education, Science and Sport, Edith Heard, EMBL Director General, Gintaras Valinčius, Director of the VU Life Sciences Center

Left to right: Stephen Knox Jones, group leader, Lina Malinauskaitė, group leader, Virginijus Šikšnys, Chairman of the Board of the VU Life Sciences Center, Edith Heard, EMBL Director General, Gintaras Valinčius, Director of Vilnius University Life Sciences Center, Urtė Neniškytė, group leader, Giancarlo Russo, group leader, Patric Pausch, group leader, Plamena Markova, EMBL Head of International Relations

During her visit Prof. E. Heard discussed the wider application of genome editing, the opportunities for the VU LSC scientists and students, and ways to attract international scientists.

During her visit, Prof. E. Heard discussed the wider application of genome editing, the opportunities for not only VU LSC but also scientists from other institutions in Lithuania to get access to both knowledge and instruments at EMBL, to develop their competences and, taking advantage of immense opportunities for networking, to increase their international visibility.

Instruct-ERIC Member

On the 1st of January 2020, Lithuania became a new member of Instruct-ERIC, which is a research infrastructure, providing open access to innovative structural biology, specifically supporting research that uses integrated approaches and technologies. The VU LSC scientists got access to the unique infrastructure and are now eligible to apply for funding to use structural biology services at all Instruct-ERIC centres, as well as training courses, internships, and R&D awards. Since 2020, Instruct-ERIC has been running the "Structure Meets Function" webinars to highlight the latest developments in structural biology in the different Instruct centres and Instruct countries. On 16 November 2021, the webinar was hosted by the VU LSC and featured by seminars of two prominent researchers: Prof. Gintaras Valinčius' seminar "Biomembranes on a chip: tethered phospholipid bilayers for protein membrane interaction studies" and Prof. Virginijus Šikšnys' seminar "Bacterial immunity: from restriction enzymes to CRISPR".

Arqus Alliance

ARQUS is European University Alliance, which brings together the Universities of Bergen, Granada, Graz, Leipzig, Lyon, Padova and Vilnius. This alliance was formally established in Brussels on 27 November 2018. Granada University is coordinating the overall organisation of the Arqus European University Alliance alongside the management of the first pilot project in order to work towards the major goals and fulfil the overall Vision and Mission of the Alliance.

On 10 October 2021, the Rectors of the Universities of Leipzig (Germany), Graz (Austria) and Bergen (Norway), which are members of the European University Alliance Arqus, visited Vilnius University Life Sciences Center.

Left to right: Prof. Martin Polaschek, Rector of the University of Graz, Prof. Beate Schücking, Rector of the University of Leipzig, Prof. Gintaras Valinčius, Director of the Vilnius University Life Sciences Center, Prof. Joachim Reidl, Vice Rector of the University of Graz, Prof. Margareth Hagen, Rector of the University of Bergen and Prof. Rimvydas Petrauskas, Rector of Vilnius University

The First Arqus Winter School 2021 *Rethinking Climate Risks*

On February 2021, the first winter school *Rethinking Climate Risks* brought together more than 65 students of Arqus Universities. During this school, students and experts from across Europe analysed and discussed Europe's role in the climate crisis following a challenge-based approach.

The VU LSC researcher Dr Arūnas Samas participated as an academic advisor in international student group and researcher Dr Alius Ulevičius conducted seminar "Climate change and habitat shifts. Who are the winners?"

Since September 2021, Dr A. Samas has been participating in the monthly remote meetings organized by the ARQUS CBP Academic Committee to plan the structure and content of the international study course for the spring semester of 2022. Along with Dr Irena Nedveckytė he participated in the international working group *Train the Trainer* on 3–4 November 2021, where the structure and content of the international study course for 2022 spring semester was discussed.

Doctoral School

DAIVA BALTRIUKIENĖ
Director of VU LSC Doctoral School

34 New PhD students in 2021

14 Defended doctoral theses

11 (in total) International PhD students

Over 130 doctoral students perform doctoral research at the VU Life Sciences Center (VU LSC) in the fields of biology, biophysics, ecology and environmental sciences, zoology, biochemistry, and biotechnology. Doctoral studies at the VU LSC are orientated towards the quality, flexible scheduling of study process and establishing suitable academic environment. Doctoral students also enhance their knowledge in various workshops, by participating in international events, academic projects as well as performing educational work. Doctoral School supports doctoral students during their doctoral training at the VU LSC by offering centralized services for PhD students, their supervisors and PhD Committees at the Life Sciences Center. The Doctoral School also focuses on increasing the international visibility and attractiveness of the PhD programmes for the undergraduates, who consider doing a PhD in the life sciences field.

PhD student community is an integral part of the VU LSC community. Student representatives are members of the Council, academic commissions, working groups of the Life Sciences Center providing student views/opinions on administration and strategic management of the Life Sciences Center. Senior graduate students suggest improvements to PhD programmes, welcome new students and consult fellow colleagues individually. The doctoral student community of the Life Sciences Center adhere to the principles of academic ethics, foster professional attitude and encourage collaboration over competition.

For more detailed information, please refer to:
https://www.gmc.vu.lt/en/doctoral-school

Information on admission, please refer to:
https://www.gmc.vu.lt/en/doctoral-school/admission

Admission contact: phd@gmc.vu.lt

A Joint Doctoral Degree

VU Life Sciences Center has signed agreements with KU Leuven (Belgium) and University of Trento (Italy) for the bi-nationally supervised doctoral thesis. The partners share the responsibilities of supervising, coordination and examining a researcher's work towards a doctoral degree. The first joint (double) degree containing a reference to the fact that the two institutions administered the doctoral procedure jointly was awarded to Gediminas Drabavičius who defended his thesis on 16 March 2021. Supervisors: Dr Giedrius Gasiūnas (Vilnius University) and Dr Dirk Daelemans (KU Leuven).

Doctoral Theses 2021

Name	*Topic*	*Supervisors*
L. Aitmanaitė	Virus compatibility in *Saccharomyces cerevisiae* LA and M virus systems	S. Serva
D. Dapkutė	Mesenchymal stem cell and cancer response to treatment with theranostic nanoparticles – towards cell therapy	R. Rotomskis
E. Denkovskienė	Construction and evaluation of inducible expression systems for controlled transfer of *Agrobacterium tumefaciens* T-DNA in plant transient expression systems	A. Ražanskienė
G. Drabavičius	Study and application of CRISPR-Cas systems	G. Gasiūnas D. Daelemans
R. Galinis	Synthesis of DNA and magnesium pyrophosphate particles and their use for protein expression *in vitro*	L. Mažutis
A. Janonienė	Exploration of carbonic anhydrase IX inhibitor influence on cancer cell migration and suitability to use in anticancer agent delivering nanosystems	V. Petrikaitė
Ž. Kapustina	The utility of modified nucleotides for high-throughput nucleic acid analysis	A. Lubys
A. Krikštaponis	Investigation of the catabolism of 7-hydroxycoumarin in *Pseudomonas mandelii* 7HK4 bacteria	R. Meškys
R. Kubiliūtė	Diagnostic and prognostic DNA methylation biomarkers of renal clear cell carcinoma	S. Jarmalaitė
M. Mickutė	The application of animal Hen1 methyltransferases for labelling and sequencing of single-stranded RNAs	G. Vilkaitis
A. Nestarenkaitė	Immune response assessment in colorectal cancer microenvironment by digital pathology analytics	A. Laurinavičienė
M. Simanavičius	The prevalence studies and diagnostics of hepatitis E virus	I. Kučinskaitė-Kodzė
I. Songailienė	Mechanisms of antiviral defence: CRISPR-Cas and toxin-antitoxin systems	V. Šikšnys
E. Šimoliūnas	Study of molecular mechanisms induced by cell-scaffold interaction	D. Baltriukienė

Bachelor and Master Studies

INGRIDA PRIGODINA LUKOŠIENĖ
Deputy Director for Studies

Studies in Numbers

1 040 Total number of bachelor & master students

827 BSc students

213 MSc students

287 New BSc students

117 New MSc students

10 International MSc students*

68 ERASMUS students

97 Graduates BSc studies

92 Graduates MSc studies

* From Armenia, India, Jordan, Nigeria, Norway, Russia, South Korea, Spain, Turkey and Uganda.

Bachelor Study Programmes

Title	*Study language*	*Number of students in 2021*
Biology	Lithuanian	130
Genetics	Lithuanian	154
Microbiology	Lithuanian	161
Molecular Biology	Lithuanian	152
Neurobiophysics	Lithuanian	119
Environmental Science and Protection	Lithuanian	42
Molecular Biotechnology	Lithuanian	69

Master Study Programmes

Title	*Study language*	*Number of students in 2021*
Environmental Studies and Management	Lithuanian	16
Biochemistry	English/ Lithuanian	24
Biodiversity	Lithuanian	21
Biophysics	English/ Lithuanian	12
Genetics	English/ Lithuanian	25
Microbiology	Lithuanian	32
Molecular Biology	English/ Lithuanian	32
Molecular Biotechnology	English	26
Neurobiology	English/ Lithuanian	25

International Study Programmes

For international students interested in studying life sciences, the LSC offers six international master's study programmes.

Biochemistry. The LSC master's programme in biochemistry provides students with in-depth knowledge of biochemistry and related sciences as well as with practical research skills. A holder of a master's degree in biochemistry knows and is able to apply modern methods and technologies of experimental biochemistry and related sciences *in vivo*, *in vitro* and *in silico*. The holder of this degree will also be able to integrate knowledge from different sciences and work in the interdisciplinary areas.

For more detailed information regarding the programme, please, refer to https://www.vu.lt/en/studies/master-studies/biochemistry

Academic contact: Prof. Saulius Serva.
Email: saulius.serva@gf.vu.lt
Admission contact: admissions@cr.vu.lt

Biophysics. A holder of a master's degree in biophysics has good knowledge of the general principles of operation and pathology in live systems, the capabilities and limitations of modern biophysical methods, principles of data analysis and planning of scientific investigation.

For more detailed information regarding the programme, please, refer to https://www.vu.lt/en/studies/master-studies/biophysics

Academic contact: Prof. Aidas Alaburda.
Email: aidas.alaburda@gf.vu.lt
Admission contact: admissions@cr.vu.lt

Genetics. The VU LSC master's programme in genetics will provide students with in-depth theoretical knowledge and good practical research skills in molecular, human, plant genetics or the genetics of microorganisms, gene engineering, cytogenetics, genotoxicology and gene informatics. A holder of a master's degree in genetics is able to carry out independent research projects, apply different modern research methods and has a good understanding of frontline issues and unsolved problems in genetics.

For more detailed information regarding the programme, please, refer to https://www.vu.lt/en/studies/master-studies/genetics

Academic contact: Prof. Juozas Lazutka.
Email: juozas.lazutka@gf.vu.lt
Admission contact: admissions@cr.vu.lt

Molecular Biology. A holder of a master's degree in molecular biology has deep knowledge in the cell structure and function of organisms of all domains of life at the molecular level, uses molecular biology methods to investigate cells and their components, applies them in research and practical work in life science-associated areas, independently identifies and solves molecular biology-related problems and their complexity in biotechnology, biomedicine, biopharmacy and environmental safety.

For more detailed information regarding the programme, please, refer to https://www.vu.lt/en/studies/master-studies/molecular-biology

Academic contact: Assoc. Prof. Aušra Sasnauskienė.
Email: ausra.sadauskaite@gf.vu.lt
Admission contact: admissions@cr.vu.lt

Neurobiology. The LSC master's programme in neurobiology will provide students with knowledge and practical skills in the areas of the neurosciences, such as electrophysiology, behaviour and psychophysiology. A holder of a master's degree in neurobiology will be able to apply modern experimental methods for investigating the nervous system and its interaction with other bodily systems, to independently solve neurobiology-related problems and their complexity in the context of modern life sciences and to work within interdisciplinary areas as well as integrate knowledge from different scientific fields.

For more detailed information regarding the programme, please, refer to https://www.vu.lt/en/studies/master-studies/neurobiology

Academic contact: Prof. Osvaldas Rukšėnas.
Email: osvaldas.ruksenas@gf.vu.lt
Admission contact: admissions@cr.vu.lt

Molecular Biotechnology. Technological Sciences, master's, two study years. The aim of this programme is to train professionals who would like to experience of what studying a doctorate might be, whilst at the same time allowing to earn a highly valuable master's level qualification for a career in industry. The uniqueness of the programme is a study based on individual interdisciplinary specialization according to student's interest through projects in laboratories as well as individual contact hours (supervising).

The graduate of this programme will be able to plan and conduct a research project, understand and construct the methodology, analyse and present the results to the scientific community and society; effectively co-operate with scientists, engineers and managers; contribute to interdisciplinary teams in solving complex tasks.

For more detailed information regarding the programme, please, refer to https://www.vu.lt/en/studies/master-studies/molecular-biotechnology

Academic contact: Dr Inga Matijošytė.
Email: inga.matijosyte@bti.vu.lt
Admission contact: admissions@cr.vu.lt

Student Scholarships

VU Life Sciences Center Scholarship

The purpose of the VU LSC scholarship is to promote personal, social, cultural and professional activities of VU LSC students and to create additional opportunities for them to improve and achieve better study results. The scholarship can be awarded for outstanding study and research results, scientific and voluntary activities, the main goal of which is to popularize science and increase people's awareness of biomedical, technological, physical sciences and achievements in their fields. The scholarship is awarded twice a year, in spring and autumn terms.

In 2021, the VU LSC scholarships were awarded to 12 BSc and MSc students:

- Austėja Balevičiūtė (MSc, Molecular Biology), Denis Baronas (MSc, Biochemistry), Džiugas Jurgutis (MSc, Biophysics), Konstanty Keda (BSc, Molecular Biology), Aurimas Kopūstas (MSc, Biochemistry), Lukas Krasauskas (MSc, Molecular Biotechnology), Ieva Lingytė (BSc, Molecular Biology), Raminta Mineikaitė (MSc, Molecular Biology), Agnė Savickaitė (MSc, Microbiology), Aivaras Vilutis (BSc, Neurobiophysics), Eglė Vitkūnaitė (BSc, Neurobiophysics), Augustinas Želvys (BSc, Molecular Biology)

and 9 doctoral students:

- Donata Dakinevičienė (Biochemistry), Lina Galinskaitė (Ecology and Environmental Sciences), Jonas Juozapaitis (Biochemistry), Kamilė Mikalauskaitė (Chemical Engineering), Vilmantas Pupkis (Biophysics), Dalia Smalakytė (Biochemistry), Monika Šimoliūnienė (Biochemistry), Mantas Žiaunys (Chemical Engineering), Emilija Žukauskaitė (Biochemistry).

President Kazys Grinius' Scholarship

On 6 July 2021, in commemorating the coronation of King Mindaugas and the National Anthem, state awards were handed at the President's of the Republic of Lithuania. The scholarships of Presidents Kazys Grinius, Antanas Smetona, Aleksandras Stulginskis, Jonas Žemaitis and Algirdas Brazauskas were awarded to students of universities and colleges who had excelled in academic, research and social activities. President Kazys Grinius' scholarship for excellent academic results was awarded to two students of Vilnius University Life Sciences Center: Kornelija Buivydaitė (BSc, Neurobiophysics) and Agnė Savickaitė (MSc, Microbiology).

Kornelija Buivydaitė

Agnė Savickaitė

Vilnius Sets up Virginijus Šikšnys' Scholarship for the Brightest Doctoral Students in the Field of Life Sciences

In order to honour Virginijus Šikšnys, distinguished professor at the Vilnius University Life Sciences Center, for his significant contribution to the discovery and development of the CRISPR-Cas9 genome editing technology, as well as for promotion of Vilnius as a world leader in the field of life sciences, Vilnius set up an international scholarship named after him.

The aim of this scholarship is to encourage young talents to continue actively working in Vilnius and it is awarded to doctoral students for internationally recognized exceptional scientific results. The amount of the annual scholarship is EUR 10,000.

Virginijus Šikšnys

The City of Vilnius undertakes to allocate them for five years in a row, with the possibility of extending this period. The scholarship is administered by the Vilnius University Life Sciences Center.

Contribution of VU Life Sciences Center to Overcome COVID-19 Pandemic in Lithuania

In 2020, humanity faced a novel coronavirus SARS-CoV-2, and the second wave struck us in 2021. The COVID-19 pandemic has heavily affected all the spheres of life from public health to economy, education and environment. In response to the COVID-19 challenges in Lithuania, the VU Life Sciences Center community joined the front lines immediately providing research infrastructure, personnel resources and knowledge aiming to find solutions and contribute to the national effort to get through the pandemic. In addition to expert activities, the LSC scientists were involved in outreach activities providing scientifically proven information on COVID-19 pandemic. These activities included communication in mass media, interviews on national TV, and lectures to the public.

Temporary Diagnostics Laboratory for COVID-19 Testing

During the first and the second outbreaks of the pandemic in Lithuania, the VU Life Sciences Center (LSC) opened the Temporary Diagnostics Laboratory for diagnostic testing of COVID-19 samples. A number of the Institute of Biosciences' laboratories were transformed into the controlled-access area in 2020, complying with all the operational and safety requirements for the biological safety level-2 laboratories, and COVID-19 diagnostic testing activities were resumed in 2021. Kristina Daniūnaitė (PhD) became the head of the laboratory, whereas Rokas Abraitis (PhD) continued his responsibilities for maintenance of technical readiness. The laboratory started functioning with over 40 permanent staff members and volunteers, assigned to sample registry, viral RNA preparation and detection, and result management groups.

In April, the laboratory passed the verification procedure for SARS-CoV-2 detection in pooled nasopharyngeal swabs (similar to the one developed at the LSC the previous year) and started receiving routine samples from educational institutions. In less than six months, 29,494 clinical samples (individual and pooled) were tested for SARS-CoV-2 at the laboratory, of which 4,367 proved positive for the infection. Due to the significant decrease in numbers of new cases in late spring, the diagnostic testing was discontinued at the end of June and was not renewed. The diagnostics ended with 50,029 tested samples in total (7,271 positive, 14.5%). Since March, most of the laboratory resources were dedicated to further development of the environmental surface testing methodology searching for the viral traces, which had proved to be highly sensitive for identification of asymptomatic infected individuals.

Kristina Daniūnaitė,
Head of Temporary Diagnostics Laboratory

The full list of members who worked at the Temporary Diagnostics Laboratory (or were otherwise involved in COVID-19 diagnostics) in 2021 is as follows: K. Daniūnaitė, R. Abraitis, R. Maleckaitė, S. Urnikytė, B. Kaminskaitė, R. Saulėnaitė, A. Mikelevičiūtė, R. Statkevičiūtė, A. Sipavičiūtė, R. Mončiunskaitė, I. Kanopaitė, K. Kasperovičiūtė, Sh. Uday Ganpule, A. Kumar Vijaya, A. Gricajeva, E. Radlinskaitė, E. Timoščenka, U. Peldžiūtė, A. Sukova, M. Šalčiūtė, E. Prosevičiūtė, I. Sabaliauskienė, K. Kriaučiūnaitė, G. Dzimitravičiūtė, V. Pukenytė, D. Šapaitė, K. Grigaitytė, Sh. Arun Prabha, L. Abraitytė, P. Semaško, A. Kvedaraitė, D. Stepanovas, P. Jučinskas, K. Limanovskaja, D. Šematovič, M. Sadauskas, J. Turčinavičienė, V. Mačiulskis, J. Žukas, L. Čekutienė, V. Bobinienė, M. Sukackas, I. Liužinaitė, R. Šveikauskas, V. Ceiko, E. Z. Sinicki.

Expert Groups:


Aurelija Žvirblienė


Edita Sužiedėlienė


Gytis Dudas

Council of Health Experts
The President of the Republic of Lithuania Gitanas Nausėda formed the Council of Health Experts, a group of COVID-19 pandemic management experts, in 2020. This Council continued its activity in 2021. The task of this Council is to analyse, evaluate and provide recommendations to the responsible authorities on stopping the spread of the coronavirus. The activities of the Council are organized in three groups: Situation Analysis and Forecasting, Public Health Measures, Organization of the Work of Health Care Institutions in order to find answers to the most important questions related to pandemic management and protection of human health. Two experts from the VU LSC are members of the Council: VU Vice-Rector for Research E. Sužiedėlienė and Head of the Department of Immunology and Cell Biology at the VU LSC A. Žvirblienė. E. Sužiedėlienė is leading the Public Health Measures Group. Both LSC scientists were awarded the Cross of the Knight of the Order of the Lithuanian Grand Duke Gediminas for their contribution to overcome the pandemic.

Working Groups of the Ministry of Health
The Ministry of Health of the Republic of Lithuania set up two special working groups: one group is in charge of vaccination issues preparing practical recommendations on COVID-19 vaccines; the second working group gives advice on COVID-19 drugs including biopharmaceuticals. Aurelija Žvirblienė, Head of the Department of Immunology and Cell Biology at the VU LSC, is a member of both working groups. She is providing expert opinion on COVID-19 vaccines and therapeutic antibodies.

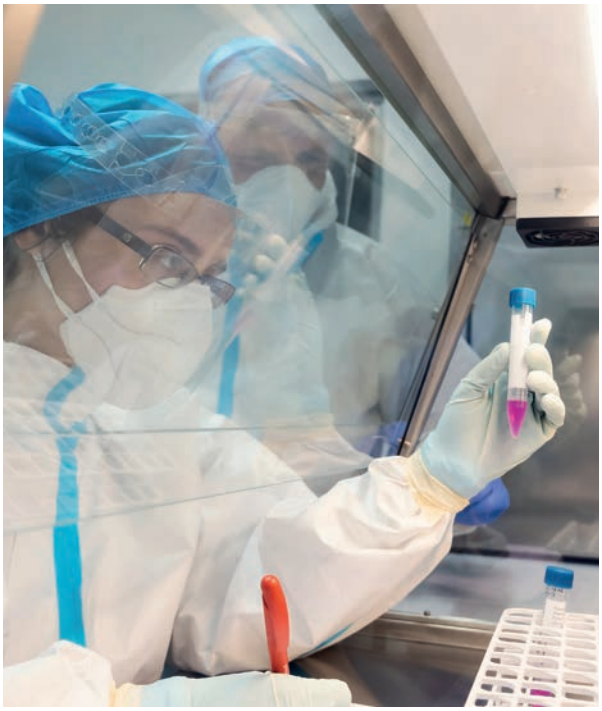
Advisory Council of Independent Experts Established by the Government of the Republic of Lithuania
Two scientists from the VU LSC, Aurelija Žvirblienė, Head of the Department of Immunology and Cell Biology at the VU LSC and Gytis Dudas, are members of the Advisory Council of the Independent Experts that was formed by the Government of the Republic of Lithuania. The Advisory Council provides evidence-based recommendations on pandemic management including COVID-19 monitoring, restrictions, special measures in healthcare and education sectors, vaccination strategies as well as other issues.

Development of the First Lithuanian Diagnostic Test for Antibodies against SARS-CoV-2 Virus Spike Protein

VU LSC researchers I. Kučinskaitė-Kodzė and M. Simanavičius contributed to the development of the first semi-quantitative COVID-19 serologic tests approved for IVD use by the State Accreditation Service. The VU LSC, UAB Baltymas and UAB Imunodiagnostika joined their efforts in developing these tests. The tests SARS-CoV-2 S IgG ELISA and SARS-CoV-2 S IgG QUANT B ELISA are produced by the UAB Imunodiagnostika and available on the market: https://imunodiagnostika.lt/en/produktai/

Environmental Surface Sample Testing for SARS-CoV-2 Traces at Preschool and Pre-Primary Education Institutions

In 2021, the environmental surface testing methodology, created at the Temporary Diagnostics (TD) Laboratory at the end of the previous year was further developed and validated in the LSC and five other institutions. The ability to identify infected individuals before the manifestation of symptoms, and, therefore, to contain new outbreaks was a promising measure to prevent viral spread at various institutions. The developed methodology was offered to the Ministry of Health and quickly found its niche among other COVID-19 detection measures taken at that time. In close collaboration with Vilnius City Municipality, the methodology was rapidly adopted for routine testing at preschool and pre-primary education institutions, where new day-by-day outbreaks posed a serious problem due to the fact that children were too small to wear face masks, as well as because of parents objection to invasive nasopharyngeal swab testing used routinely for their kids. Looking for SARS-CoV-2 traces on surfaces became an attractive alternative enabling not only to avoid direct children testing, but also allowing the sampling to be performed by public health specialists instead of nurses. The Laboratory conducted on-line training sessions on surface sampling and logistics for various institutions around the country. In April, routine surface testing was initiated in Vilnius County and the first batch of samples was received at the LSC. Until September, the Temporary Diagnostics Laboratory was the only laboratory conducting surface testing. During that period (except summer holidays), over 9,000 surface samples were tested at the LSC. The methodology gained even larger attention later in autumn when the prevalence of COVID-19 started to increase again. By the end of 2021, the Laboratory analysed over 22,600 surface samples of preschool institutions (mainly from Vilnius, Utena, Panevėžys, Alytus and Šiauliai counties) and the testing is being continued in 2022.

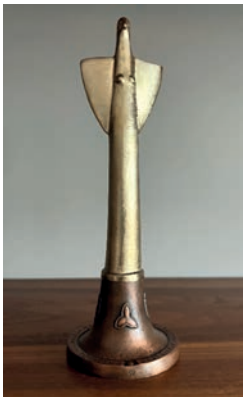


International Awards

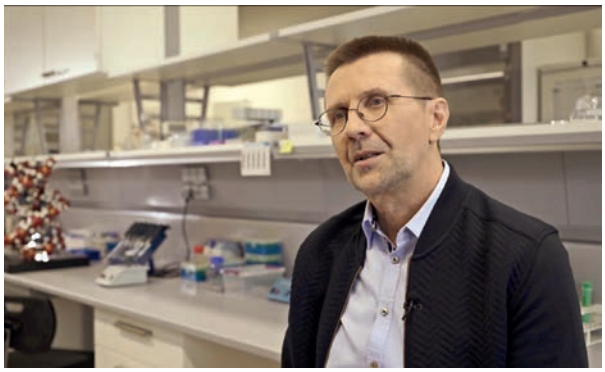
The Baltic Assembly Prize for Literature, the Arts and Science

In 2021, the Baltic Assembly Science Prize for outstanding achievements in the field of biomedical sciences – pioneering research in CRISPR-Cas9 genome editing – was awarded to Prof. Virginijus Šikšnys.

The Baltic Assembly Prize is awarded for achievements in literature, the arts and science and consists of a monetary prize, a certificate and a statuette, which are awarded annually during the Session of the Baltic Assembly. The Baltic Assembly is an international cooperation organisation of the parliaments of Lithuania, Estonia and Latvia founded in 1991. The Assembly's session is its supreme body. In 2021, Lithuania held the presidency of the Baltic Assembly.



The Baltic Assembly Science Award Statuette



Virginijus Šikšnys

L'Oréal Baltic *For Women in Science* Fellowship Programme

The L'Oréal Baltic *For Women in Science* Programme is designed to encourage the contribution of women pursuing research careers in Latvia, Lithuania and Estonia in the fields of life and physical sciences, environmental sciences, material sciences, mathematics, computer and information sciences. This programme is implemented in cooperation with the Baltic Academies of Sciences and the UNESCO National Commissions. Seven fellowships of 6 000 EUR are granted annually.

In 2021, Joana Smirnovienė, PhD student of the VU LSC, was awarded the L'Oreal Baltic program *For Women in Science* fellowship for her research that focuses on the development of innovative pharmaceuticals for the treatment of cancer and obesity.

Joana Smirnovienė

Gold Medal in International Directed Evolution Competition

The team *Vilnius GMC* formed by two PhD students of the VU LSC, Jonas Juozapaitis and Lorenzo Camisi, won the very first gold medal of the iDEC 2021 (International Directed Evolution Competition). iDEC 2021 is a new international competition in the field of molecular biology, which aims to promote the use of directed evolution techniques to develop new biotechnological products, to improve a protein, a genome or a biochemical pathway in an organism.

The project 'Ultra-high Throughput Evolution of a Bacterial Endolysin as a New Antimicrobial Agent' was carried out using microfluidic technology developed by the VU LSC Prof. Linas Mažutis in collaboration with Harvard University scientists proposing a solution to reprogram a protein used by pathogenic bacteria for reproduction and turn it into a new type of antibacterial agent against one of the most dangerous pathogens *Staphylococcus aureus*. If this bacterium were to acquire complete resistance to currently known antibiotics, it would lead to a mortality rate of more than 80% of those infected. The winners were also awarded the Most Potential Tool Prize and, together with two other teams, the Best Community Building Prize. The team also distinguished themselves in the categories of the Screening Assay Award, Best Presentation and Best Target Molecule.

Lorenzo Camisi, Jonas Juozapaitis

The Gold Medal in International Genetically Engineered Machine Competition in 2021

International Genetically Engineered Machine Competition (iGEM) of synthetic biology took place in Paris on 4–14 November, where the Vilnius-Lithuania iGEM team formed of Vilnius University students gained worldwide recognition by winning the gold medal; they were also nominated for 12 additional prizes and won two of them, for the best measurement and for educational activities.

The Vilnius-Lithuania iGEM 2021 team's project *AmeBye* was designed to prevent the spread of infectious amoebiasis. Throughout the competition cycle, the young scientists developed naringenin-synthesizing probiotics for the prevention of the infection as well as a diagnostic test.

The Vilnius-Lithuania iGEM team has been participating in the iGEM competition since 2015.

Since 2015, the VU students' team Vilnius-Lithuania iGEM has proved to be the best in the international competition of synthetic biology iGEM. In 2017 and 2020, the team won the Grand Prix of the competition against 250 teams of best world universities. In 2015, 2016, 2018 and 2019, the Vilnius-Lithuania iGEM team won gold medals, and in 2018, the undergraduate team was awarded a bronze medal.

The iGEM team 2021: Bernadeta Aleksandravičiūtė, Radvilas Bendorys, Ita Čiutaitė, Gintarė Petraitytė, Ieva Pudžiuvelytė, Jokūbas Putrius, Karolis Sabutis, Dominykas Špelveris, Guoda Taraškevičiūtė and Greta Zaburaitė.

Instructors: Rimvydė Čepaitė and Saulius Lipkevičius.

Primary PI: Dr Rolandas Meškys, Distinguished Professor of the VU Life Sciences Center.

The Vilnius-Lithuania iGEM team 2021

National Awards

Medals of the Order of the Lithuanian Grand Duke Gediminas

On 6 July 2021, on the Statehood Day, the President of the Republic of Lithuania Gitanas Nausėda presented the Medal of the Order of the Lithuanian Grand Duke Gediminas for the services to Lithuania and for the promotion of Lithuania's name in the world as well as for active management of coronavirus pandemic. Prof. Edita Sužiedėlienė, the Vice-Rector of Vilnius University, a coordinator of the Public Health Measures Group of the Health Expert Council of the President of the Republic of Lithuania and Prof. Aurelija Žvirblienė, Research Professor of Vilnius University Life Sciences Center, a member of the expert groups on COVID-19 vaccination, initiated by the President, the Government and the Ministry of Health of the Republic of Lithuania, received this medal.

Aurelija Žvirblienė, Gitanas Nausėda, President of the Republic of Lithuania, Gintautas Žvirblis

Edita Sužiedėlienė, Gitanas Nausėda, President of the Republic of Lithuania, Kęstutis Sužiedėlis

Lithuanian Science Prize

The Lithuanian Science Prize 2020 in the field of physical sciences was given to Gediminas Niaura, Research Professor of the VU Life Sciences Center and Professor of the Faculty of Physics, together with Professor Albertas Malinauskas of the Center for Physical Sciences and Technology for the cycle of works 'Research into the Molecular Structure and Functionality of Materials by Vibrational Spectroscopy Methods (2005–2019)'. The Lithuanian Science Prizes are awarded annually since 1993 for fundamental and applied scientific research and applied scientific activities of significance to Lithuania.

Gediminas Niaura

St. Christopher's Awards

The Statuette of St. Christopher is an award established in 1998 and given annually to 10 residents or organizations in the city of Vilnius for their merits in the areas of culture, science, art, sports, social and other fields. St. Christopher is the patron of the city of Vilnius and all the travelers. Traditionally, the Statuettes of St. Christopher are awarded by the Mayor of Vilnius.

Daumantas Matulis, Professor of the VU Life Sciences Center, was awarded The Statuette of St. Christopher 2020 for contribution to science and propagation of Vilnius in the world.

Daumantas Matulis

Competition of the Lithuanian Academy of Sciences to Encourage Science Promotion Projects

Ramunė Grikšienė, Associate Professor of the VU LSC, took the 2nd place for the cycle of seminars 'Scientists in Your Room' in the category of the best science promotion. The researcher organized 14 virtual events, during which 11 researchers working in Lithuania and abroad made presentations. A Facebook page 'Scientists in Your Room' has been created to inform about these events and spread scientific knowledge.

The Lithuanian Academy of Sciences arranged the competition striving to encourage organizations and individuals to spread knowledge about science and technology, increase the attractiveness of researcher's profession, promote the dialogue between researchers and society, help to better understand scientific information.

Ramunė Grikšienė

The Global Lithuanian Leaders Award 2021

For Solid Voice in Global Science Community the Vilnius-Lithuania iGEM team received the Global Lithuanian Leaders Award, which is given to Lithuanians and Lithuania-related individuals who have been contributing to the prosperity and global standing of Lithuania.

Winners of Competition *The Best Doctoral Thesis in 2020*

Two young scientists from the VU Life Sciences Center became laureates of *The Best Doctoral Thesis 2020* competition: Kristina Šnipaitienė and Tomas Šneideris. The aim of this competition is to encourage PhD students to carry out scientific activities of the highest quality and relevance to society, to foster inter-institutional and interdisciplinary cooperation and to prepare high-level theses that will be recognized worldwide. 71 research papers were submitted to the competition organized by the Lithuanian Union of Young Scientists and only 10 best theses' authors were recognized as the winners of the competition awarded by the President of the Republic of Lithuania Gitanas Nausėda.

Kristina Šnipaitienė was awarded for her doctoral thesis in the field of biology 'RNA Studies of Body Fluids for the Diagnosis and Prognosis of Prostate Cancer'. The scientific supervisor of her doctoral thesis is Prof. Sonata Jarmalaitė.

Tomas Šneideris

Kristina Šnipaitienė

Tomas Šneideris was awarded for his doctoral thesis in biochemistry 'Study of the Formation and Self-Replication Characteristics of Protein Amyloid Fibrils'. Scientific supervisor is Research Prof. Vytautas Smirnovas.

Competition *The Best Master's Thesis 2021*

The Lithuanian Academy of Young Scientists together with the Ministry of Foreign Affairs arranges competition of the best master's theses pursuing the encouragement of the second level (MSc) students to write high quality papers relevant to society and industry. In 2021, 78 papers were submitted. Seven authors of *The Best Master's Thesis 2021* competition became the winners, including three graduates of the VU LSC: Denis Baronas, Justina Žvirblytė and Giedrė Skliutė.

Denis Baronas was awarded for the best thesis in the field of technological sciences 'Exploring the Binding Mechanism of Sulfonamides Bearing an Ester Group with Human Carbonic Anhydrases'. Supervisor: Dr Asta Zubrienė.

Justina Žvirblytė was awarded for the best work in the field of medicine and health sciences 'Study of Transcription of Single Cells in Healthy and Cancerous Human Kidney Tissues'. Supervisor: Prof. Linas Mažutis.

Giedrė Skliutė

Justina Žvirblytė

Denis Baronas

Giedrė Skliutė's thesis '*Ex vivo* Research of Stem Cells of Endometrium Origin' was recognized as the best in the field of natural sciences. Supervisor: Prof. Rūta Navakauskienė.

Lithuanian Academy of Sciences' Competition of University Students' Research

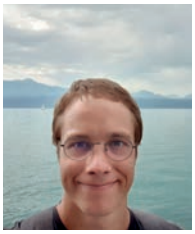
In 2021, the Lithuanian Academy of Sciences' prize for university students' research went to the MSc student Laura Klimkaitė of the Life Sciences Center for her work 'Opportunistic Pathogen *Stenotrophomonas maltophilia*: Identification and Characterization of Type 2 Toxin-Antitoxin Systems'. Supervisor: Julija Armalytė.

Laurita Klimkaitė

Winners of the 14th Lithuanian Young Scientists Conference *BIOFUTURE: Perspectives of Natural and Life Sciences*

Two young scientists of the VU LSC, Milda Mickutė and Algimantas Kriščiūnas, were awarded for the best presentations of the 14th Lithuanian Young Scientists Conference *BIOFUTURE: Perspectives of Natural and Life Sciences*!

Milda Mickutė



Algimantas Krikščiūnas

Awards of the State Lithuanian Language Commission for Fostering the Lithuanian Language

Since 2016, the State Lithuanian Language Commission gives award for fostering the Lithuanian language, for significant achievements in developing Lithuanian terminology, promoting scientific language and linguistic education of society. In 2021, the sculpture 'Snail' and diploma were given to Ernestas Kutorga, professor of the Institute of Biosciences of the VU Life Sciences Center, for the preparation and publication of book 6 'Lithuanian Mushrooms. Inoperative Discomycetes' of the multi-volume publication 'Lithuanian Mushrooms'.

Ernestas Kutorga. Photo of the State Commission of the Lithuanian Language

Letter of Thanks from the Minister of Environment of the Republic of Lithuania

The fourth edition of the Red Data Book of Lithuania was published in 2021. It provides research data on 566 protected species of animals (225 species), plants (224) and fungi (117, including lichens). All species have been evaluated against the criteria of the International Union for Conservation of Nature and classified into categories. The voluminous Red Data Book consists of 683 pages illustrated with photographs of organisms and distribution maps, and weighs even 2.57 kg. In total, 3 thousand copies have been published. The electronic version of the Red Data Book is available online: https://gamtosknyga.lt/leidinys/lietuvos-raudonoji-knyga/.

This publication of the Ministry of Environment has been prepared by almost 50 scientists and naturalists. The Minister of Environment awarded all the authors of this study with the Letter of Thanks for personal contribution to the preparation of the Red Data Book of Lithuania and the protection of our country's biodiversity. Researchers from the VU LSC who participated in the preparation of the Red Data Book of Lithuania: Arbačiauskas K., Budrys E., Bukelskis E., Dagys M., Kaupinis A., Kutorga E., Naujalis J. R., Prigodina Lukošienė I., Rasimavičius M., Skujienė G.

Vilnius University Awards

Rector's Science Award

In 2021, Dr Rūta Navakauskienė, Research Professor of the VU Life Sciences Center Institute of Biotechnology, received Rector's Science Award for significant scientific achievements.

In the category of young researchers, the Rector's Science Award for outstanding scientific achievements in 2021 was given to two VU Life Sciences Center community members: associate professor Renata Gudiukaitė and research assistant Greta Bigelytė.

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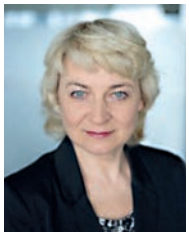
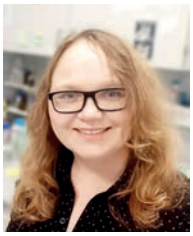

Rūta Navakauskienė


Renata Gudiukaitė


Greta Bigelytė

The Best Lecturer of 2021

Assoc. professor Renata Gudiukaitė was elected as the best lecturer of the VU Life Sciences Center.

LSC Letters of Thanks

Marking the end of the teaching year 2020/2021, letters of thanks were handed to volunteer students and employees who carried out SARS-CoV-2 coronavirus tests in the temporarily arranged diagnostic testing laboratory of the VU Life Sciences Center. This volunteer initiative of the LSC scientists and researchers contributed significantly to faster diagnosis of COVID-19, lightened the load of other laboratories and shortened the time the patients had to wait for answers.

VU Awards for Significant Research Achievements in 2020

A considerable number of the Life Sciences Center researchers were awarded for significant research achievements in 2020:

- research assistant Inga Songailienė, doctoral student Jonas Juozapaitis, senior researcher Dr Giedrė Tamulaitienė, senior researcher Audronė Rukšėnaitė, research professor Dr Giedrius Sasnauskas, research professors Dr Česlovas Venclovas (the category of distinguished professor) and Dr Virginijus Šikšnys (the category of distinguished professor) for the article 'HEPN-MNT toxin-antitoxin system: the HEPN ribonuclease is neutralized by OligoAMPylation' published in *Molecular Cell*, Cambridge, MA: Cell Press [in the category of the best publication in the fields of nature, medicine and health and technology];

From the left: A. Rukšėnaitė, Giedrius Sasnauskas, Inga Songailienė, Česlovas Venclovas, Giedrė Tamulaitienė

Virginijus Šikšnys

Jonas Juozapaitis

- research professor Dr Rolandas Meškys (the category of distinguished professor) and research assistant Dr Dalius Ratautas for the article 'Real-time glucose monitoring system containing enzymatic sensor and enzymatic reference electrodes' published in *Biosensors and Bioelectronics*, Oxford: Elsevier Ltd. [in the category of the best publication in the fields of nature, medicine and health and technology];

Rolandas Meškys

Dalius Ratautas

- research professor Dr Habil. Saulius Klimašauskas (the category of distinguished professor), research professor Dr Giedrius Vilkaitis and doctoral student Milda Mickutė for applied work 'Analysis of Single-Stranded RNA' [in the category of the best applied work].

Saulius Klimašauskas

Giedrius Vilkaitis

Milda Mickutė

Guests

Visit of Edith Heard, Director General of the European Molecular Biology Laboratory (EMBL), and Plamena Markova, Head of International Relations of EMBL

Visit of Aušrinė Armonaitė, Minister of Economy and Innovation of the Republic of Lithuania

Visit of Arancha González Laya, Minister for Foreign Affairs, European Union and Cooperation of the Kingdom of Spain

Visit of the Rectors of the Universities of Leipzig (Germany), Graz (Austria) and Bergen (Norway), members of the Arqus European University Alliance

Visit of the delegation from Alsace including Greta Komur-Thilloy, Dean of the Faculty of Philology and Humanities, Christelle Dekaite, Head of the Chemical and Polymer Research Group and member of the Rector's Office of the University of Haute-Alsace, Anne-Sophie Schuller, a Researcher in the Laboratory of Photochemistry and Macromolecular Engineering

Visit of Eva Zažímalová, President of the Czech Academy of Sciences

Visit of Taiwan business delegation

Visit of Federico Pollano, Senior Vice President Business Development, and Karolin Gebhardt, Strategic Project Assistant to the CEO of German company Renstchler Biopharma

Scientific Events

Scientists at the VU Life Sciences Center Have Discovered a New Gene Editing Tool

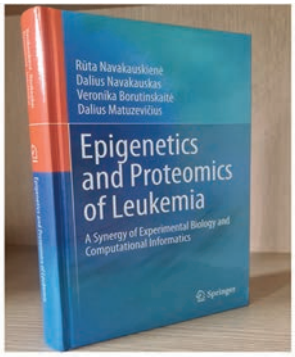
The prestigious scientific journal *Nature* has published an article entitled 'Transposon-associated TnpB is a programmable RNA-guided DNA endonuclease' by scientists from Vilnius University (VU) Life Sciences Center. This study expanded understanding of transposition mechanisms by highlighting the role of TnpB in transposition, experimentally confirmed that TnpB is a functional progenitor of CRISPR-Cas nucleases and established TnpB as a prototype of a new system for genome editing.

All the authors are or have been working at the VU Life Sciences Center, namely, Dr Tautvydas Karvelis, Gytis Druteika, Greta Bigelytė, Karolina Budrė, Rimantė Žedaveinytė, Dr Arūnas Šilanskas, Dr Darius Kazlauskas, Prof. Dr Česlovas Venclovas, Prof. Dr. Virginijus Šikšnys.

It was the first time that *Nature* has published a paper by a team of solely Lithuanian scientists.

Monograph Epigenetics and Proteomics in Leukaemia – A Synergy of Experimental Biology and Computational Informatics

Prof. Dr Rūta Navakauskienė and Dr Veronika Borutinskaitė, together with their colleagues from VILNIUS TECH University, Prof. Dalius Navakauskas and Dr Dalius Matuzevičius, have published a monograph Epigenetics and Proteomics in Leukaemia – A Synergy of Experimental Biology and Computational Informatics.



The COINS 2021

The COINS conference, organized by Vilnius University students since 2004, was held on 27 February 2021. Every year, this international event attracts many researchers to the VU LSC from all over the world, including Nobel Prize winners. Due to the pandemic, The COINS 2021 was virtual, and the presentations were given by representatives of Lithuania's scientific elite such as Prof. Virginijus Šikšnys, winner of the prestigious Kavli Prize and also the runner-up for the Nobel Prize in Chemistry in 2021. In panel discussions, Dr Gintaras Valinčius, Director of VU LSC, Agnė Vaitkevičienė, Vice President of the Lithuanian Biotechnology Association, and other representatives in the field shared their views on the contribution of the state in promoting innovation in life sciences and the importance of education in creating innovations.

Science Day by Thermo Fisher Scientific

On 10 November, Thermo Fisher Scientific organized its traditional annual event, the Thermo Fisher Science Day for the 11th time. Due to the pandemic, this time it was a virtual event with the latest scientific presentations. The guest speaker was Karen Nelson, Chief Scientific Officer at Thermo Fisher Scientific, a member of the US National Academy of Sciences, author of 3 books and more than 220 scientific publications. Traditionally, the Vilnius iGEM team, which is participating in the world's largest synthetic biology competition in the USA, also presented its latest project at the conference.

A special platform opened up the possibility not only to listen to presentations, to learn about new products and technologies used in life sciences, but also to ask questions or share ideas.

Invited Speakers

Speaker	*Institution*	*Title*
Jonathan Arias	University of Oslo	Harvesting iPS cell and genome editing technologies for cell-based therapeutics and disease modelling
Pijus Brazauskas	Evox Therapeutics, Oxford	Transcriptional regulation by gene body residing 5hmC and exosome therapy to treat *Argininosuccinic aciduria*
Rokas Grigaitis	University of Vienna	Regulation of DNA helicases in homologous recombination
Andrej Gorbatenko	Icahn School of Medicine at Mount Sinai, New York	Functional genetics identify novel factors of detachment mediated cell death and breast cancer
Stephen Jones	University of Texas, Austin	Massively parallel kinetic profiling of natural and engineered CRISPR nucleases
Denis Kainov	Norwegian University of Science and Technology	Q&A regarding SARS-CoV-2
Miglė Kazlauskienė	University of Zurich	RNA-associated machineries: from bacterial CRISPR-Cas to human m6A-mRNA modification
Prashant Kumar	International Tech Park, Bangalore	The emerging roles of liquid biopsy in diagnosis, prognosis and treatment monitoring in cancer
Lina Malinauskaitė	Cambridge Biomedical Campus	GABA receptors nanobodies - a new path to pain relief
Algirdas Mikšys	Regensburg University	A structural look into the stressosome complex and its role in the Gram-positive general stress response
Jorūnė Sakalauskaitė	GLOBE Institute, University of Copenhagen	Shell palaeoproteomics: from biomineralisation to characterisation of ancient proteins preserved in archaeological mollusc shells
Patrick Pausch	University of California, Berkeley	How a hypercompact CRISPR-Cas nuclease cuts double-stranded DNA
Federico Pollano	Renstchler Biopharma	Trends & Disruption in Pharma industry: Which developments have the potential to massively impact our customers' business?
Giancarlo Russo	ETH Zurich	Ten years of research and support in a -omics core facility: an overview
Kazimier Trebacz	Maria Curie-Sklodowska University in Lublin	Long-distance signalling in plants: electrophysiological and molecular approach
Aleksej Zelezniak	Chalmers University of Technology	What machines can teach us about biology? Bridging machine intelligence and synthetic biology for understanding and engineering of microbial systems

Community Events

Sculpture the *DNA Arch*

A colourful sculpture the *DNA Arch* was installed by the Vilnius University's Life Sciences Center as a tribute to Prof. Virginijus Šikšnys and other scientists who have made significant contributions to the Nobel Prize-worthy CRISPR-Cas9 gene editing method development ('gene scissors' technology).

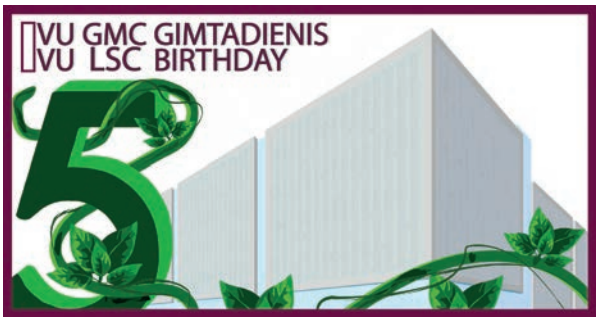
Sculpture by artist Eglė Žvirblytė

5-Year Anniversary of the VU LSC

In October 2021, the VU Life Sciences Center celebrated the fifth anniversary of its establishment. On this occasion, a number of events took place throughout the week of 4-8 October. The birthday program started on Monday with the discussion on "Life Sciences Center in 2026", with the participation of Gintautas Jakštas, Vice-Minister of Education, Science and Sport of the Republic of Lithuania, student representative Ieva Lingytė, Dr Urtė Neniškytė, Dr Juozas Nainys, science journalist Goda Raibytė, Prof. Virginijus Šikšnys, PhD student Monika Šimoliūnienė, Lithuanian Biotechnology Association Vice-President Agnė Vaitkevičienė, Prof. Česlovas Venclovas, and Prof. Gintaras Valinčius as a moderator.

On Tuesday, the VU LSC community planted about 500 crocuses near the main entrance to the Life Sciences Center.

On Wednesday, students organized orienteering game inside the VU LSC building. More than 60 community members



participated in this game solving intriguing and catchy clues in 12-stop routes.

On Thursday, PhD students organized a living library where they had a possibility to ask professors and other VU LSC staff members questions and listen to different stories in a cosy setting.

Throughout the week, students organized an OpenLab: 72

Left to right: Gintaras Valinčius (moderator), Česlovas Venclovas, PhD student Monika Šimoliūnienė, LBTA Vice-President Agnė Vaitkevičienė, Virginijus Šikšnys, Urtė Neniškytė, student representative Ieva Lingytė, Juozas Nainys, Gintautas Jakštas, Vice-Minister of Education, Science and Sport of the Republic of Lithuania

tours to the research laboratories. 28 laboratories signed up for the event with the desire to present their research and open their doors to colleagues and students. The event goal was to be better acquainted with the research carried out in each lab, identify common interests, collaboration opportunities and as it comes to bachelor and master students, an opportunity to learn more about research areas for their internship. About 590 community members registered for the tours.

On Friday, we had the closing ceremony of the VU LSC birthday week with a birthday cake, concert and dances.

Opening of New Academic Year (Renovatio Studiorum)

On the first autumn day, September the 1st, the celebration ceremony of the new study year traditionally brought together the large community of the VU LSC. As the new academic year begins, traditions are being followed: all the students, both academic and administrative staff of the VU LSC observed the solemn raising of the Vilnius University flag and participated in the traditional parade of the Vilnius University students, professors, lecturers and alumni starting from the Seimas (Parliament) of the Republic of Lithuania marching down the main street of Vilnius, Gediminas avenue, heading towards the central historical building of Vilnius University.

Diploma Award Ceremony

On 29 June 2021, the community of the Life Sciences Center held a diploma ceremony for graduates of the VU LSC BSc and MSc degree programs. 97 students gained bachelor's degree, two of them received *Cum Laude* diplomas for excellent study results and 11 were awarded *Magna Cum Laude* diplomas for excellent study and scientific achievements. Master's degree was conferred on 92 graduates, 6 of them *Cum Laude* diplomas for excellent study results and 13 *Magna Cum Laude* diplomas for excellent study and scientific achievements.

Public Engagement

Project *Hygiene of Smart Phones* for the Public

Renata Gudiukaitė, scientist of the VU LSC, in cooperation with *Bite Lietuva* Company conducted a research of public interest *Hygiene of Smart Phones* testing the level of cleanness of various surfaces showing the spread of microorganisms on everyday objects for the first time in Lithuania. To assess and compare the level of cleanness of different objects 20 more samples of surfaces frequently touched by people were taken (dustbin lid, refuelling pistol, library lift button, traffic light control button, credit card reader, banknotes). Personal surfaces such as bankcard, computer mouse, phone charger were also tested. Research showed that smart phone was the dirtiest according to the number of bacteria on the surface. It was dirtier more than twice compared to a bench of public transport stop ranking second.

Augmented Reality Project *6th SinBio Sense*

In 2021, the Vilnius-Lithuania iGEM (Global Synthetic Biology Competition) team of Vilnius University students launched an innovative, ongoing project, aiming to acquaint the public with synthetic biology and to make this discipline as understandable to everyone as possible. Using augmented reality models, the developers of the platform visualized invisible cellular processes and other points of interest on the smart phone screen. Models and all the relevant information can also be found on the project page: https://igem-vilnius-ar.com/

Installation *MicroMoment*

Vilnius-Lithuania iGEM-2021 team in cooperation with artist Jolita Vaitkutė created an artistic-educational installation *MicroMoment* consisting of almost 500 tightly closed Petri dishes containing microorganism colonies collected in different sites of Vilnius. While studying the installation viewers could see microorganisms, found in various sites of Vilnius city, gain visual perception about the surrounding environment as well as learn about the usage of microorganisms in synthetic biology and relations with every one of us.

Installation *MicroMoments* by Jolita Vaitkutė, photo by J. Auškelis

International Microorganism Day

The 17th of September is the International Microorganism Day (IMD), celebrated in Lithuania and internationally each year. This day the microbiologists from the LSC invited children, teachers, and families to celebrate. The aim of the event was to teach the communities about the microorganisms in their environment by introducing the beauty of the micro-world and showing how beneficial microorganisms are in daily life.

During the event, our scientists shared their experience and knowledge in the fields of microbiology and microbial biotechnology. The IMD was held online and gathered more than 500 participants from different regions of Lithuania.

Lithuanian Science Festival *Spaceship Earth*

Science festival *Spaceship Earth* is an annual event in Lithuania. Over the years, the science festival has become the largest science-promoting event in the country, spreading over more than 10 cities and districts. Every September, more than 30,000 participants visit various events (lectures, demonstrations, excursions, exhibitions). VU Life Sciences Center researchers and doctoral students invited schoolchildren and children

to 13 public lectures and discussions about life sciences and biotechnology.

Life Science Day for Schoolchildren (European Biotech Week)

The 9th European Biotech Week took place from September 27 to October 03 2021 (virtually). Biotech National Associations, in collaboration with companies, academic and governmental institutions, science museums and the media, organise events across Europe to celebrate this innovative technology. The Life Sciences Center (VU LSC) invited secondary school children to attend two on-line seminars on life sciences: "Epigenetics: how

genes are regulated" and "Bacteria against viruses – continuous arms race". Children had an opportunity to learn about biotechnology studies in Lithuania and got answers about the opportunities and challenges of biotechnology.

Mobile Bioclass

The Mobile Bioclass is a mobile laboratory in Lithuania. It aims at promoting biosciences among schoolchildren and inspiring them to pursue careers in sciences. During the Bioclass, pupils get a chance to become scientists, work with real scientific instruments, familiarize themselves with up-to-date methods used in modern molecular biology and conduct hands-on experiments related to DNR in their classrooms. The Mobile Bioclass is a joint project of the company Thermo Fisher

Scientific Baltics and Vilnius University taking place since 2011. The Mobile Bioclass visited more than 50 percent of the secondary schools in 70 cities of Lithuania.

National Student Academy and Extramural School of Young Biochemists

The Life Sciences Center collaborates with the National Student Academy and the School of Young Biochemists. Both organizations focus on secondary school 9–12 class schoolchildren. Through both distance learning and residential events, our scientists provide the pupils of both schools with knowledge in biochemistry, genetics, molecular biology, microbiology, and biotechnology to popularize research and practical skills of pupils, who have ability to understand research design and conduct scientific research. The Extramural School was founded in 1978 and until now, more than 1,600 students have finished this school.

Acts on Welfare of Wild Animals

The Ministry of Environment striving to improve legal acts concerning welfare of wild animals formed a working group to deal with the issues of wild animals' welfare in 2017. Dr Grita Skujienė of the VU LSC takes part in the group's work. In 2021, the group looked over the requirements of keeping wild animals in captivity and presented suggestions for their amendments. The working group has already compiled a list of wild animals species allowed to keep and/or breed in captivity as well as a list of domesticated animals.

Gift for Schools – Book about the Legendary Scientist Maria Curie

Before Christmas, almost 200 gymnasiums in the country's regions received a gift: book "Mary Curie – A Woman Out of Her Time". The book was published in Lithuanian and the donation campaign for schools was initiated by the BASNET Forum of the Baltic Association of Women Scientists. VU Life Sciences Center together with the Ministry of Education, Science and Sport, the French Embassy in Lithuania, VU Center for Nuclear and Elementary Particle Physics (CERN) and UAB Cureline Baltic supported the donation of this book to schools.

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List of Patents

GRANTED US PATENTS

1. RNA-directed DNA cleavage by the Cas9-crRNA complex (US9637739B2; US10844378B2)
2. Systems and methods for barcoding nucleic acids (US10596541B2; US11052368B2)*
3. System and method for a biomimetic fluid processing (US10343163B2; US10710073B2; US9795965B1)*
4. Programmable RNA shredding by the type III-A CRISPR-Cas system of Streptococcus Thermophilus (US10385336B2)
5. Derivatization of biomolecules by covalent coupling of non-cofactor compounds using methyltransferases (US8822146B2)
6. Conversion of alpha-hydroxyalkylated residues in biomolecules using methyltransferases (US8889352B2; US9505797B2)
7. Fluorinated benzenesulfonamides as inhibitors of carbonic anhydrase (US9725467B2)
8. Nucleic acid production and sequence analysis (US9347093B2; US9988673B2)
9. New s-adenosyl-L-methionine analogues with extended activated groups for transfer by methyltransferases (US8008007B2)*
10. Process for the production of monoclonal antibodies using chimeric VLPS (US7919314B2)
11. 5-Aryl-4(5-substituted 2,4-dihydraxyphenyl)-1,2,3-thiadiazoles as inhibitors of HSP90 chaperone and the intermediates for production thereof (US8314132B2)
12. Analysis of single-stranded RNA (US11008605B2)

GRANTED EU PATENTS

1. RNA-directed DNA cleavage by the Cas9-crRNA complex (EP2828386B1)
2. Systems and methods for barcoding nucleic acids (EP3134536B1; EP3299469B1)*
3. System and method for a biomimetic fluid processing (EP2941642B1)*
4. Programmable RNA shredding by the type III-A CRISPR-Cas system of Streptococcus Thermophilus (EP3189140B1)
5. Conversion of alpha-hydroxyalkylated residues in biomolecules using methyltransferases (EP2414527B1)
6. Derivatization of biomolecules by covalent coupling of non-cofactor compounds using methyltransferases (EP2414528B1)
7. Fluorinated benzenesulfonamides as inhibitors of carbonic anhydrase (EP2914583B1)
8. Nucleic acid production and sequence analysis (EP2776575B1)
9. Production of selenoproteins (selprot) (EP3019194B1)
10. Analysis of single-stranded RNA (EP3271478B1)
11. System and method for synthesis of DNA particles and use thereof (EP3402594B1)*
12. 5-Aryl-4(5-substituted 2,4-dihydraxyphenyl)-1,2,3-thiadiazoles as inhibitors of HSP90 chaperone and the intermediates for production thereof (EP2268626B1)
13. New s-adenosyl-L-methionine analogues with extended activated groups for transfer by methyltransferases (EP1874790B1)*
14. Benzimidazo [1,2-C][1,2,3] thiadiazol-7-sulfonamides as inhibitors of carbonic anhydrase and the intermediates for production thereof (EP2054420B1)

GRANTED JP PATENTS

1. RNA-directed DNA cleavage by the Cas9-crRNA complex (JP6423338B2)
2. System and method for a biomimetic fluid processing (JP6429794B2)*
3. New s-adenosyl-L methionine analogues with extended activated groups for transfer by methyltransferases (JP08008007B2)*
4. Systems and methods for barcoding nucleic acids (JP6853667B2)*

PATENT APPLICATIONS

1. RNA-directed DNA cleavage by the Cas9-crRNA complex (US20150291961A1; US20180187195A1; JP2019030321A; JP2021019617A)
2. Systems and methods for barcoding nucleic acids (US20150298091A1; EP3456846A1; US20210379555A1)*
3. System and method for a biomimetic fluid processing (US20200316597A1)*
4. System and method for producing target biological substances (EP3712612A1)*
5. Selective inhibitors of carbonic anhydrase (US20180222856A1; EP3328833A1)
6. System and method for synthesis of DNA particles and use thereof (US20190002943A1)*
7. Production of cyclic adenylates and their use as allosteric regulators (EP3630966A1); US20210130799A1
8. Methods for the identification and characterization of double-strand break sites and compositions and uses thereof (US20210147909A1; EP3790993A1)*
9. Characterization of prostate cancer using DNA methylation assay (PCT/IB2019/056204)
10. N 4-modified cytidine nucleotides and their use (EP3681897A1; US20200270295A1)
11. Systems and methods for encapsulation and multi-step processing of biological samples (WO2020255108A1; US20200400538A1)*
12. Method for generating functional protein sequences with generative adversarial networks (WO2021059066A1)*
13. Methods and compositions for non-invasive prenatal diagnosis through targeted covalent labelling of unmodified genomic sites (WO2021198726A1)
14. Clear cell renal cell carcinoma biomarkers and uses thereof (US63128874)*
15. Carbonic anhydrase inhibitors synthesized on interconnecting linker chains (PCT/IB2021/061310)
16. Antibodies For Photoactive Protein Manipulation (EP21187460.7)
17. Catalytic biomolecule activity recording into DNA sequence (EP3864168A1*; US20210355486A1*)

Key Performance Indicators	2021
New patent applications filed in 2021	11
Total number of EU and US patents	33
US patents granted in 2021	2
EU patents granted in 2021	–
JP patents granted in 2021	1
Licenses (new in 2021)	16 (2)

* Jointly owned patent with a foreign research organization and/or company

Cooperation

RESEARCH INSTITUTIONS

Aarhus University (Denmark)
AdventHealth for Children (USA)
Auckland University of Technology (New Zealand)
August Kirchenstein Institute of Microbiology and Virology (Latvia)
Australian Animal Health Laboratory - CSIRO (Australia)
Autonomous University of Madrid (Spain)
Bangor University (UK)
Barcelona Supercomputing Center (Spain)
Beijing Sports University, China
Bern University (Switzerland)
Bigelow Institute (USA)
Bordeaux University (France)
Boston University (USA)
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Cambridge University (UK)
Cancer Center and Institute of Oncology (Poland)
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Tartu University (Estonia)
Telethon Institute of Genetics and Medicine (Italy)
Tokyo Institute of Technology
The Florey Institute of Neuroscience and Mental Health, Australia
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INTERNATIONAL INDUSTRY COLLABORATIONS

Abcam AG (UK), ArcDia (Finland), BS Biotechna (Poland), Bruker Daltonik GmbH (Germany), CardeaBio (US), DuPont (US), Experimentica (Finland), Fibenol (Estonia), Kalon Biological/ Clin-Tech Ltd (UK), Novartis (Switzerland), Polypure (Norway), Ramidus AB (Sweden), Santa Cruz Biotechnology Inc. (US), serYmun Yeast (Germany), Synthon Chemicals (Germany), ThermoFisher Scientific (US).

NATIONAL INDUSTRY COLLABORATIONS

Baltymas, Bioanalizės sistemos, BioenergyLT, Biomatter Design, Biorro, CasZyme, Certumtech, Ekorama, Elymus, Experimentica, Ferentis, Genie Biotech EU, Imunodiagnostika, Laboratorija 1, Lipidohms, Nagenus, Nanodiagnostika, Naujoji Ringuva, Nomads, Pienas LT, Placenta, Prodentum, Profarma, Roquette Amilina, Sanobiotech, ThermoFisher Scientific Baltic, 3D Creative, Vilniaus Ventos puslaidininkiai.

COMPANIES FOUNDED BY LSC RESEARCHERS

Baltymas, Bioanalizės sistemos, Caszyme, Droplet Genomics, Enzymics, IMD technologies, Lipidohms, Nomads, Platelet BioGenesis, Platformina, Profarma, Sekos, ThermoPharma Baltic, Ubique calculus, Virgo Sanitas.

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