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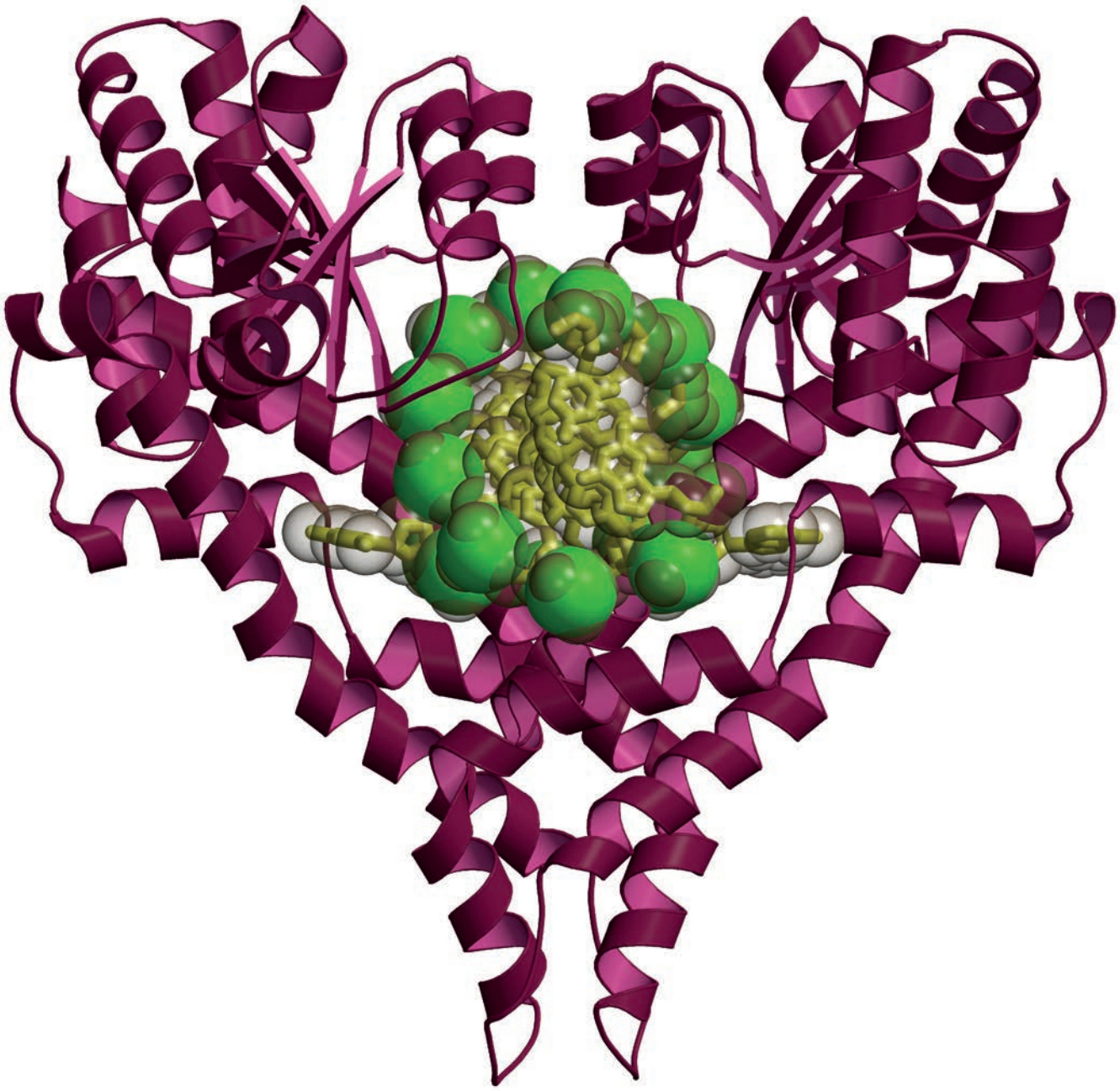
Life Sciences Center



Annual Report 2020

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FOREWORD

Year 2020 was both challenging and inspiring. Unexperienced in modern times, the deadly pandemic spread fast across the globe reaching Lithuania in early March. Initially it seemed the paralysis and major disruption of the most of our academic activities at the Life Sciences Center of Vilnius University are imminent. In mid-March when the Government announced nationwide quarantine, the lectures and student lab practices were halted and the access to research laboratories was restricted. In these circumstances, our community demonstrated strong determination and resilience not to let the academic life stop. Within several days the remote forms of lectures started, and the access to experimental facilities were renewed for students graduating in 2020.

At the beginning of COVID-19 pandemic, Lithuania as other countries experienced a shortage of COVID-19 testing capacities. Within a week, the Temporary Diagnostic Testing Laboratory was set up at the Life Sciences Center. After recruiting a team of volunteers and obtaining appropriate certificates from the Government, the PCR-based testing started in April. Throughout the year, more than 20 000 samples with nearly 3 000 diagnosed SARS-Cov-2-positive were tested in our Center. During the pandemic, our community members were actively consulting the Ministry of Health of the Republic of Lithuania as well as the President. On request from the Ministry of Health, the scientists at the Life Sciences Center prepared and validated the group testing protocols for COVID-19 patients. For the first time the testing of surfaces was developed to track the asymptomatic COVID-19 patients. First experimental tests of workplaces at the Life Sciences Center revealed the effectiveness of the methodology to prevent the infection in organisations and public spaces. This contactless technique was later adopted for COVID-19 testing in kindergartens. For significant contributions in the fight against COVID-19 the Life Sciences Center community was awarded letters of gratitude by the Senate and Rector of Vilnius University and honorable awards by the President and the Government of the Republic of Lithuania.

One of the most important academic events in 2020 was signing of the agreement between Vilnius University Life Sciences Center and the European Molecular Biology Laboratory (EMBL). The Life Sciences Center became the 7th remote partnership institution of EMBL. According to the agreement the VU LSC-EMBL Partnership Institute for Genome Editing will be established. The funding of the Partnership Institute was secured from the Ministry of Education, Science and Sport to start six research groups at the Life Sciences Center specializing in various fields and applications of CRISPR gene editing systems.

This year witnessed exceptional achievements of our students at iGEM international competition in synthetic biology. Addressing an important problem of modern fish farming, which faces a high risk of fish infections our students along with their colleagues from other University departments prepared and remotely presented their project at the annual iGEM

Giant Jamboree which this year was carried out remotely. For the second time our students won the Grand Prize of the iGEM competition. This historic victory proves again and again the creativity and academic energy of the students at the Life Sciences Center. The President the Republic of Lithuania awarded our students the historical presidential flag.

We are very proud of our women researchers at the Life Sciences Center. This year two of them, Dr Rima Bydvytytė and Dominyka Dapkutė were granted L'Oréal-UNESCO Baltic *For Women in Science* fellowships. Women are becoming more and more active in seeking for management positions at the Life Sciences Center. Dr Eglė Lastauskienė was appointed the Director of the Institute of Biosciences, which is our major operational unit involved in studies. This appointment reflects the efforts of our community to ensure gender equality in top management positions at the Life Sciences Center.

I wish to thank all our academic community for the academic year full of unseen challenges. This year will remind us that all challenges may also provide opportunities to realise our talents and energy for the benefit of society. To take advantage of those opportunities we need to continue standing firmly on our basic academic values: dedication to excellence in science, academic freedom, collegiality and inclusiveness, openness to the world and non-discrimination, respect for societal needs and public engagement. These values, I believe, will lead us towards new success stories in 2021.

Gintaras Valinčius
Director

RESEARCH DESCRIPTIONS

Biomolecular Structure & Function

Cell & Molecular Biology

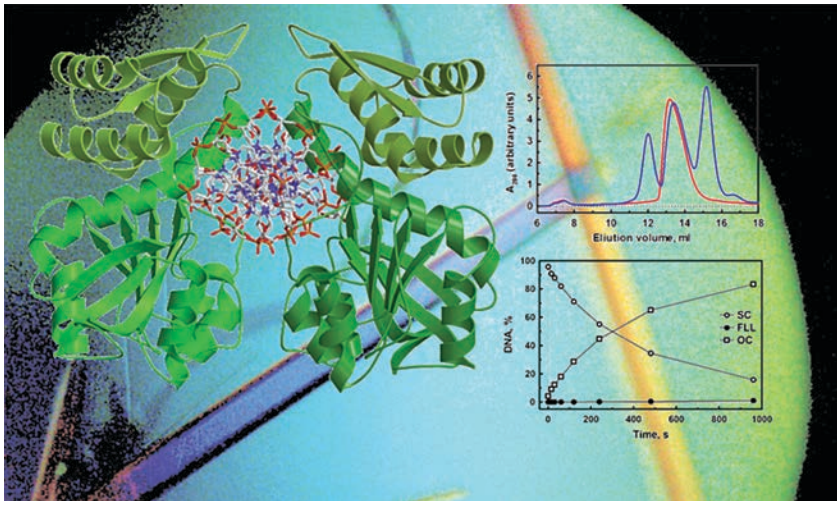
Ecosystems & Biodiversity

Molecular Biotechnologies

Molecular Mechanisms of Disease

Neurobiology

VIRGINIJUS ŠIKŠNYS
Distinguished Professor
Head, Department of Protein-Nucleic Acids Interactions
Institute of Biotechnology
Email: virginijus.siksnys@bti.vu.lt
Phone: +370 5 223 4354



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 systems, in collaboration with Dr. D. Wigley (Imperial College London), Dr. R. Seidel (Universität Leipzig), Dr. M. D. Szczelkun (Bristol University), Dr. J. Young (Corteva), Dr. C. Venclovas (Vilnius University), Dr. M. Bochtler (IUCMB), Drs. K. Makarova and E. Koonin (NIH). We continue to explore molecular mechanisms behind cyclic oligoadenylate signalling pathway discovered by us in 2017 and other proteins related to CRISPR-Cas or other antiviral defence systems.

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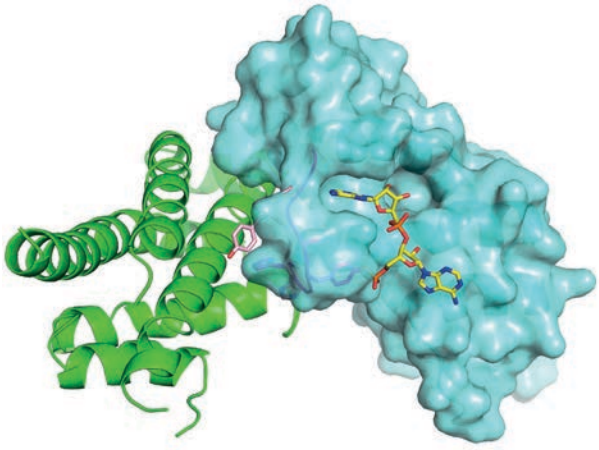
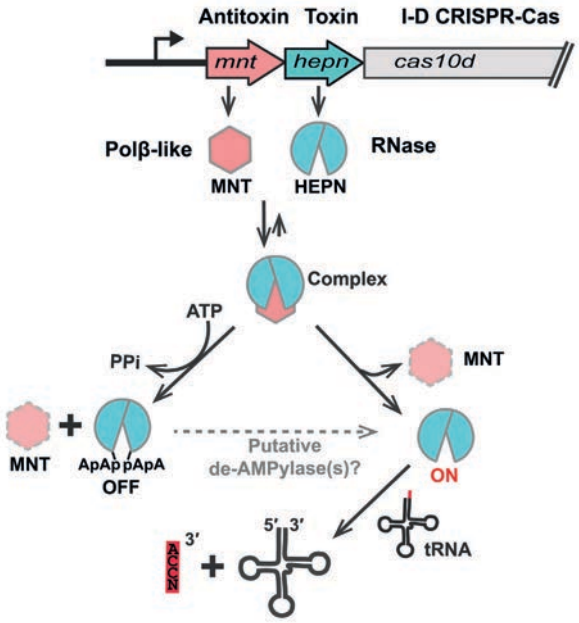
Novel Toxin-Antitoxin System Related to I-D CRISPR-Cas System

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).



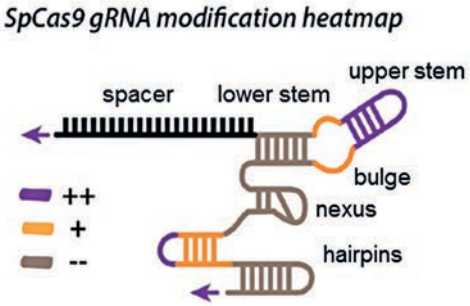
Crystal structure of di_AMPylated HEPN ribonuclease



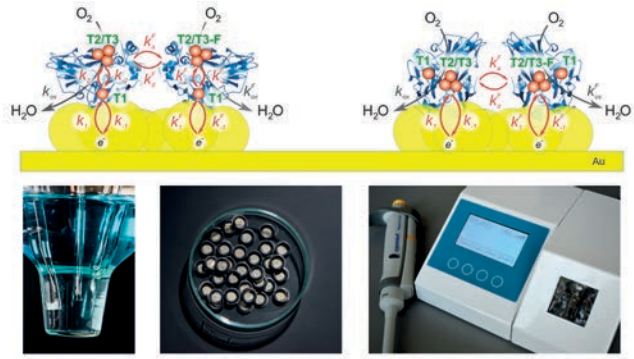
Proposed mechanism of action of A. flos-aquae HEPN-MNT toxin-antitoxin system

Studies of 5′ Modifications to CRISPR–Cas9 Guide RNA

A key aim in exploiting CRISPR–Cas is guide RNA (gRNA) engineering to introduce additional functionalities, ranging from individual nucleotide changes that increase efficiency of on-target binding to the inclusion of larger functional RNA aptamers or ribonucleoproteins (RNPs). Cas9–gRNA interactions are crucial for complex assembly, but several distinct regions of the gRNA are amenable to modification. We used *in vitro* ensemble and single-molecule assays to assess the impact of gRNA structural alterations on RNP complex formation, R-loop dynamics, and endonuclease activity. Our results indicate that RNP formation was unaffected by any of our modifications. R-loop formation and DNA cleavage activity were also essentially unaffected by modification of the Upper Stem, first Hairpin and 3′ end. In contrast, we found that 5′ additions of only two or three nucleotides could reduce R-loop formation and cleavage activity of the RuvC domain relative to a single nucleotide addition. Such modifications are a common by-product of *in vitro* transcribed gRNA. We also observed that addition of a 20 nt RNA hairpin to the 5′ end of a gRNA still supported RNP formation but produced a stable ~9 bp R-loop that could not activate DNA cleavage. Consideration of these observations will assist in successful gRNA design (Mullally et al. *Nucleic Acids Res.* 2020, 48: 6811–6823).



gRNA heatmap showing gRNA regions which tolerate (purple), partially tolerate (orange) and do not tolerate (grey) modification



MARIUS DAGYS
Senior Researcher
Head, Department of Bioanalysis
Institute of Biochemistry
Email: marius.dagys@bchi.vu.lt
Phone: +370 613 91030

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 principal 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, a number of 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 [1–3]. Our team is also developing bioreactor systems, where wasteful saccharide substrates are selectively oxidised and high-value oxidation products are produced. For such an approach, we utilize bi-enzymatic reaction with biosensor-based microprocessor-controlled substrate dispensing. In order 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. Recently, molecularly imprinted polymers based on polypyrrole and polyaniline preparations have intensively been studied 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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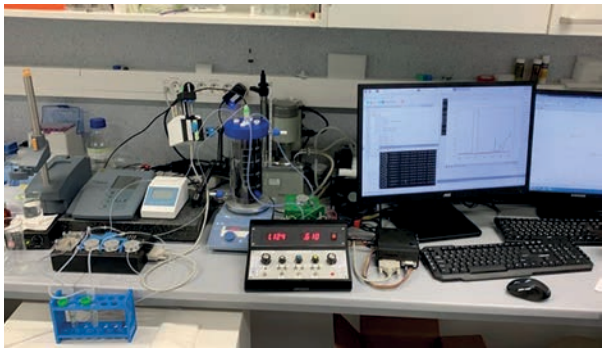
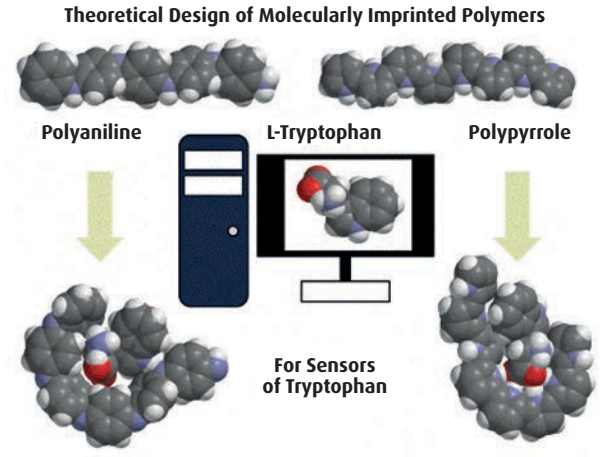
Dehydrogenases in Custom Sensor-Controlled Bioreactors

The use of the dehydrogenases to oxidize substrates in bioreactors is very attractive due to their broad substrate specificity and high catalytic activity. However, the application requires an effective mediated enzyme reoxidation method. The mediator in regeneration scheme must be highly reactive with the enzyme to regenerate; all forms of the mediator must be stable, nontoxic and cheap. The oxidized mediator form is produced in reaction with heme peroxidase, which exhibits high catalytic activity at pH 7.0 and broad substrate specificity. Peroxidase uses hydrogen peroxide as co-substrate to oxidase mediators. To avoid the hydrogen peroxide-induced enzyme inactivation, the addition of hydrogen peroxide to the reactor mixture was performed in very small doses of 40 nL by using the syringe pump developed by our team. The rate of dosage was controlled by analysing the data of our custom highly-sensitive hydrogen peroxide and optical, oxidized mediator-form sensors, all combined into microcontroller-coordinated control algorithm. As of today, the turnover number of PQQ glucose dehydrogenase in model reactor reaches ~1x10^7, which means one can produce a valuable product at ~0,65 Eur/g and expect to sell it for about a hundred times more (RCL grant No. 01.2.2-LMT-K-718-01-0019).



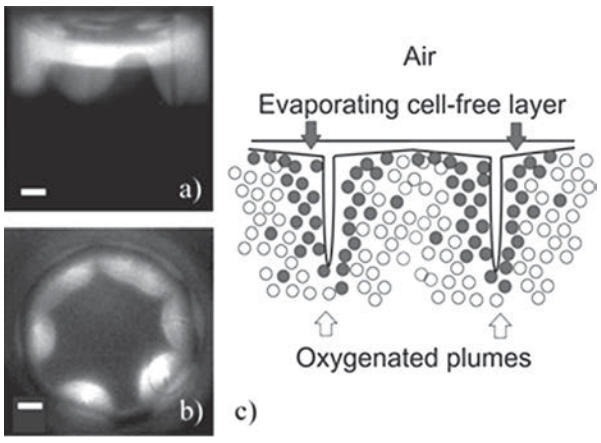
Theoretical Design of Molecularly Imprinted Polymers for Analysis

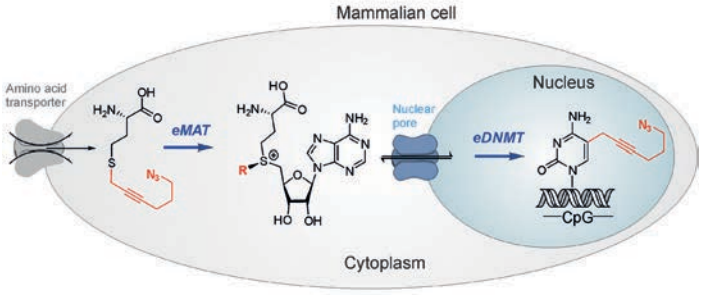
Creation of molecularly imprinted polymers (MIPs) as the supramolecular systems with tailor-made binding sites complementary to template molecules in shape, size and functional groups is an important task for the analytical, physical and theoretical chemistries. In this work, the polypyrrole and polyaniline-based host - guest MIPs were theoretically studied for the detection of tryptophan. These simulations showed that polyaniline is not suitable for the selective detection of tryptophan due to high flexibility of its chains and low energy of intermolecular interactions. In contrast, the polypyrrole-based hosts can be used to detect tryptophan, because all these simulated forms shaped the bow-shaped inner cavity with the strongly coordinated target molecule. Moreover, the insights 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. Analysis of the experimental data together with mathematical modelling suggests the following interpretation of pattern formation (right figure: a) and b) show typical side-view and top-view images of cylindrical samples (bar – 1 mm), the scheme of a system that forms spatiotemporal patterns is shown in c). The evaporation- and settling-driven instability of the surface layer results in formation of oxygenated plumes. In the vicinity of the plumes, active cells (grey circles) 'aggregate' and 'grow' at the expense of passive cells (white circles). These studies were partly funded by RCL grant No. S-MIP-17-98.





SAULIUS KLIMAŠAUSKAS
Distinguished Professor
Head, Department of Biological DNA Modification
Institute of Biotechnology
Email: saulius.klimasauskas@bti.vu.lt
Phone: +370 5 223 4350

GIEDRIUS VILKAITIS
Research Professor
Department of Biological DNA Modification
Institute of Biotechnology
Email: giedrius.vilkaitis@bti.vu.lt
Phone: +370 5 223 4372

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 [1].

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 [2,3].

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 [3,4]. 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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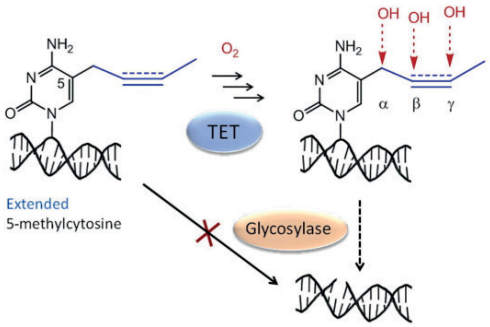
Enzymatic Hydroxylation and Excision of Extended 5-Methylcytosine Analogues

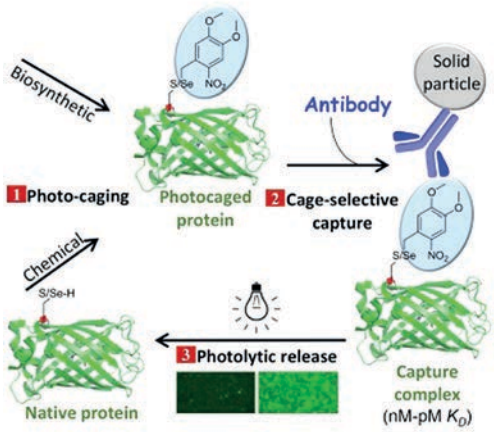
Methylation of cytosine to 5-methylcytosine (mC) is a prevalent reversible epigenetic mark in vertebrates established by DNA methyltransferases (MTases); the methylation mark can be actively erased via a multi-step demethylation mechanism involving oxidation by Ten-eleven translocation (TET) enzyme family dioxygenases, excision of the latter oxidation products by thymine DNA (TDG) or Nei-like 1 (NEIL1) glycosylases followed by base excision repair to restore the unmodified state. Here we probed the activity of the mouse TET1 (mTET1) and Naegleria gruberi TET (nTET) oxygenases with DNA substrates containing extended derivatives of the 5-methylcytosine carrying linear carbon chains and adjacent unsaturated carbon-carbon bonds. We found that the nTET and mTET1 enzymes were active on modified mC residues in single-stranded and double-stranded DNA *in vitro*, while the extent of the reactions diminished with the size of the extended group. Iterative rounds of nTET hydroxylations



of ssDNA proceeded with high stereo specificity and included not only the natural alpha position but also the adjoining carbon atom in the extended side chain. The regioselectivity of hydroxylation was broken when the reactive carbon was adjacent to an sp^1 or sp^2 system. We also found that NEIL1 but not TDG was active with bulky TET-oxidation products. These findings provide important insights into the mechanism of these biologically important enzymatic reactions (Tomkuvienė et al. *J. Mol. Biol.* 2020, 423: 6157–6167).

Photocage-Selective Capture and Light-Controlled Release of Target Proteins

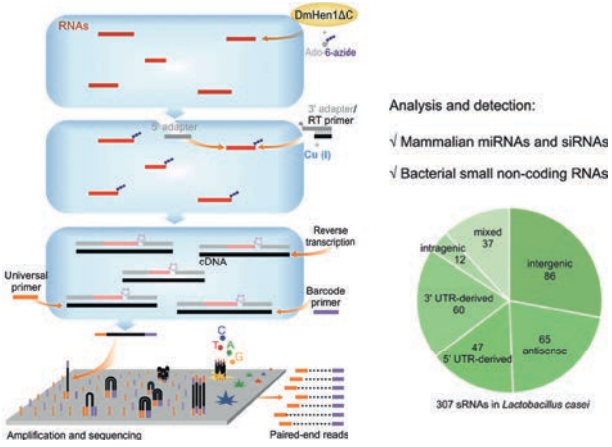
Photochemical transformations enable exquisite spatio-temporal control over biochemical processes, however, methods for reliable manipulations of biomolecules tagged with biocompatible photo-to-sensitive reporters are lacking. We went on to create a high affinity binder specific to a photolytically removable caging group. We utilized chemical modification or genetically-encoded incorporation of noncanonical amino acids to produce proteins with photocaged cysteine or selenocysteine residues which were used for raising a high affinity monoclonal antibody against a small photoremovable tag, 4,5-dimethoxy-2-nitrobenzyl (DMNB) group. Employing the produced photocage-selective binder, we demonstrate selective detection and immunoprecipitation of a series of DMNB-caged model proteins and DNA cytosine-5 methyltransferase enzymes from complex biological mixtures. The proposed orthogonal strategy permits photocage-selective capture and light-controlled traceless release of target proteins



for a myriad of applications in nanoscale assays (Rakauskaitė et al. *iScience*. 2020, 23(12): 101833; Klimašauskas et al., LT2020539).

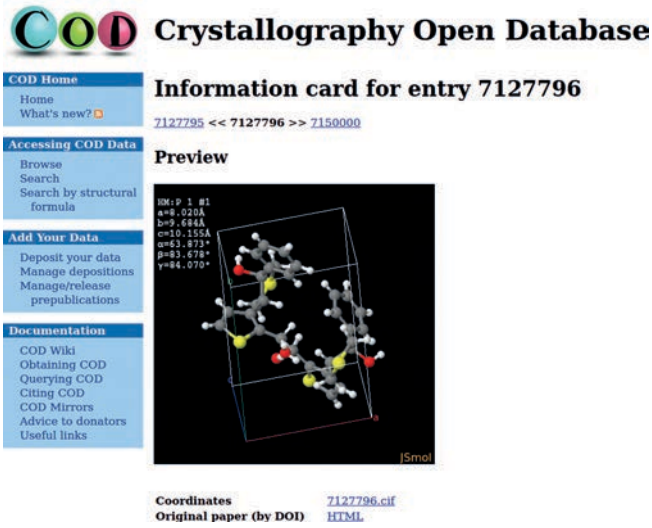
Hen1 Methyltransferase-Directed RNA Capture and Sequencing of miRNAs and Bacterial Small RNAs in Probiotic *Lactobacillus casei*

Targeted installation of designer chemical moieties on biopolymers provides an orthogonal means for their visualization, manipulation and sequence analysis. Although high-throughput RNA sequencing is a widely used method for transcriptome analysis, certain steps, such as 3′ adapter ligation in strand-specific RNA sequencing, remain challenging despite numerous optimizations. Here we remedy this limitation by adapting two small RNA 2′-O-methyltransferases, ssRNA-specific DmHen1 and dsRNA modifying AtHEN1, for orthogonal chemo-enzymatic click tethering of a 3′ sequencing adapter that supports cDNA production by reverse transcription of the tagged RNA. We show by profiling a reference miRNA pool and the small RNA transcriptome of probiotic *Lactobacillus casei* BL23 that the developed methyltransferase-captured 3′ RNA sequencing technique, meCap-seq, can advance analysis of eukaryotic and prokaryotic ssRNA pools. Our findings provide a valuable resource for studies of the regulatory RNome in *Lactobacilli* and pave the



way to developing novel transcriptome and epitranscriptome profiling approaches *in vitro* and inside living cells (EP3271478 B1; Mickutė et al., *submitted*).

SAULIUS GRAŽULIS
Research Professor
Department of Protein - DNA Interactions
Institute of Biotechnology
Email: saulius.grazulis@bti.vu.lt, grazulis@ibt.lt
Phone: +370 684 49802



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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1. Vaitkus, A., Merkys, A. & Gražulis, S. Validation of the Crystallography Open Database using the CIF framework. *Journal of Applied Crystallography*. 2021, accepted.
2. Gražulis, S., Merkys, A., Vaitkus, A., Chateigner, D., Lutterotti, L., Moeck, P. et al., Le Bail, A. Crystallography open database: history, development, and perspectives. In: O. Isayev, A. Tropsha, & S. Curtarolo (Eds.), Materials Informatics. 2019, 1–39. Wiley. doi:10.1002/9783527802265.ch1 .
3. Mendili, Y. E., Vaitkus, A., Merkys, A., Gražulis, S., Chateigner, D., Mathevet, F. et al., Guen, M. L. Raman Open Database: first interconnected Raman–X-ray diffraction open-access resource for material identification. *Journal of Applied Crystallography*. 2019, 52(3): 618–625. doi:10.1107/s1600576719004229 .
4. Quirós, M., Gražulis, S., Girdzijauskaitė, S., Merkys, A. & Vaitkus, A. Using smiles strings for the description of chemical connectivity in the crystallography open database. *Journal of Cheminformatics*. 2018, 10(23). doi:10.1186/s13321-018-0279-6.
5. Merkys, A., Mounet, N., Cepellotti, A., Marzari, N., Gražulis, S., Pizzi, G. A posteriori metadata from automated provenance tracking: integration of AiiDA and TCOD. *Journal of Cheminformatics*. 2017, 9(1): 56. doi:10.1186/s13321-017-0242-y.

Crystallographic Data Validation

The crystallographic data validation topic concerns collection, analysis and validation of crystallographic information in the COD. As experimental crystallographic data is not directly usable in computational chemistry analyses, additional information and assumptions have to be employed to augment the crystallographic data with chemical annotations in fully automated manner. Analysis of the results derived by such processes leads to the identification of outliers, which may be genuine either due to the problems with computation workflows or the data itself. Identification of the latter is crucial to increase the quality of both the crystallographic and the chemical data in the COD as well as other bodies of experimental crystallographic data (project leader - A. Merkys).

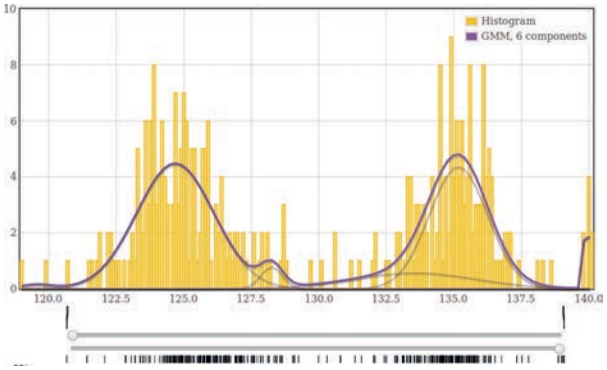


Fig. 1. Distribution of c(cCH)2(H)-c(cCH)2(H)-c(cCH)2(H) bond angles in the COD data.

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. These descriptors were later used to link a large portion of the COD to the PubChem database (project leader - A. Vaitkus).

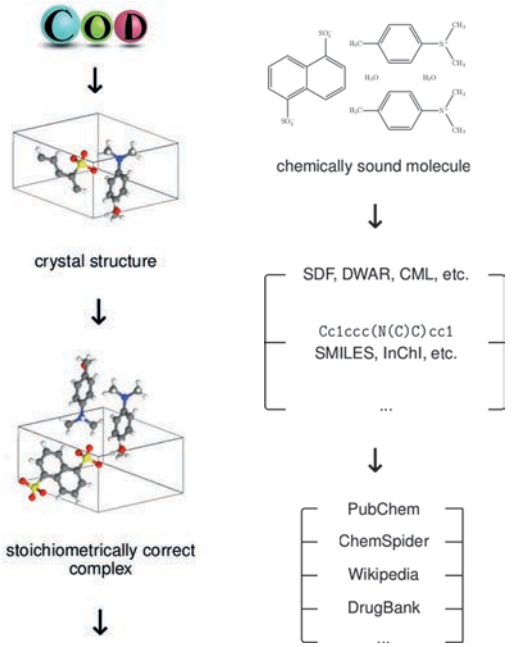


Fig. 2. Schema of the automated pipeline used to derive chemical information from the COD.

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 enables to study side-chains regardless of unobserved angles or modified amino acids. The flexibility of the method enhances the study of possible side-chain positions and their potential interactions with the ligands (project leader - A. Grybauskas).

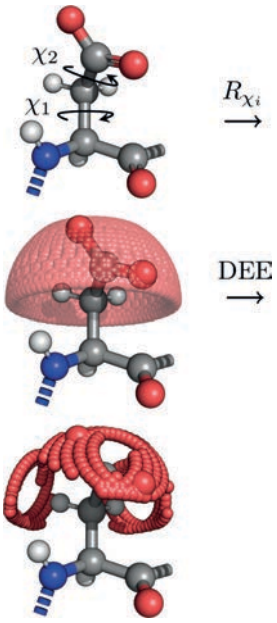
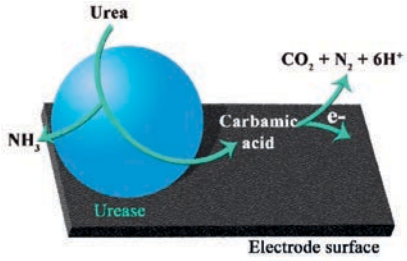
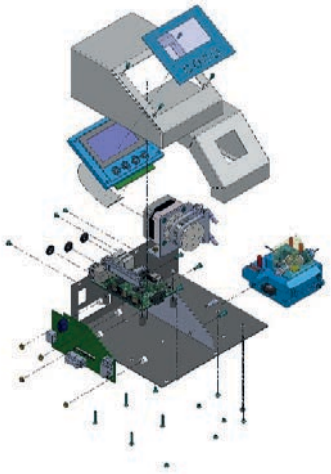


Fig.3. Rotamer generation steps: first, the energy values of all angles are calculated until they reach certain threshold (dead-end elimination), then the lowest energy rotamers are kept.





JULIJA RAZUMIENĖ
Research Professor
Department of Bioanalysis
Institute of Biochemistry
Email: julija.razumiene@gmc.vu.lt
Phone: +370 670 61893

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 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 ofnanomaterial-based glucose biosensors, which could operate with high precision and accuracy in clinically-relevant fluids – human serum [1] and blood [3]. This type of biosensors could be miniaturized and implanted in patients' bodies and be used for real-time monitoring of glucose. Additionally, we have developed biosensors for low-level glucose measurements and demonstrated that those biosensors could be used for monitoring the stress level of juvenile trout fish [2]. Clinically relevant projects in our department are carried out in co-operation with Vilnius University Hospital *Santaros Klinikos* and are related to the diagnosis and prognosis of the clinical outcome of patients undergoing renal replacement therapy as well as of those with other specific conditions, e.g. acute pancreatitis [4].

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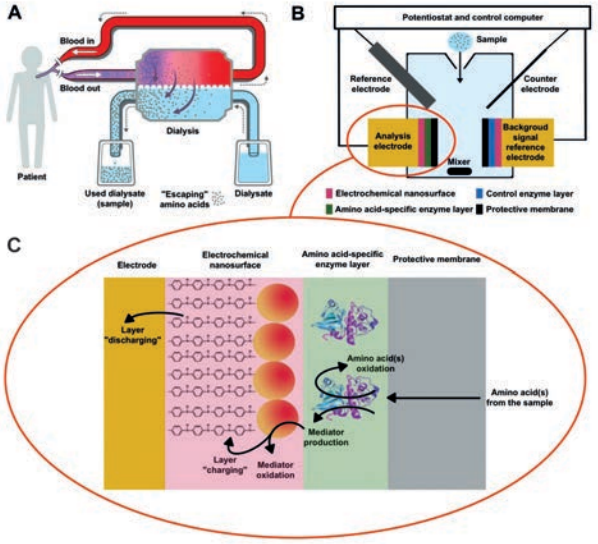
1. Ratautas, D. et al. Real-time glucose monitoring system containing enzymatic sensor and enzymatic reference electrodes. *Biosensors & Bioelectronics*. 2020, 164: 112338.
2. Makaras, T., Razumienė, J. et al. A new approach of stress evaluation in fish using β-d-Glucose measurement in fish holding-water. *Ecological Indicators*. 2020, 109: 105829.
3. Gineitytė, J. et al. Highly efficient direct electron transfer bioanode containing glucose dehydrogenase operating in human blood. Journal of *Power Sources*. 2019, 441: 227163.
4. Razumienė, J. et al. The synergy of thermally reduced graphene oxide in amperometric urea biosensor: application for medical technologies. *Sensors*. 2020, 20: 4496.

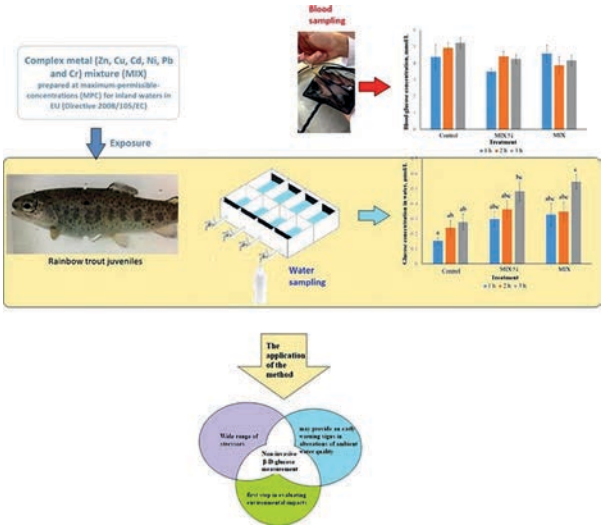
Amino Acids' Biosensor in Clinical Applications

We are developing an electrochemical biosensor platform for the fast, accurate and cheap detection as well as quantification of total and specific (glutamate, glutamine, lysine) amino acids present in biologically relevant fluids – dialysate buffer after haemodialysis and human serum/blood. The analysis of the amino acids in biological fluids for clinical applications is an unresolved challenge worldwide, since the concentration levels of amino acids are very low (typically 1–100 µmol/l or less) and create challenges. The major applicable methods for amino acid analysis in clinical diagnostics involve either commercial colorimetric (e.g., ELISA) kits or chromatographic amino acid analysers, which are costly, time-consuming and require highly trained scientific personnel to operate. For such reasons the analysis of amino acid in hospitals for clinical diagnostics and monitoring is not a usual routine procedure. Consequently, the extreme diagnostic potential of amino acids present in biological fluids is not fully utilized and may be overlooked (RCL grant No. 01.2.2-LMT-K-718-03-0005 and S-EJPRD-20-1).



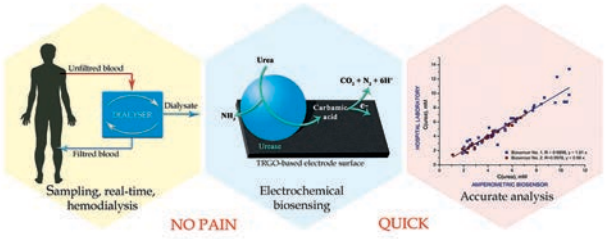
Biosensors for Fish Stress Level Control

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 [2]. The developed biosensor has several advantages over conventional methods, i.e. a wide linear range, high sensitivity, good selectivity, long-term stability and ability to act in non-pre-treated turbid media. 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).



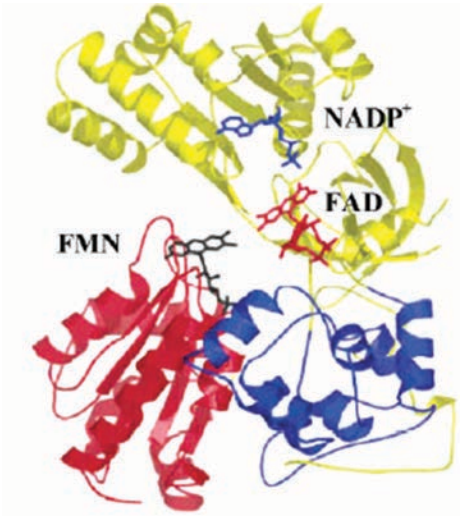
Urea Biosensors with Thermally Reduced Graphene Oxide

We discovered that the synergy of the electrode based on thermally reduced graphene oxide (TRGO) nanoparticles in combination with urease allowed the development of a promising urea biosensor for clinical trials. According to the data of recently reported (2015-2019) urea biosensors, the features of TRGO-based urea biosensor include advanced analytical characteristics such as good sensitivity, wide linear range, long storage and operational stability for the accomplishment of more than 300 samples of clinical trials without significant change. Low-cost design, good reproducibility and fast response time allowed applying the biosensor for monitoring of urea levels in real samples such as urine, blood and dialysate collected during the haemodialysis (HD) procedure. The accuracy of the biosensor action



was validated by approved methods on a base of a large number of measurements.

The experiments confirmed that urea measurement in urine and in spent dialysate possess a great potential as a tool for evaluation of dialysis adequacy as well as a step leading to point-of-care non-invasive technologies (RCL grant No. 01.2.2-LMT-K-718-01-0025).



NARIMANTAS ČĖNAS
Research Professor
Head, Department of Xenobiotics Biochemistry
Institute of Biochemistry
Email: narimantas.cenas@bchi.vu.lt
Phone: +370 5 223 4392

Mechanisms of Flavoenzyme Redox Reactions

Flavoenzymes contain flavinmononucleotide (FMN) or flavinadenindinucleotide (FAD) in their active centres. The distinctive feature of flavoenzymes is their ability to transform single-electron transfer into a two-electron one. They play important roles in biological oxidation-reduction, hydroxylation, transhydrogenation, antioxidant protection, redox signalling, and other processes. Flavoenzymes also 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 2017–2020 were as follows: a) characterization of interaction mechanism of quinones, nitroaromatics and aromatic N-oxides with possible target enzymes in bacteria (S. aureus flavohemoglobin, collaboration with Dr L. Baciou and F. Lederer, Université Paris-Sud, France), 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 *E. coli* nitroreductase A and other nitroreductases (collaboration with Dr D. F. Ackerley, Victoria University of Wellington, New Zealand); c) evaluation of nitroaromatic compounds as inhibitors for *Plasmodium falciparum* glutathione reductase and *Trypanosoma congolense* trypanothione reductase in the context of development of antiplasmodial and antitrypanosomal agents (collaboration with Dr E. Davioud-Charvet, Université de Strasbourg, France, and Dr J. S. Blanchard, Albert Einstein College of Medicine, NY, USA); d) continuation of synthesis, studies of enzymatic single- and two-electron reduction and mammalian cell culture 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 2020, we are continuing the studies of a potent antiplasmodial agent, 1,4-naphthoquinone plasmodione, and its derivatives (in collaboration with Dr E. Davioud-Charvet, Lithuanian-French Programme "Gilibert", No. S-LZ-19-4). In addition, we have carried out the studies for antibacterial activity of aromatic di-N-oxide compounds used as single agents and in combination with conventional antibiotics, and participated in the studies of antibacterial photodynamic therapy.

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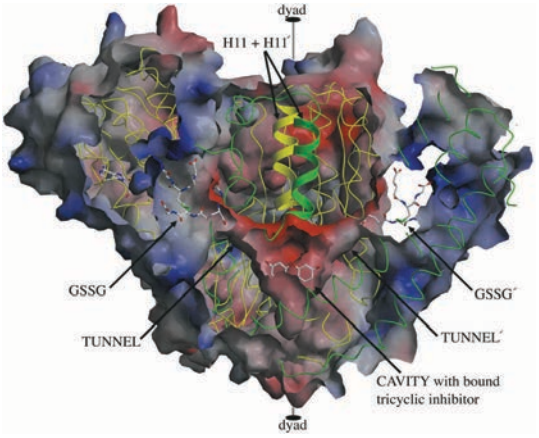
1. Nemeikaitė-Čėnienė, A., Šarlauskas, J., Misevičienė, L., Marozienė, A., Jonušienė, V., Lesanavičius, M., Čėnas, N. Aerobic cytotoxicity of aromatic N-oxides: the role of NAD(P)H:quinone oxidoreductase (NQO1). *Int. J. Molec. Sci.* 2020, 21: 8754.
2. Lesanavičius, M., Aliverti, A., Šarlauskas, J., Čėnas, N. Reactions of Plasmodium falciparum ferredoxin:NADP+ oxidoreductase with redox cycling xenobiotics: a mechanistic study. *Int. J. Molec. Sci.* 2020, 21: 3234.
3. Nemeikaitė-Čėnienė, A., Šarlauskas, J., Jonušienė, V., Marozienė, A., Misevičienė, L., Yantsevich, A. V., Čėnas, N. Kinetics of flavoenzyme-catalyzed reduction of tirapazamine derivatives: implications for their prooxidant cytotoxicity. *Int. J. Molec. Sci.* 2019, 20: 4602.
4. Marozienė, A., Lesanavičius, M., Davioud-Charvet, E., Aliverti, A., Grellier, P., Šarlauskas, J., Čėnas, N. Antiplasmodial activity of nitroaromatic compounds: correlation with their reduction potential and inhibitory action on *Plasmodium falciparum* glutathione reductase. *Molecules*. 2019, 24: 4509.
5. Moussaoui, M., Misevičienė, L., Anusevičius, Ž., Marozienė, A., Lederer, F., Baciou, L., Čėnas, N. Quinones and nitroaromatic compounds as subversive substrates of Staphylococcus aureus flavohemoglobin. *Free Radic*. Biol. Med. 2018, 123: 107–115.

Mechanisms of Antiplasmodial and Antitrypanosomal *in vitro* Activity of Nitroaromatic Compounds

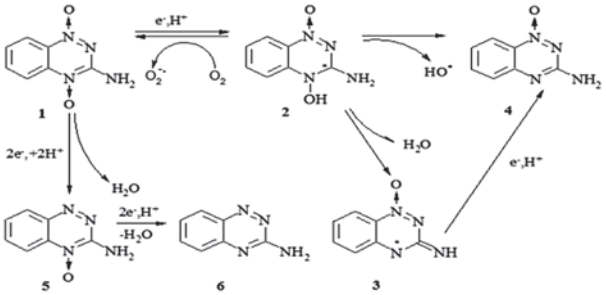
We found that the *in vitro* antiplasmodial activity of a series of nitrobenzenes and nitrofurans increases with their single-electron reduction potential (E^1_7), log D, and their ability to inhibit *Plasmodium falciparum*, but not human erythrocyte glutathione reductase. Ferredoxin:NADP+ oxidoreductase is expected to be the most active enzymatic reductant of nitroaromatics in parasite (Marozienė et al. *Molecules*. 2019, 24: 4509). Similarly, the *in vitro* activity of a series of 5-vinylquinoline-substituted nitrofurans against *Trypanosoma b. brucei* exhibited parallelism with their inhibitory activity against trypanothione reductase (Benitez et al. *Chemija*. 2020, 31: 111–117).



Structure of erythrocyte glutathione reductase with indicated domains of binding of substrates and inhibitors (Sarma et al. *J. Mol. Biol*. 2003, 328: 893–907)

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

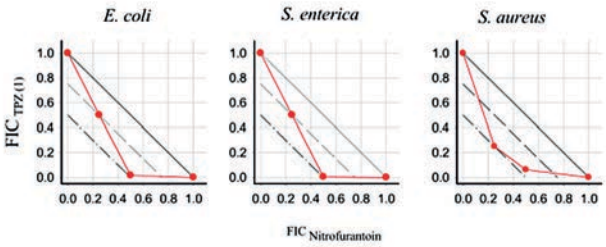
3-Amino-1,2,4-benzotriazine-1,4-dioxide (tirapazamine, TPZ) is an anticancer agent selective for hypoxic cells. However, some of its derivatives may exert significant toxicity to normal (oxic) cells. We found that the aerobic cytotoxicity of a number of TPZ derivatives is well above that of quinones 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).



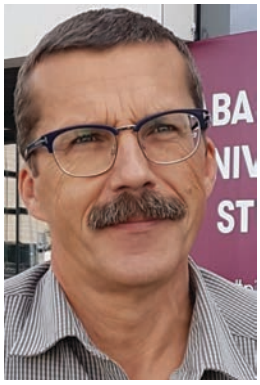
Scheme of single-electron reduction, redox cycling, and formation of metabolites of tirapazamine (1)

Sensitization of *S. enterica* Strain with 5-Aminolevulinic Acid (ALA) -induced Porphyrins

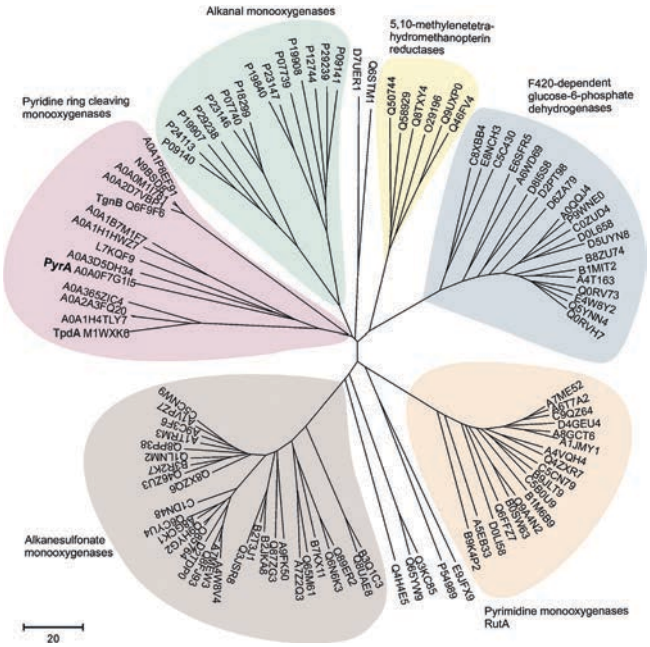
Photodynamic therapy (PDT) of bacteria represents an alternative to antibiotic therapy. The efficacy of treatment is dependent on the interaction of photosensitizers with bacterial wall or intracellular accumulation. Various types of endogenous porphyrins were generated in *S. enterica* after incubation with 5-ALA, a precursor of heme. The revealed time-dependent changes in compositions of intra- and extracellular porphyrins implied that ALA-induced sensitization triggers a complex protective mechanisms of bacteria (Polmickaitė-Smirnova et al. *Photochem. Photobiol. Sci*. 2019, 18: 2730).



Isobolograms of combinatory action of TPZ and NFT (Polmickaitė-Smirnova et al. 2020)



ROLANDAS MEŠKYS
Distinguished Professor
Head, Department of Molecular Microbiology and Biotechnology
Institute of Biochemistry
Email: rolandas.meskys@bchi.vu.lt
Phone: +370 5 223 4386



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 oxygenases active towards indole, pyridine and 4-hydroxypyridine as a primary substrate have been characterized, genetically modified and applied for development of biocatalytic processes [1–4]. Screening for novel enzymes is also carried out by applying metagenomic techniques – effective selection systems combined with tailored substrates [5].

More than 160 of differently modified nucleotides play a crucial role in various biological processes. Also, various modified nucleotides are used as promising building blocks for programmable changes of nucleic acids. The biosynthetic pathways of many modified nucleotides including N^4-acetylcytosine derivatives are well understood, but the catabolism or salvage of those compounds are only scarcely studied. For the first time, we show that in *E. coli* the ASCH domain-containing protein YqfB, which has a unique Thr-Lys-Glu catalytic triad, catalyses the hydrolysis of N^4-acetylcytidine. In addition, novel N^4 amino acid-acylated and alkylated 2'-deoxycytidine analogues were synthesized.

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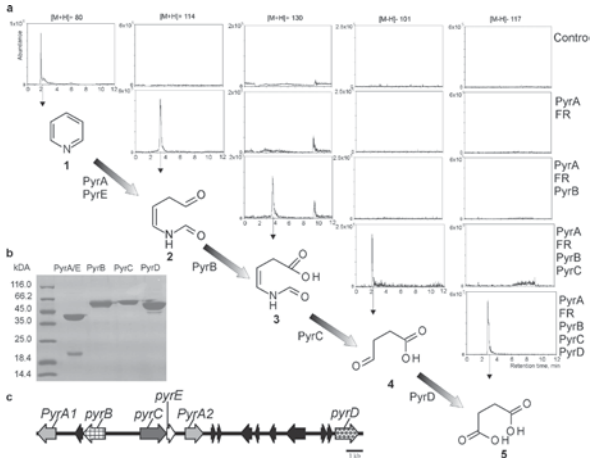
1. Časaitė et al. Microbial degradation of pyridine: a complete pathway deciphered in *Arthrobacter* sp. 68b. *Appl. Environ. Microbiol.* 2020, 86: e00902-20.
2. Sadauskas et al. Bioconversion of biologically active indole derivatives with indole-3-acetic acid-degrading enzymes from *Caballeronia glathei* DSM50014. *Biomolecules*. 2020, 10: 663.
3. Vaitekūnas et al. Biochemical and genetic analysis of 4-hydroxypyridine catabolism in *Arthrobacter* sp. strain IN13. *Microorganisms*. 2020, 8: 888.
4. Sadauskas et al. Enzymatic synthesis of novel water-soluble indigoid compounds. *Dyes Pigm*. 2020, 173: 107882.
5. Urbelienė et al. A rapid method for the selection of amidohydrolases from metagenomic libraries by applying synthetic nucleosides and a uridine auxotrophic host. *Catalysts.* 2020, 10: 445.

Microbial Degradation of Pyridine: A Complete Pathway Deciphered in *Arthrobacter* sp. 68b

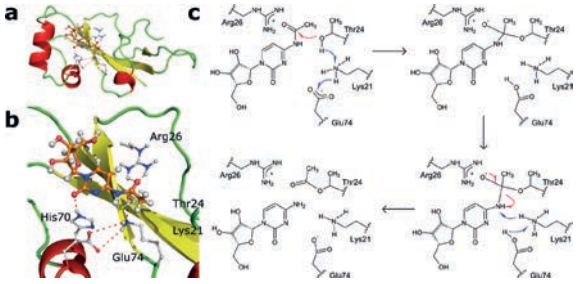
Pyridine and its derivatives constitute majority of heterocyclic aromatic compounds that occur largely as a result of human activities and contribute to the environmental pollution. It is known that they can be degraded by various bacteria in the environment, however, the degradation of unsubstituted pyridine has not yet been completely resolved. Here, we present data on the pyridine catabolic pathway in *Arthrobacter* sp. 68b at the level of genes, enzymes and metabolites. The *pyr* genes cluster, responsible for the degradation of pyridine, was identified in a catabolic plasmid p2MP. The pathway of pyridine metabolism consisted of four enzymatic steps and ended by formation of succinic acid. The first step in the degradation of pyridine proceeds through a direct ring cleavage catalysed by a two-component flavin-dependent monooxygenase system, encoded by *pyrA* and *pyrE* genes. The genes *pyrB*, *pyrC*, and *pyrD* were found to encode (*Z*)-*N*-(4-oxobut-1-enyl)formamide dehydrogenase, amidohydrolase, and succinate semialdehyde dehydrogenase, respectively. These enzymes participate in the subsequent steps of pyridine degradation (Časaitė et al. *Appl. Environ. Microbiol*. 2020, 86: e00902-20).



***In vitro* reconstruction of the pyridine degradation pathway**
(**a**) The analysis of pyridine metabolites by LC-MS and the metabolic pathway of pyridine. Samples were analysed in the positive or negative ionization mode; the extracted ion chromatograms correspond to the quasimolecular ions of (1) pyridine (m/z=80 $[M+H]^+$), metabolite 2 – (*Z*)-*N*-(4-oxobut-1-enyl)formamide (m/z=114 $[M+H]^+$), metabolite 3 – (*Z*)-4-formamidobut-3-enoic acid (m/z=130 $[M+H]^+$), 4 – succinic acid semialdehyde (m/z=101 $[M-H]^-$), and 5 – succinate (m/z=117 $[M-H]^-$);
(**b**) purified proteins participating in pyridine pathway;
(**c**) *pyr* gene cluster in the p2MP plasmid. PyrA – pyridine monooxygenase, PyrE and FR – flavin reductase, PyrB – (*Z*)-*N*-(4-oxobut-1-enyl)formamide dehydrogenase, PyrC – amidohydrolase, PyrD – succinate semialdehyde dehydrogenase.

YqfB Protein from *Escherichia coli*: Atypical Amidohydrolase Active towards N^4-acylcytosine Derivatives

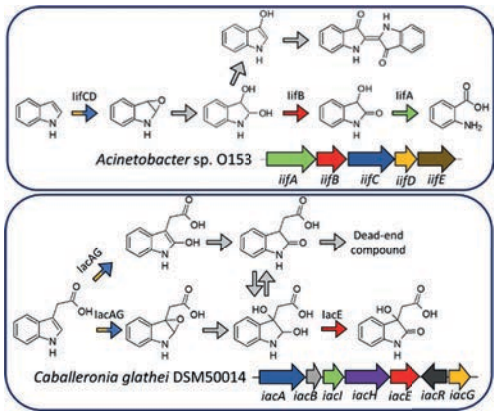
Human activating signal cointegrator homology (ASCH) domain-containing proteins are widespread and diverse but, at present, the vast majority of those proteins have no function assigned to them. This study demonstrates that the 103-amino acid hypothetical protein YqfB from *E. coli* is a unique ASCH domain-containing amidohydrolase responsible for the catabolism of N^4-acetylcytidine (ac4C). YqfB has several interesting and unique features: (i) it is the smallest monomeric amidohydrolase, (ii) it is active towards structurally different N^4-acylated cytosines/cytidines, iii) it has a very high activity rate (kcat/Km up to 2.8×10^6 $M^{-1}s^{-1}$) and (iv) it contains a unique Thr-Lys-Glu catalytic triad and Arg acting as an oxyanion hole. YqfB ability to hydrolyse various N^4-acylated cytosines and cytidines not only sheds light on the long-standing mystery of how ac4C is catabolized in bacteria, but also expands our knowledge of the structural diversity within the active sites of amidohydrolases. (Stanislauskienė et al. *Sci. Rep*. 2020, 10: 788).



Predicted structure of the active centre of YqfB and proposed catalytic mechanism of ac4C hydrolysis
(**a**) The overall structure of the proposed enzyme-substrate complex.
(**b**) A detailed view of Yqfb active centre with bound ac4C, as generated by MD simulation; dashed lines correspond to hydrogen bonds.
(**c**) The acylation step of ac4C hydrolysis catalysed by YqfB.

Bioconversion of Biologically Active Indole Derivatives with Indole-3-Acetic Acid-Degrading Enzymes from *Caballeronia glathei* DSM50014

A plant auxin hormone indole-3-acetic acid (IAA) can be assimilated by bacteria as an energy and carbon source, although no degradation has been reported for indole-3-propionic acid and indole-3-butyric acid. *Caballeronia glathei* possesses a full *iac* gene cluster and is able to use IAA as a sole source of carbon and energy. Next, IacE is responsible for the conversion of 2-oxoindole-3-acetic acid intermediate into the central intermediate 3-hydroxy-2-oxindole-3-acetic acid (DOAA). Finally, IacA and IacE were shown to convert a wide range of indole derivatives, including indole-3-propionic acid and indole-3-butyric acid, into corresponding DOAA homologs. This work provides novel insights into Iac-mediated IAA degradation and demonstrates the versatility and substrate scope of IacA and IacE enzymes. (Sadauskas et al. *Biomolecules*. 2020, 10: 663).



Comparison of biodegradation of indole in *Acinetobacter* sp. strain 0153 and biodegradation of IAA in *Caballeronia glathei* DSM50014 (M. Sadauskas, PhD thesis). Grey reaction arrows indicate spontaneous reactions. Arrows with identical colours indicate genes and proteins with similar predicted functions.

ČESLOVAS VENCLOVAS
Distinguished Professor
Head, Department of Bioinformatics
Institute of Biotechnology
Email: ceslovas.venclovas@bti.vu.lt
Phone: +370 5 223 4368
Website: http://bioinformatics.lt

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. The increasing computer power and the flood of biological data make computational prediction of 3D structure of proteins and their complexes an important alternative to experiments. Computational methods are also indispensable in the analysis or prediction of interaction sites even in the case of experimentally solved structures. However, computational methods have their own challenges. Computational structure prediction works best when related structures (templates) are available. Therefore, the detection of remote homology is one of the major impediments. The reliable estimation of the accuracy of predicted structures is another important problem. More efficient methods for the analysis and prediction of protein binding sites are also badly needed.

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, for comparative protein structure modelling, and for 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 (http://bioinformatics.lt/software).

2) Application of computational methods to biological problems. In this research direction, we have been 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. In addition, 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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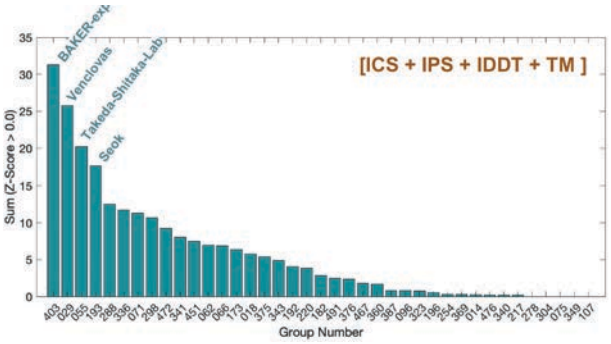
Predicting Structures of Protein Assemblies in Global CASP and CAPRI Experiments

Measuring progress in ability to predict 3D structures of protein complexes is the goal of CAPRI experiments and, lately, one of the major aims of CASP experiments. In summer of 2020, we participated in both CASP14 and CAPRI experiments that were executed in parallel. In both experiments, we tested the performance of our template-based modelling, free docking and hybrid modelling protocols. Among key components of these protocols were the latest versions of PPI3D and VoroMQA methods, developed in our group. The PPI3D web server enables searching, analysing and modelling protein complexes, whereas VoroMQA allows estimation of protein structure quality. Independent assessors found our CAPRI results (group 'Venclovas') to be at the top jointly with the results of the other two groups. According to CASP assessment, our group was second. CASP and CAPRI experiments were performed on different test sets, and assessment methods were somewhat different. Despite slight differences in overall ranking, our group continued to be one of the leading groups in the modelling of protein complexes. The results of the joint CASP14-CAPRI experiment are to be published in a special issue of *Proteins*.

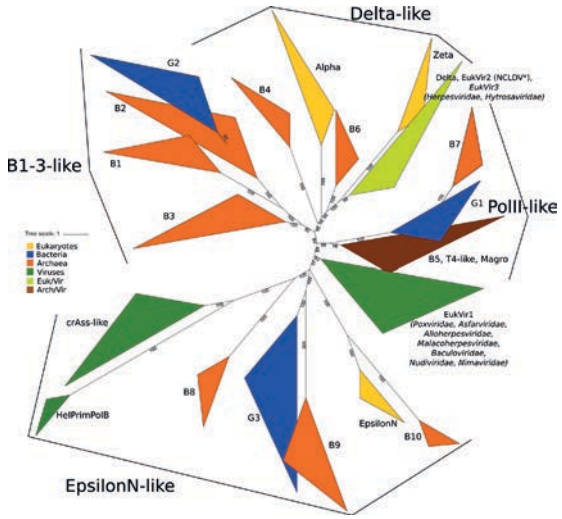
Rank	Group	Performance
1	Seok	9/4**
	Venclovas, *Baker*	8/1***/3**
4	Zou, Chang	8/3**
	MDOCKPP	7/1***/2**
7	Pierce, Kihara	7/3**



Group rankings in modelling of protein assemblies in CAPRI (top), and CASP14 (bottom), presented at the virtual CASP14-CAPRI meeting in Nov 30–Dec 4, 2020.

Uncovering Convoluted Evolutionary History of Human Replicative Polymerases

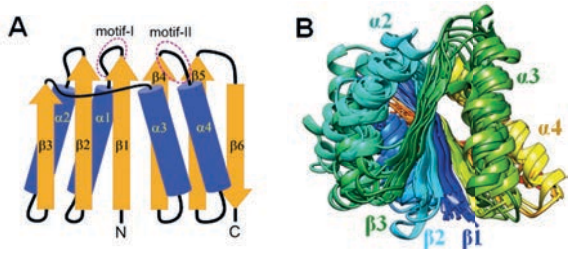
B-family DNA polymerases (PolBs) are the most common replicases, present in all domains of life and in many DNA viruses. Despite extensive research, origins and evolution of PolBs remain enigmatic. To unravel the evolutionary history of PolBs, we performed comprehensive computational analysis of these proteins originating from archaea, bacteria, eukaryotes and viruses. As a result, we defined and characterized six new groups of archaeal PolBs and a new group of bacterial PolBs, which appears to be related to the catalytically active N-terminal module of the eukaryotic Polε. We also uncovered the similarity of the catalytically inactive Polε C-terminal module to Polα. Finally, we discovered that two novel groups of archaeal PolBs have C-terminal metal-binding domains, closely related to those present in eukaryotic Polα and Polε. Collectively, the results of this study allowed us to propose a scenario for the evolution of human and other eukaryotic PolBs.



Phylogenetic tree of B-family DNA polymerases
(Kazlauskas et al. *Nucleic Acids Res*. 2020, 48: 10142–10156).

Surveying CARF and SAVED Proteins, Key Players in Antivirus Defence of Prokaryotes

Proteins possessing CARF and SAVED domains are key components of cyclic oligonucleotide-based antiphage signalling systems (CBASS) that, upon activation, induce cell dormancy or death. Most CARF proteins belong to a CBASS built into type III CRISPR–Cas systems. The CARF domain binds cyclic oligoA (cOA) synthesized by the Cas10 polymerase-cyclase and allosterically activates the effector, typically a promiscuous ribonuclease. Some CARF domains also function as ring nucleases that cleave cOA thereby terminating signal transduction. Due to the extreme sequence divergence and the diversity of domain architectures of CARF domain-containing proteins, CARF domains are often overlooked or misannotated in genome analyses. Therefore, we performed a comprehensive analysis of the CARF and SAVED domains encoded in bacterial and archaeal genomes. Based on this analysis, we proposed a classification of CARF and SAVED proteins, predicted several families of novel ring nucleases, and provided new insights into the organization of the cOA signalling pathway (Makarova et al. *Nucleic Acids Res*. 2020, 48: 8828–8847).



CARF domain structures (A) Topology of the CARF fold (B) Superposition of multiple CARF domain structures coloured by chain progression.

GINTARAS VALINČIUS
Research Professor
Head, Department of Bioelectrochemistry and Biospectroscopy
Institute of Biochemistry
Email: gintaras.valincius@gmc.vu.lt
Phone: +370 675 33278

Protein Structure and Interactions in Phospholipid Membranes

The molecular organisation and function of biological membranes are essential to the 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 MPs arise 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 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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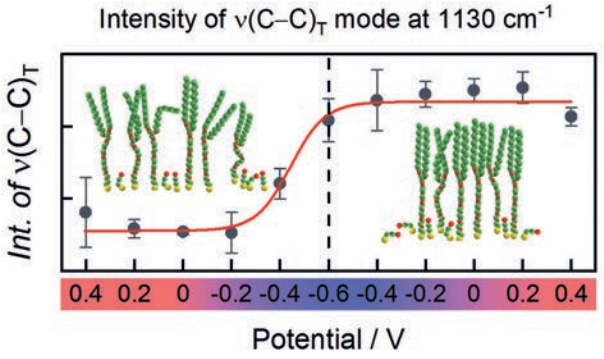
Properties and Function of Tethered Bilayer Membranes

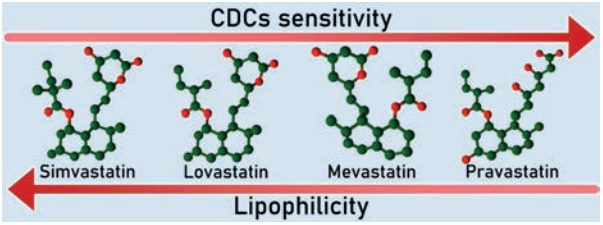
Properties and function of tethered bilayer membranes (tBLM), a biomimetic model of phospholipid membrane, depend on the structure of the self-assembled lipid-like monolayer that anchors tBLM to the surface. Electrode potential that can be adjusted via an external source is a physical variable, which can be used to fine-tune structure and possibly the function of tBLMs. In this project, we explored the potential-induced structural changes in monolayers composed of widely-used in tBLM design lipid-like long-chain WC14 molecules and short-chain hydrophilic backfiller 2 mercaptoethanol by the electrochemical surface-enhanced Raman spectroscopy. To infer the electric potential-induced changes in monolayer, the metal–adsorbate (i.e. Au–S and Au–O) spectral bands and C–C stretching vibrational mode of WC14's alkyl chains in all-trans configuration near 1130 cm-1 were analysed. Negative electric potential promotes the mobility of anchor molecules on the surface by decreasing the strength of metal–adsorbate bonding. At the same time, water pushes the hydrophobic WC14's chains into phase-segregated clusters and in such a way minimizes the system's



energy. The clustering of molecular anchors has a detrimental effect on the integrity of tBLMs and their electrical insulation, therefore we propose the spectral band near 1130 cm-1 as a predictor of the functional properties of tBLMs. In general, our findings explain detrimental effects of negative going electric polarization of tBLMs empirically observed by a number of researchers before. This work was published in Talaikis et al. *J. Phys. Chem. C*. 2020, 124: 19033–19045.

Pleiotropic Effects of Statins via Interaction with the Lipid Bilayers

Statins are selective inhibitors of cholesterol biosynthesis, used worldwide for cholesterol lowering in the primary and secondary prevention of cardiovascular diseases. Clinical trial studies indicated that the observed general benefits of statins appear to be greater than what might be expected from changes just in lipid levels, suggesting effects beyond cholesterol lowering (i.e. pleiotropic effects). Using a combined approach based on biophysical and biological methods, we demonstrate that lipophilic, but not hydrophilic statins are capable of reducing the damage caused by pneumolysin, a toxin of the cholesterol-dependent cytolysins (CDCs) family. This protection correlates with statins' lipophilicity (expressed by the log P value) and capacity to interact with the lipid bilayer. Our data suggest that lipophilic statins associate with membranes and interfere with the ability of CDCs to bind to membrane cholesterol, influencing membrane lipid rafts



organization. The ability to influence membrane lipid structure is one of the reported cholesterol-independent effects of statins. Evaluation of the capacity of statins to modulate membrane properties is an essential step for developing a correct therapeutic approach for cardiovascular diseases as well as for understanding the potential of this class of drugs in cancer therapy to increase tumour response to cytotoxic agents. The work was published in Penkauskas et al. *BBA-Biomembranes*. 2020, 1862(9): 183306.

Clusters of Transmembrane Protein Pores Observed by Electrochemical Impedance Spectroscopy

Cluster formation is a widely-observed phenomenon, which results in unique sets of properties both in physical, chemical, and biological domains of nature. By invoking the Voronoi tessellation concept we demonstrate the possibility to distinguish between random and sparsely clustered patterns both in computer-generated and real-world systems using one single parameter σ, the standard deviation of the normalized Voronoi sector areas distribution. For random systems, σ≈0.54, and for clustered patterns σ>0.54. Because of a specific structure and dielectric properties of tethered bilayers, they can be characterized by an alternating current technique, electrochemical impedance spectroscopy (EIS). EIS measures macroscopic parameters of systems, and it is not a structural technique *per se*. However, we found the EIS spectra-derived quantitative metric ζ to be a diagnostic parameter that allows assessment of the distribution type (homogeneous, random or clustered) of defects at nanometre level. One of the most interesting findings of the current study is the fact that the EIS derived ζ parameter is sensitive to an average size of the defects, thus, enabling a purely electrochemical methodology to access fine structural information such as the size of incomplete protein pores in phospholipid bilayers. Overall, our results demonstrate a fundamental property of the macroscopic technique, electrochemical impedance spectroscopy, to probe structural arrangement of defects with sizes between 0.5 nm to 25.5 nm located in a thin, 2 nm thick phospholipid dielectric layer. This project is a part of collaboration efforts with Dr Tadas Meškauskas' group from the Faculty of Mathematics and Informatics at Vilnius University. It was published in Raila et al. *Electrochimica Acta*, 2020, 364: 137–179.

GINTAUTAS TAMULAITIS
Research Professor
Department of Protein-Nucleic Acids Interactions
Institute of Biotechnology
Email: gintautas.tamulaitis@bti.vu.lt
Phone: +370 5 223 4357

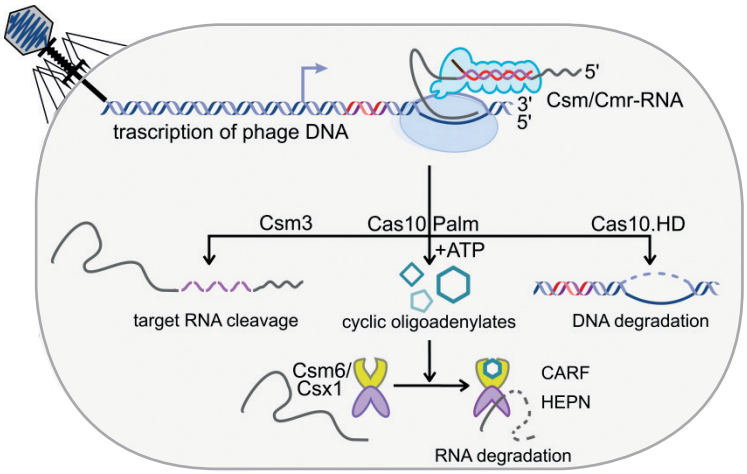


Fig. 1. Type III CRISPR-Cas immunity mechanism.

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 cyclic AMP (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 second messengers for biofilm formation, virulence and regulation of bacterial cell cycle. Recent studies in bacteria have reported that various cyclic oligonucleotides are used as signalling molecules in bacterial antiviral defence systems of Type III CRISPR-Cas and CBASS (cyclic-oligonucleotide-based anti-phage signalling systems). 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] (Fig. 1). 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 (cAn)-dependent immunity pathway [3–5]. In response to the viral RNA binding the Csm/Cmr complex synthesizes cAn molecules of various ring size (n=2–6) [4,5]. The cA4 or cA6 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 [4,5]. Bioinformatics analysis revealed that the sensor CARF and SAVED (a divergent version of CARF) domains are found fused with different enzymatic or non-enzymatic effector domains of Type III CRISPR-Cas associated or CRISPR-Cas unrelated proteins. We aim to understand the molecular and structural mechanisms by which CARF/SAVED domain-containing proteins are involved in bacterial immunity or possibly other cell processes.

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cA_n Synthesis *in Vivo*

We have previously showed that Type III-A Csm complex from *Streptococcus thermophilus* (StCsm) synthesizes *in vitro* cA_ns from ATP. cA_3 was identified as the major reaction product; however, only the minute cA_6 product activated StCsm6 ribonuclease. To get an insight into StCsm6 activation mechanisms in the cell, we examined cA_n metabolites produced *in vivo* in the heterologous *Escherichia coli* host expressing the *S. thermophilus* type III-A CRISPR-Cas system. To monitor amount of the cA_n produced in *E. coli* we employed HPLC-MS technique. HPLC-MS analysis revealed that *in vivo* the StCsm effector complex predominantly produces cA_5 and cA_6 in response to exogenic nucleic acids that derive either from plasmid or phage (Fig. 2) (Smalakyte et al. *Nucleic Acids Research*. 2020, 48(16): 9204–9217). The amount of cA_5 and cA_6 detected in *E. coli* is comparable to the amount of the other second messengers, such as c-di-AMP metabolite present in the *Streptococcus suis* host.

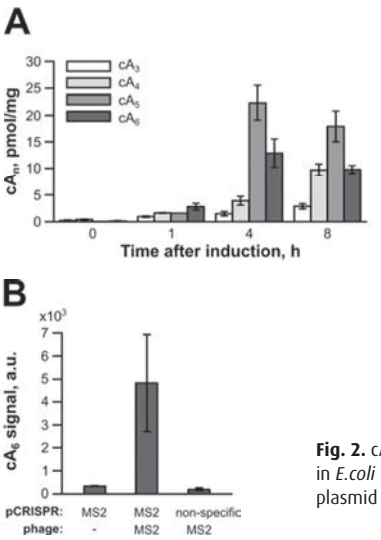


Fig. 2. cA_n production by StCsm in *E.coli* in response to exogenic plasmid (A) or phage (B).

Regulation of cA_6-Signalling Pathway

To avoid host damage the cA_n-signalling pathway must be tightly regulated. Previously we demonstrated that cA_n synthesis is controlled through target RNA degradation by Csm3 subunit of Csm complex. However, the synthesized cA_6 or cA_4 may continue to activate Csm6 nuclease. A family of specialized enzymes called ring nucleases which are composed of a sole CARF domain and degrades cA_4 has been identified in archaea. To explore how cA_6 is degraded in the cell we focused on the well-characterized *S. thermophilus* type III-A CRISPR-Cas system. By dissecting StCsm6 domains we demonstrated that both CARF and HEPN domains act as ring nucleases that degrade cA_ns to switch-off signalling (Fig. 3) (Smalakyte et al. *Nucleic Acids Research*. 2020, 48(16): 9204–9217). CARF ring nuclease converts cA_6 to the A_3>p product. HEPN domain, which typically degrades RNA, also shows ring nuclease activity and indiscriminately degrades cA_6 or other cA_ns down to A>p. We proposed that concerted action of both ring nucleases enables self-regulation of the ribonuclease activity in the HEPN domain and eliminates all cA_n secondary messengers in the cell when viral infection is combated by a coordinated action of Csm complex and the cA_6-activated Csm6 ribonuclease.

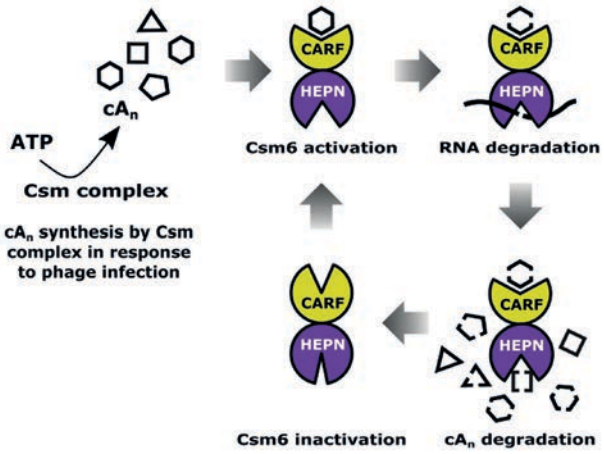
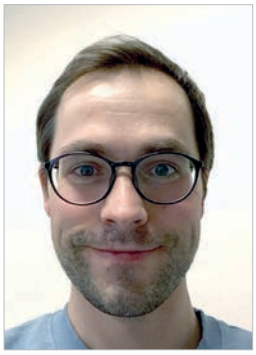


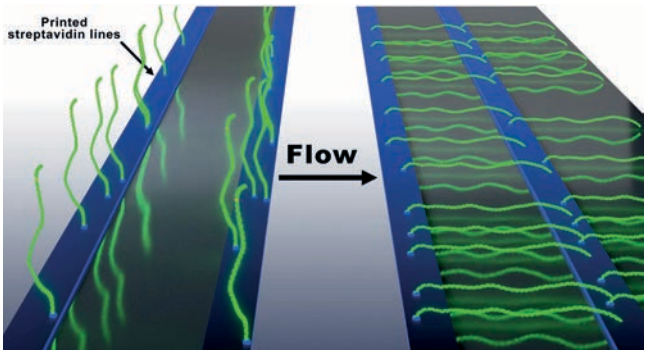
Fig. 3. CARF and HEPN domains of StCsm6 act as ring nucleases in regulation of cA_6-signalling pathway.

RNA Knockdown in Zebrafish Using Csm Ribonuclease

Csm/Cmr complex itself is able to cleave the recognized viral transcripts (Fig. 1). To explore type III-A CRISPR-Cas system as a molecular tool in eukaryotes we have studied the StCsm complex-targeted RNA knockdown in embryonic development of zebrafish in collaboration with Prof. M. Bochtler (International Institute of Molecular and Cell Biology, Warsaw, Poland) (RCL grant No. S-LL-18/07). Targeting and control StCsm complexes were purified as intact ribonucleoproteins from *E. coli* extracts. StCsm injections were administered into the yolk of 1-cell stage embryos, and the phenotype of zebrafish was then monitored at later time points (Fig. 4). We have demonstrated that the StCsm complex specifically silenced both the reporter system and endogenous transcripts that in the embryo are written off in the early stages of its development (Fricke et al. *CRISPR Journal*. 2020, 3(4): 299–313).

Fig. 4. Zebrafish single-eye-phenotype (oep) image observed after injection into 1-cell embryos of oep-targeted wild-type (WT) or ribonuclease-mutant StCsm complexes.





MARIJONAS TUTKUS
Researcher
Department of Protein-Nucleic Acids Interactions
Institute of Biotechnology
Email: marijonas.tutkus@gmc.vu.lt
Phone: +370 627 16095

MINDAUGAS ZAREMBA
Research Professor
Department of Protein-Nucleic Acids Interactions
Institute of Biotechnology
Email: mindaugas.zaremba@bti.vu.lt
Phone: +370 5 223 4357

Single-Molecule Studies of Protein and DNA Interactions

Real-time monitoring of single-proteins on nucleic acid (NA) substrates is an essential tool for studying NA-interacting proteins allowing better mechanistic understanding. To follow individual proteins on a NA molecule, one or both ends of NA molecule that are extended by an external force are attached to the surface. Over the past 20 years, a number of such methods emerged: tethered particle motion (TPM), optical or magnetic tweezers (OTs or MTs), flow-stretch assays and various combinations of these methods. Stretched NAs are a common means to investigate the dynamics of protein-NA interactions. Several experimental strategies are employed to extend and align NA molecules (e.g., NA combing or through hydrodynamic flow). The hydrodynamic flow method allows attaching NA molecules to the glass surface, where they are stretched by a shear hydrodynamic flow. The flow can be applied to extend single-end tethered NA, or deployed to allow the specific double-end tethering. Anchoring the NA onto a lipid bilayer and a diffusion barrier etched on the microscope slide causes the alignment of the NA moving under a buffer flow. This method, known as DNA curtains, allows imaging of many DNA molecules in parallel.

Recently our team developed an alternative assay to the original DNA Curtains that we termed the "Soft" DNA Curtains (LRC grant No. S-MIP-17-59). We fabricated streptavidin patterns (i.e., line-features) on the modified coverslip surface that can be utilized to assemble stably immobilized biotinylated DNA arrays. The application of hydrodynamic buffer flow allows extension of the immobilized DNA molecules along the surface of the flow cell channel. We also fabricated the uniformly oriented double-tethered DNA Curtains using heterologous labelling of the DNA by biotin and digoxigenin. We increased the stability of the immobilized DNA molecules using a more stable alternative to sAv called traptavidin as an ink for the fabrication of protein templates.

The main goal of our research topic is to apply the developed platform for studies of DNA targeting mechanisms of diverse CRISPR-Cas systems family (LRC grant No. S-MIP-20-55), novel molecular-tools – prokaryotic Argonaute (pAgo) proteins (LRC grant No. 09.3.3-LMT-K-712-19-0113) and various restriction endonucleases. Our newest publication on the "Soft" DNA Curtains [1] and its continuation [2] received a broad interest of scientists from various fields.

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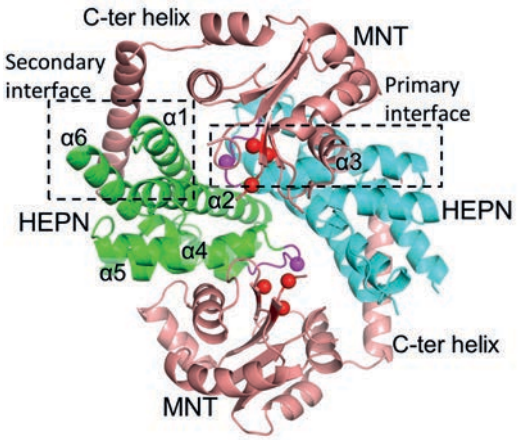

GIEDRIUS SASNAUSKAS
Research Professor
Department of Protein-Nucleic Acids Interactions
Institute of Biotechnology
Email: gsasnaus@ibt.lt
Phone: +370 5 2234443

GIEDRĖ TAMULAITIENĖ
Senior Researcher
Department of Protein-Nucleic Acids Interactions
Institute of Biotechnology
Email: eigie@ibt.lt
Phone: +370 5 2234357

Structural Biochemistry

Protein X-ray crystallography is the primary technique 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 is currently being 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.

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DAUMANTAS MATULIS
Research Professor
Head, Department of
Biothermodynamics and Drug Design
Institute of Biotechnology
Email: daumantas.matulis@bti.vu.lt
Phone: +370 5 223 4364

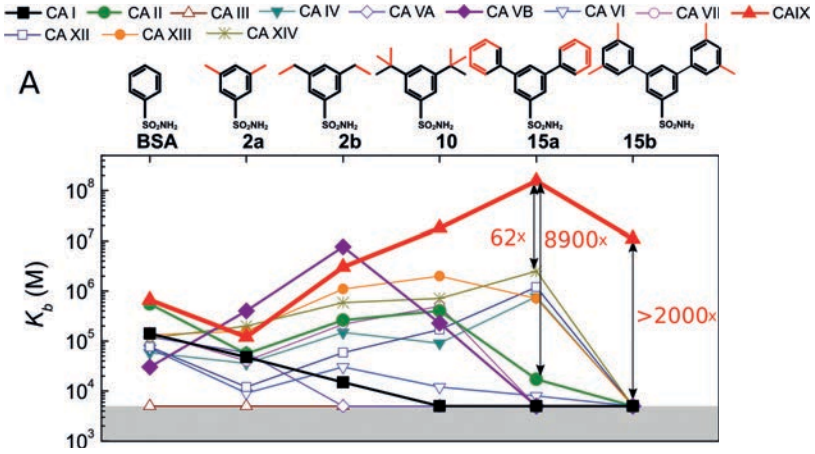

Fig. 1

Structure and Thermodynamics for Drug Design

Rational drug design attempts to discover a ligand that would bind a disease target protein with high affinity and selectivity over unintended targets to avoid toxicity. Despite significant efforts, the underlying physical forces that determine the protein-ligand recognition are still rather poorly understood.

To help design compounds and predict their affinity to target proteins, we are assembling datasets, where chemical compounds binding to proteins would be characterized, including

(a) the X-ray crystallographic structures of protein-ligand complexes,
(b) the thermodynamics of interaction including the enthalpy, entropy, Gibbs energy, volume, heat capacity and other thermodynamic parameter changes upon binding,
(c) the kinetics of the same protein-ligand binding, including the on- and off-rates.

We are primarily focused on the human family of twelve catalytically active carbonic anhydrase isoforms as a disease protein-target. These enzymes have essentially the same fold and a highly similar shape of the active site suitable for the testing of isoform selectivity.

The group of scientists come from various backgrounds including molecular biologists, biochemists, organic chemists, biophysicists, physicists, computer modellers, biologists and pharmacists. Organic synthesis scientists design and perform the synthesis of novel compounds, molecular and cellular biologists perform the cloning, expression (both in bacterial and in human cell cultures) and purification of target proteins, biothermodynamicists determine the energetics of binding between the synthesized compounds and the target proteins by isothermal titration calorimetry or thermal shift and search for structure-energetics correlations, crystallographers determine the X-ray crystallographic structures of protein-compound complexes, *in silico* modellers perform compound docking, and the pharmaceutical scientists perform development studies of the effect of compounds in various biological systems including cancer cell cultures, zebrafish and mice.

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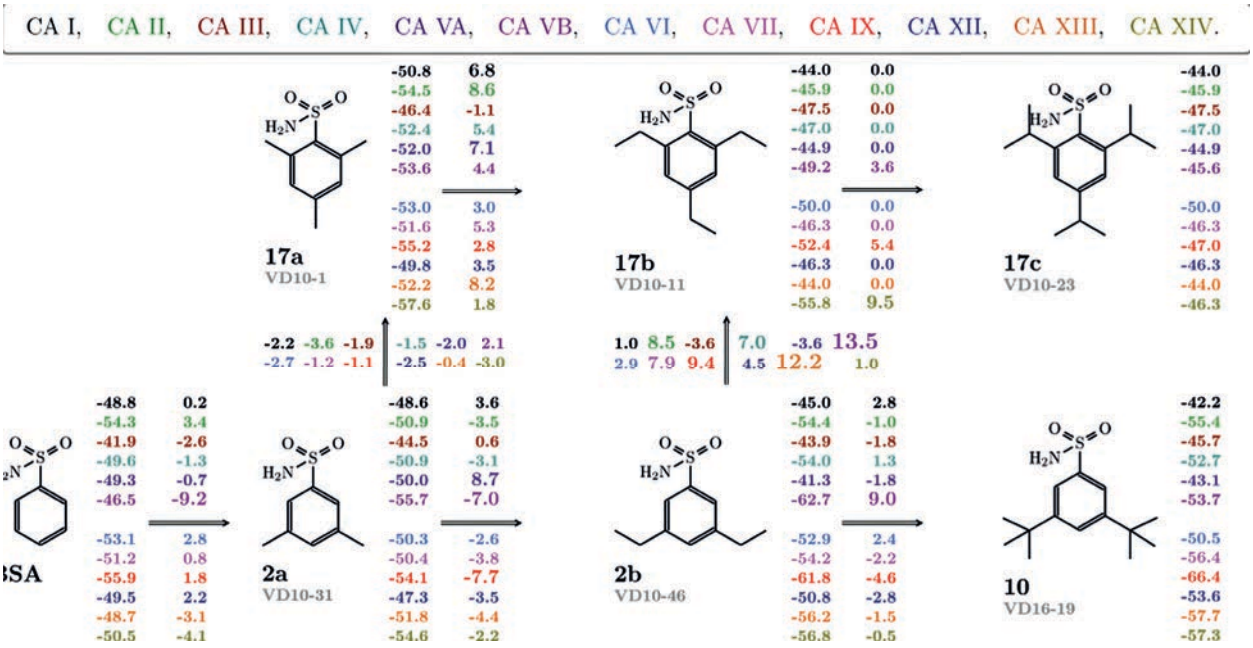

Fig. 2

Compound Binding to Proteins via 'Induced Fit' or 'Lock-and-Key'

Compounds bind to proteins via different mechanisms ranging from 'induced fit' to 'lock-and-key' modes. The induced fit emphasizes the fact that ligands may alter the protein structure and dynamics upon binding. The ligand-bound and ligand-free states of a protein may differ significantly. The lock-and-key principle is applied when the protein is conformationally rigid and does not visually change its structure upon ligand binding. This seems to us to be the case despite variation in the literature and it is important to design ligands that would fit the active site pocket of the disease target protein as closely as possible.

We have designed a series of compounds with enlarged sizes until they could not fit in the active site and the affinity dropped thousand-fold upon addition of a minor substituent such as a methyl group (Fig. 1). A series with a substituted hydrophobic group of increasingly larger size was used. If the protein is rigid, there ought to be a limiting size after which the compound cannot fit into the binding site. This size limit should vary for every isoform and could be employed to search for isoform-selective inhibitors. Additionally, the size of the ligand would prevent it from binding to isoforms that are of vital importance and should not be targeted.

The map of correlations between the compound chemical structures and the intrinsic standard Gibbs energy changes upon compound binding to all 12 CA isoforms shows the energies next to the chemical structures (Fig. 2). Differences in the energies upon binding comparing chemically similar compounds are shown next to the arrows connecting the compounds. Colours represent the 12 catalytically active human CA isoforms.

X-ray crystallographic analysis of several compounds bound to CA II, CA IX and CA XII essentially confirmed that the most successful ligands nearly completely filled the binding site. X-ray crystallography confirmed that the selectivity of **15a** towards CA IX was due to the optimal size of the compound and the binding site, e.g. due to the decreased size of residue 131, which has changed from Phe in many CA isoforms to Val in CA IX.

Docking of all compounds to all CA isoforms was employed to test the extent to which we could predict the structural positions and the binding energetic (Fig. 3). The docking calculations were able to correctly reproduce the binding modes of larger ligands, such as **24**, **10**, and **15a**, with mixed success in reproducing the binding modes of the smaller ligands. For the important CA IX and CA XII isoforms, the docking essentially confirmed conclusions drawn from the X-ray structures. Thus, it has a predictive capability that can be used to design better drugs.

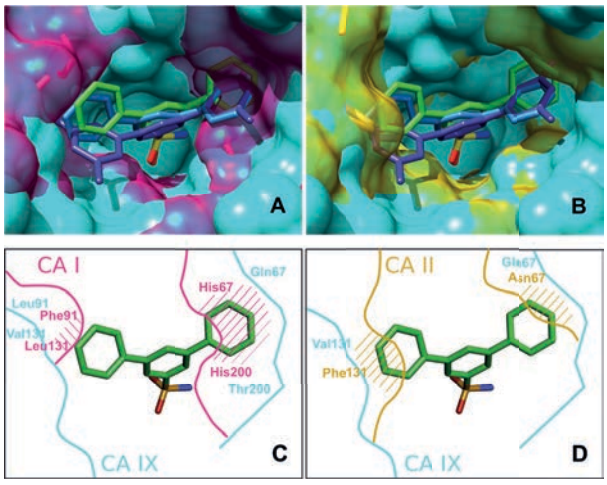

Fig. 3

NOMEDA KUISIENĖ
Professor
Department of Microbiology and Biotechnology
Institute of Biosciences
Email: nomeda.kuisiene@gf.vu.lt
Phone: +370 652 00495

Applications of the 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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Biosynthesis Genes of Bioactive Compounds: Evaluation of Diversity and Expression Analysis in the Unique Environment

The identification of novel compounds with antibacterial, antifungal, anticancer, antiviral, antidiabetic, antiprotozoal and other bioactivities represents an important field of modern biomedical research. Microorganisms are the main targets in this research because of their high potential to produce these bioactive compounds. Bioactive compounds can be difficult to identify phenotypically because of a few reasons: the amount of these compounds can be beyond the detection limits; certain experimental conditions can be inappropriate for the induction of the biosynthesis of these compounds; the coding genes of bioactive compounds can be silent etc. The problem can be solved, and the real potential of bioactivity can be determined through the analysis of biosynthesis genes and not via that of the bioactive compounds themselves. The aim of the current project is to reveal the diversity and prevalence of bioactive compound biosynthesis genes in the bacteria of the deepest cave of the Earth, the Krubera-Voronja Cave. Polyketide synthase, nonribosomal peptide synthetase and bacteriocin biosynthesis genes were under investigation in this project (Lukoseviciute et al. *Microbial Ecology*. 2020; Bukelskis et al. *Frontiers in Microbiology.* 2019).

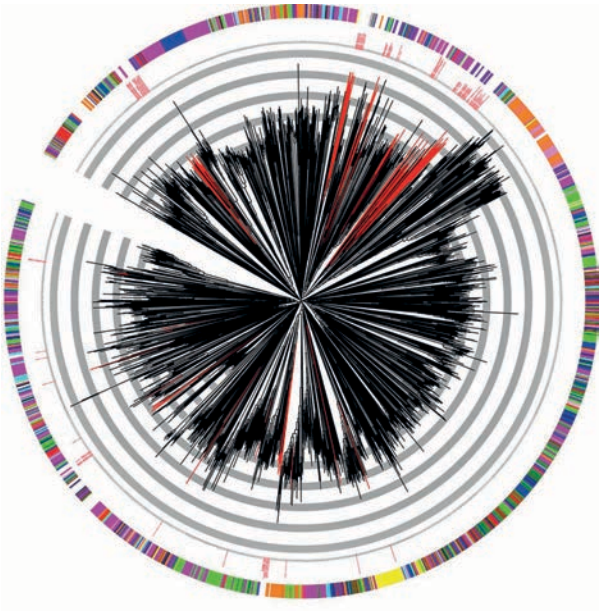

Fig. 1. Phylogenetic diversity of the adenylation domains of the nonribosomal peptide synthetases in the metagenome of Krubera-Voronja Cave.

Identification, Expression and Characterization of Bacterial Collagen-Like Proteins

During the last decade, a large number of collagen-like proteins have been identified in bacteria mainly through an *in silico* analysis. Only a few bacterial collagen-like proteins have been expressed in *Escherichia coli*. It was shown that these recombinant bacterial proteins adopt a classical triple-helix conformation and exhibit high thermal stability. The amino acid composition of bacterial collagen-like proteins varies from species to species, and from protein to protein, conferring the different characteristics to these proteins. Collagen-like proteins can be produced in large quantities by recombinant methods, and the construction of proteins with the desirable characteristics can also be carried out. Therefore, bacterial collagen-like proteins represent an excellent source for the design of new biomaterials with the desirable structural properties and functions. The identification, expression and characterization of bacterial collagen-like proteins represent a highly attractive and important area of research work in the fields of regenerative medicine and biotechnology (Kananavičiūtė et. al. *Genomics*. 2020).

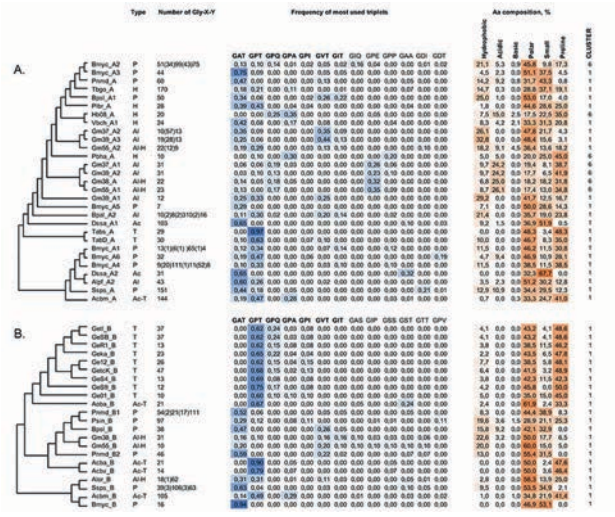

Fig. 2. Collagen-like sequence proteins having BclA_C (A) and Exospore_TM domains (B).

Molecular Epidemiology of Pathogenic Bacteria in Lithuanian Healthcare Institutions

Bacterial resistance to antimicrobial agents plays an important role in healthcare institutions nowadays. Spread of multidrug resistant bacteria can occur during inter- and intra-hospital transmissions among patients and hospital personnel. One of the highest rates of resistance in healthcare institutions is observed in *Acinetobacter spp*. isolates, which causes outbreaks around the world and is highly adaptable to changes both in the environment and in the use of antibiotics. These characteristics lead to a high rate of occurrence of multidrug-resistant *Acinetobacter spp*. cases in the environment.

Methods of molecular epidemiology, such as virulence factors determination, resistance genes distribution and genotyping, are used to better understand the antimicrobial resistance patterns of *Acinetobacter spp*. and can be used to strengthen the control of multidrug resistant infections in healthcare institutions and to prevent potential outbreaks of this pathogen in the future (Kirtikliene et al. *Microbial Drug Resistance*. 2019; Tratulyte et al. *European Journal of Clinical Microbiology & Infectious Diseases*. 2019).

LILIJA KALĖDIENĖ
Professor
Head, Department of Microbiology and Biotechnology
Institute of Biosciences
Email: lilija.kalediene@gf.vu.lt
Phone: +370 5 239 8205

Applied and Environmental Microbiology

Although the potential for 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 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. If the enzyme is immobilized properly, it can be considered as a special type of formulation of its properties such as activity, stability, selectivity, purity, and others. Therefore, it is important to examine new types of immobilization sorbents. 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 coumpounds 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 research 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.

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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Microbial biofilms are widespread in the environment and form on biotic and abiotic surfaces if constant moisture is present. 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 mostly originating from life-supporting devices, equipment collected in pre-flight conditions and crewmembers. Resilient biofilms pose a higher risk to crewmembers' health and the material integrity of the spacecraft than planktonic cells. Moreover, biofilms in space conditions are characterized by faster formation and acquisition of resistance to chemical and physical control methods than under the same conditions on Earth, making most decontamination methods unsafe. Thus, biofilm control methods that are safe for confined, closed-loop systems such as spacecraft are of high demand. Visible-light irradiation technology - antimicrobial photoinactivation (API) based on natural PSs such as riboflavin (RF) and chlorophyllin - are being developed by the research group, and the technology shows promising results for its use in the mentioned area (confined, closed-loop facilities such as spacecraft and others). API belongs to a multitarget process; therefore, bacteria do not develop resistance, moreover, the process exhibits a very rapid microbial killing.

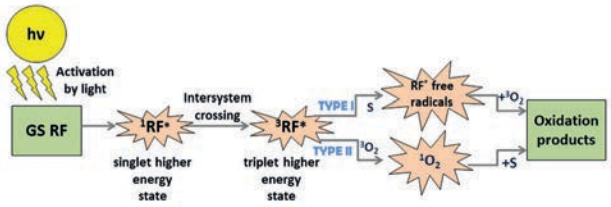


Fig. 1. Example of natural PSs-based API mechanism; upon activation by light, RF is excited to a singlet state of higher energy ($^1RF^*$), followed by intersystem crossing to an excited triplet state ($^3RF^*$). $^3RF^*$ can be involved in a photosensitized oxidation process of two types. Type I: RF transfers the energy to a substrate (S) and generates RF^* free radicals interacting with molecular oxygen in the ground state (GS) to yield oxidation products. Type II: RF transfers the energy to molecular oxygen in the ground state to generate the more reactive $^1O_2$. The process leads to the final products harmful to microbes.

Biocatalysts exerting activity against ester bonds have a broad range of applications in modern biotechnology. Some of the most industrially relevant enzymes of this type are lipolytic. A novel bacterial hormone-sensitive lipase-like (bHSL) family homologue, designated EstAG1, was discovered by mining gDNA of bacteria isolated from fat contaminated soil. EstAG1 was hyperactivated by organic solvents implicating that it could be an industrially applicable enzyme for the organic synthesis of valuable products such as biodiesel, flavour and aroma esters etc. Based on low EstAG1 amino acid sequence identities to closest homologues, by unique catalytic amino acid peculiarities in the sequence and phylogenetic analysis the enzyme belongs to a new family of bacterial lipolytic enzymes.

Fig. 2. Application possibilities of bacterial lipolytic enzymes.

The genome of the thermophilic bacterium, *Aeribacillus pallidus* 8, encodes the bacteriocin pallidocin belonging to a small class of glycocins and is posttranslationally modified by S-linked glucose on a specific Cys residue. In this study, the pallidocin biosynthetic machinery was expressed in *E. coli* to achieve its biosynthesis and modification. The characterized biosynthetic machinery was employed to produce two other glycopeptides Hyp1 and Hyp2. Heterologous expression of a glycocin biosynthetic gene cluster with S-glycosyltransferase provides a good tool for production of hypothetical glycocins encoded by various bacterial genomes and allows rapid in vivo screening.

Moreover, geobacillin 26 from *Geobacillus stearothermophilus* 15 was thoroughly investigated. Our study suggests that this bacteriocin is not a cell wall hydrolyser as most of high molecular weight bacteriocins and has no amino acid sequence similarities to other known function proteins. No other class III bacteriocin from a thermophilic bacterium has been reported and well characterized before.

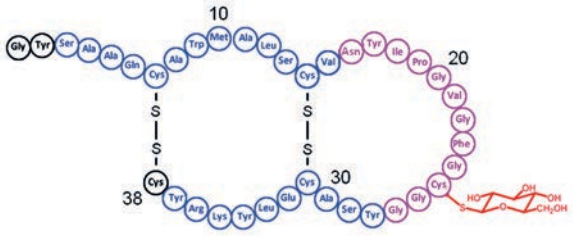


Fig. 3. Proposed structure of pallidocin. The α-helical structure shown in blue, coil structure in purple.

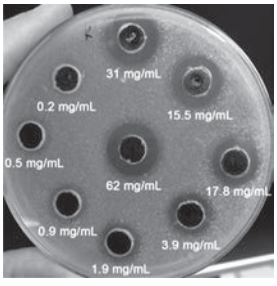
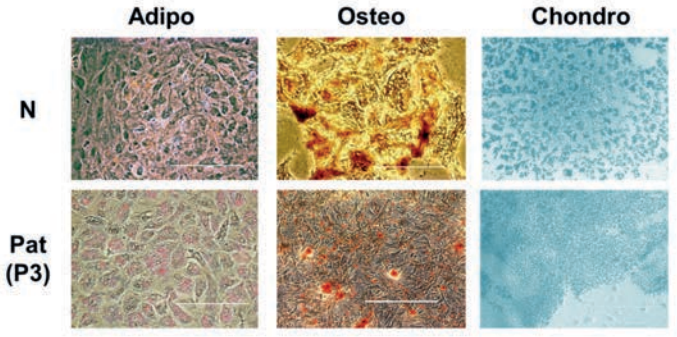


Fig. 4. Antibacterial activity of Geo26-His. NB-agar medium inoculated with sensitive strain P. genomospecies1 NUB36187.



RŪTA NAVAKAUSKIENĖ
Research Professor
Head, Department of Molecular Cell Biology
Institute of Biochemistry
Email: ruta.navakauskiene@bchi.vu.lt
Phone: +370 5 223 4409

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.

Human amniotic fluid-derived stem cells (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 study we investigated the differentiation potential and assessed epigenetic factors involved in tissue-specific differentiation.

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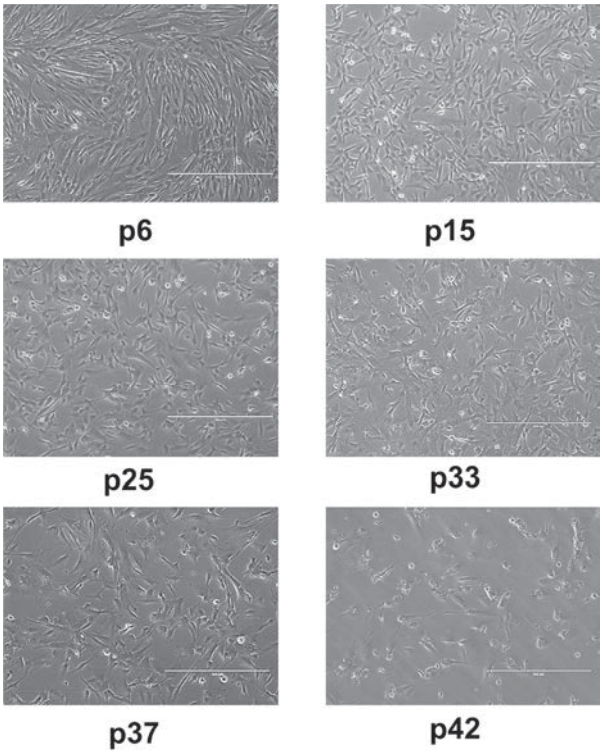
The Impact of AFSCs on Long-Term Cultivation Capacity and Differentiation Potential

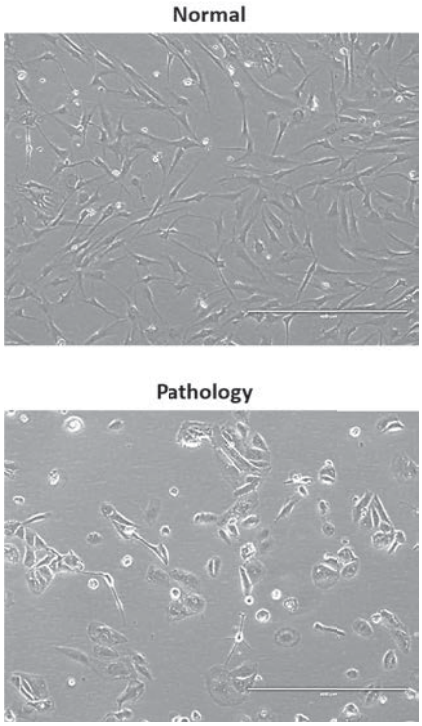
It is crucial to investigate whether the main characteristics of SCs are stably maintained during the long-term cultivation *in vitro* and whether the passage number of AFSCs culture can have an impact on the future clinical applications. Therefore, we examined the proliferation, differentiation, death and senescence of amniotic-fluid derived stem cells during the long-term culturing *in vitro*. AFSCs were expanded up to 42 passages and maintained their stemness and mesenchymal characteristics. Alterations in morphology, the expression of pluripotency genes and cell surface markers concomitant with senescence initiation as well as reduced cardiomyogenic differentiation potential were detected at the late passages. These results provide useful insights into the potential use of AFSCs for bio-banking and universal applications requiring large amounts of cells or repeated infusions (Gasiūnienė, Valatkaitė & Navakauskienė, 2020).



AFSCs morphology from different passages of long-term cultivation

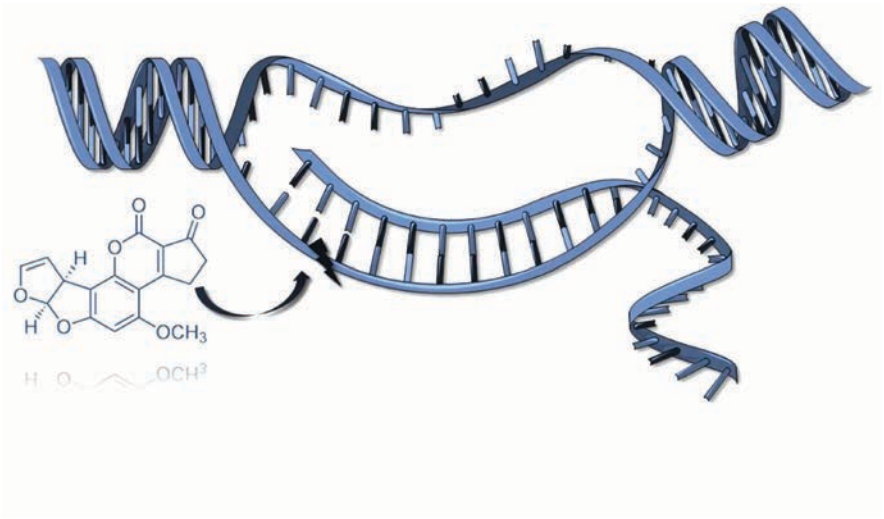
Metabolic and Neurogenic Potential of AFSCs from Normal vs Foetus Affected Gestations

Although AFSCs are widely researched, their analysis mainly involves SCs obtained from normal foetus unaffected gestations. However, in clinical setting, the knowledge about AFSCs from normal gestations would be poorly translational, as AFSCs from normal and foetus diseased gestations may differ in their differentiation and metabolic potential. Therefore, in this study the metabolic, neurogenic and neurotrophic potential of AFSCs, obtained from foetus affected gestations with polyhydramnios, in comparison with foetus unaffected gestations, were investigated. Results demonstrated that these cells are similar in gene expression levels of stemness markers. However, they do differ in expression of certain cell surface markers. In addition, AFSCs from "Normal" and "Pathology" groups were found to be different in oxidative phosphorylation rate, as well as in the level of ATP and reactive oxygen species production. AFSCs from normal gestations were found to be more prone to neurogenic differentiation. Overall, these observations provide supplementary insights into the neurogenic potential of AFSCs obtained from foetus unaffected vs foetus affected gestations (VU funded research No. MSF-LMT-3/2020).



Morphology of AFSCs obtained from healthy and foetus diseased gestations upon neural differentiation induction

JUOZAS LAZUTKA
Professor
Head, Department of Botany and Genetics
Institute of Biosciences
Email: juozas.lazutka@gf.vu.lt
Phone: +370 5 239 8257



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. 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. Such knowledge is especially relevant to ultrashort UV pulses, because radiation in the UV range has the greatest DNA-damaging potential.

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. In collaboration with a large international group of researchers, 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. In collaboration with industrial partners (Light Conversion Ltd., CC *Akių Gydytojų Praktika*), 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 such a parameter as beam delivery to the target was also revealed.

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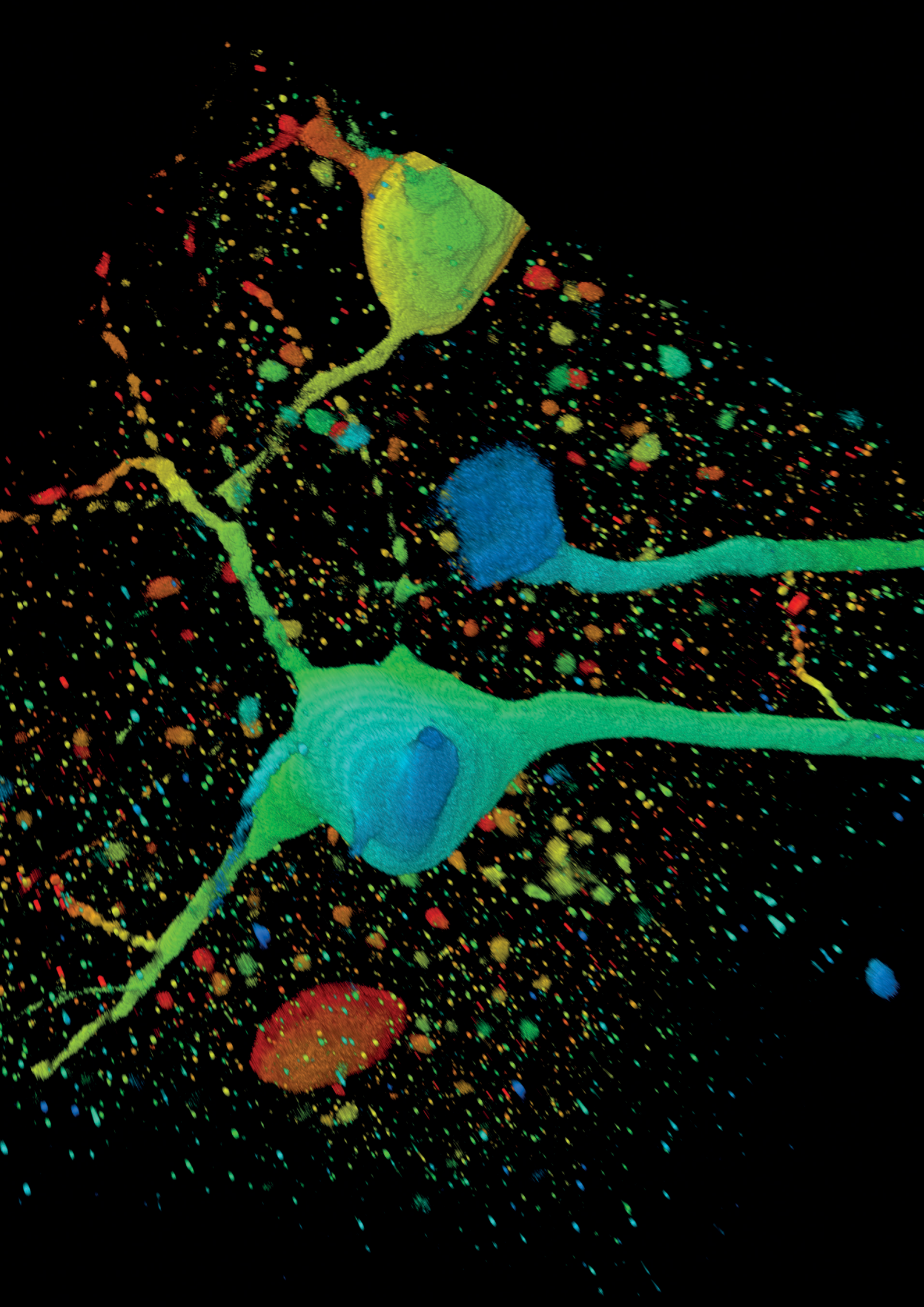
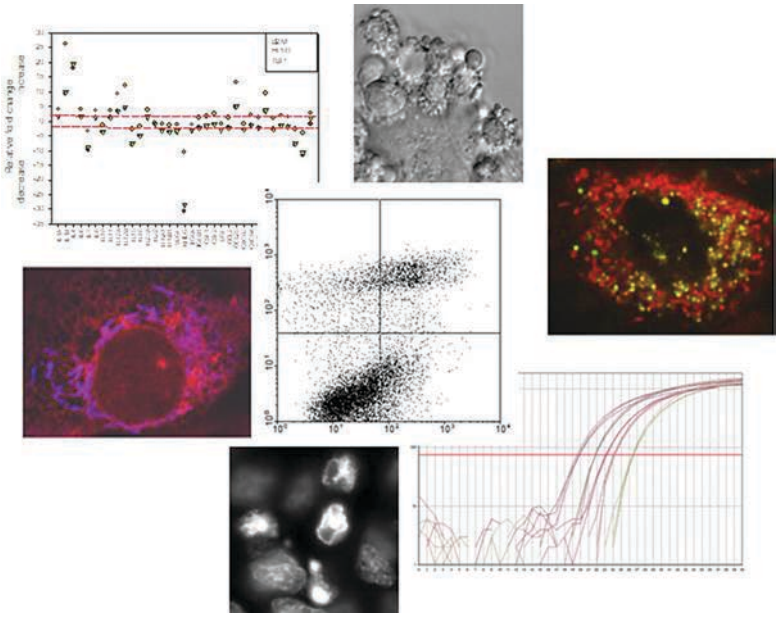


AUŠRA SASNAUSKIENĖ
Associate Professor
Department of Biochemistry and Molecular Biology
Institute of Biosciences
Email: ausra.sadauskaite@gf.vu.lt
Phone: +370 5 239 8225



Molecular Mechanisms of Cell Death and Survival

Acquired chemoresistance is a major limitation for successful anti-cancer therapy. Some mechanisms of cell chemoresistance are well known: alterations in drug transport and metabolism, modification of drug targets, activation of DNA repair or changes in apoptosis induction. Deeper understanding of chemoresistant cell physiology, in particular cell survival signalling, autophagy and cell death pathways, suggests new possible targets to overcome chemoresistance. We focus our research on molecular mechanisms responsible for cellular chemoresistance of colorectal cancer cells, in particular on the alterations in pathways of cell survival signalling and autophagy. The latter may have both cancer-promoting and cancer-suppressing effects. One focus of our group is to reveal the changes in autophagic machinery in chemoresistant vs chemosensitive cells.

Inflammation and antitumor immunity are important determinants of colorectal cancer progression; it is mediated by cytokine signalling. We have demonstrated that cell stimulation with exogenous interleukin-1 alpha (IL-1α) increased 5-fluorouracil (5-FU) cytotoxicity in both chemosensitive and chemoresistant colorectal cancer cell lines [1]. It was the result of increased cell death, and not of cell cycle arrest. 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.

We have determined that interleukin-8 (IL-8) and its receptor CXCR2 are upregulated in the chemoresistant colorectal cancer cells. However, chemoresistant cells remained sensitive to blockade of the CXCR2 pathway; it reduced the cell number. IL-1α alpha was found to stimulate production of IL-8 [2].

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.

In 2020, the researchers of our group together with the scientists from the Institute of Biochemistry participated in two projects: 1) evaluation of cytotoxicity of aromatic nitrocompounds and N-oxides (DOTSUT-34/09.33-LMT-K712-01-0058), 2) elucidation of the mechanisms of bacteriophage-derived nanotube entry to colorectal cancer cells (S-SEN 20-4). We have also implemented a project (MSF-JM-2/2020) with researchers from the Institute of Biomedical Sciences of the Faculty of Medicine, dedicated to molecular mechanisms of pathogenesis of rare genetic diseases. During the year 2020, an invited review article concerning the changes in Notch signalling pathway in endometrial cancer was prepared [4].

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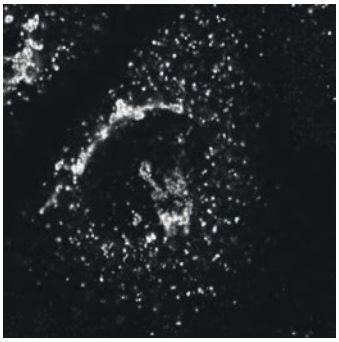

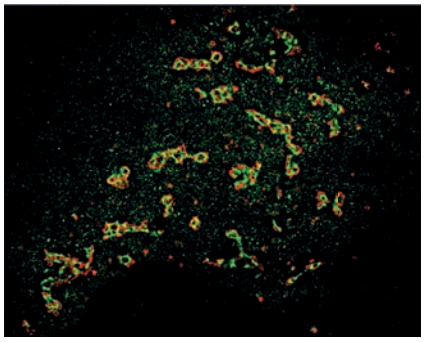

VYTAUTĖ STARKUVIENĖ-ERFLE
Professor
Institute of Biosciences
Email: vytaute.starkuviene-erfle@gmc.vu.lt
Phone: +49 176 3431 0625

Molecular Mechanisms of Intracellular Trafficking

Intracellular trafficking distributes newly synthesized and endocytosed material to diverse cellular destinations and, by doing so, ensures cellular homeostasis. The functionality of the secretory trafficking and endocytosis are regulated in a highly complex manner with hundreds of molecular machineries and multiple pathways acting simultaneously. A precise coordination of transport carriers' formation, directionality of their movement, fusion to the acceptor membranes and the morphology of intracellular organelles is tightly regulated in a temporal and spatial manner. Trafficking is also closely linked to multitude of other cellular processes: autophagy, cell death, regulation of transcription and translation. Deregulation of cargo trafficking leads to ever-increasing list of such diseases as cancer, cardio-vascular or neurodegenerative ones. Regulation of trafficking as a cellular response function to changes in the surrounding is little understood, and I am especially interested to understand this relation in detail.

To dissect the complexity of trafficking and signalling pathways, we use fluorescent microscopy-based assays, cell biology and biochemistry techniques to identify novel intracellular and extracellular regulators, inter-connections among them. We modify gene, transcript and protein expression function by geneediting, RNA interference and antibody-mediated approaches, respectively. We develop techniques to perform these experiments in highresolution and on a largescale, thereby, extracting high-content information from varying biological scales.

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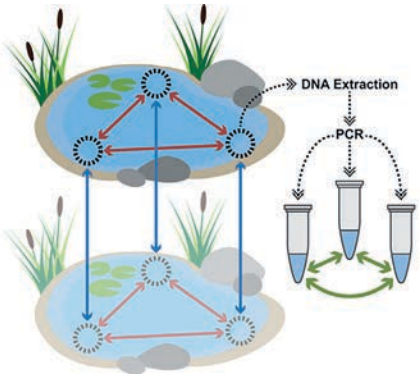

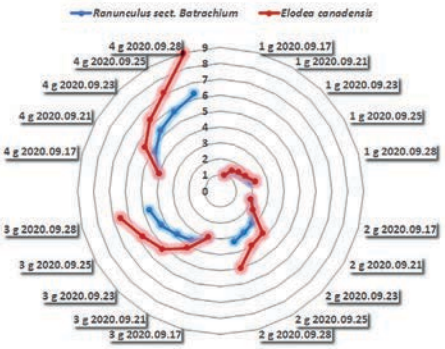

DONATAS ŽVINGILA
Professor
Department of Botany and Genetics
Institute of Biosciences
Email: donatas.zvingila@gf.vu.lt
Phone: +370 5 239 8252

Plant Polymorphism, Genome Stability and Its Changing Factors

Plants as model systems are widely used in molecular-genetic, developmental and environmental studies. The progress of molecular marker techniques and the sequencing of the Arabidopsis genome began the era of plant genomics. 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. Study of lines derived from different cross-combinations confirmed the triggering effect of *tweaky* mutations on the induction of genetic instability, which may occur due to pleiotropic auxin action in *tw* mutants on the expression of genes related to developmental processes [1]. Another aspect of our investigation concerns plant evolution and ecology with particular interest on the phenomena of invasiveness and hybridization. Our study confirmed the hybridogenic origin of distinctive *Batrachium* genotypes [2] and the role of the multiple introductions on the invasiveness of *Bunias orientalis* in two climatically different zones [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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Genetic Diversity of Aquatic *Ranunculus* (*Batrachium*, *Ranunculaceae*) in One River Basin Caused by Hybridization

Aquatic *Ranunculus* (sect. *Batrachium*) include both homophyllous and heterophyllous plants. The development of floating leaves may be induced by genetic mechanisms or/and environmental conditions, and this fact complicates the morphologically based identification of species. DNA-based studies provide the opportunity to expand the knowledge of this complicated group. We studied heterophyllous *Ranunculus* with well-developed capillary and intermediate leaves and visually homophyllous plants with capillary leaves with the aim to evaluate their genetic polymorphism and taxonomic status: whether the plants with well-developed and weakly expressed intermediate leaves belong to different forms (taxa) or they just express morphological variation of one or two taxa in a specific, highly variable river environment. The molecular analysis did not reveal any inter simple sequence repeat (ISSR) polymorphism associated with the development of intermediate leaves. Analysis of nuclear ribosomal internal transcribed spacers ITS1–2 sequences revealed several ribotypes, which indicated the genetic heterogeneity of the plants studied and indirectly confirmed

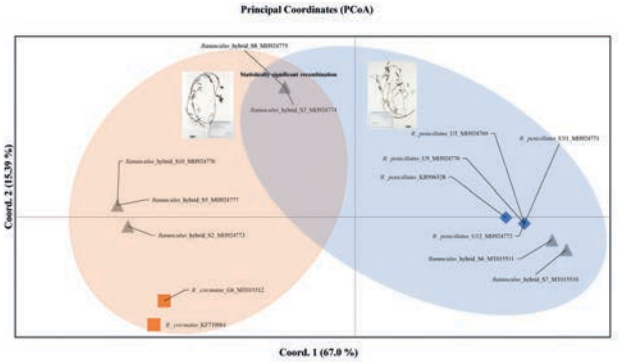

Fig. 1. Principal coordinate analysis of supposed parental species (*R. circinatus* (orange) and *R. penicillatus* (blue) and *Ranunculus* hybrid from the Skroblus River (grey).

the hybrid origin of some of them. Hybrid sterile plants between *R. circinatus* and *R. penicillatus* were discovered in the Skroblus River; however, identification of the parental species was impeded by the polymorphism detected. For this reason, cytological studies were performed and allowed confirmation of this hybrid (Butkuvienė et al. *Plants.* 2020, 9: 1455).

Genetic Structure of Lithuanian *Nuphar lutea* River Populations

Currently, in Europe, increasing attention is being paid to the genetic diversity of aquatic macrophytes. Insufficient information exists about the river plants of the Baltic States. Our study aimed to evaluate the genetic diversity of *Nuphar lutea* individuals growing in the Lithuanian watercourses. The populations were studied in the river catchments of Lithuania: the Nemunas, the Venta and the Lielupė. The genetic structure of the populations was evaluated at microsatellite loci. The population genetic data of *N. lutea* were analysed by multiple tests, including hierarchical analysis of molecular variance (AMOVA), principal coordinate analysis (PCoA) and the Mantel test. The observed (H_0) and expected (H_E) heterozygosity values per population were at the corresponding intervals: 0.242–0.655 and 0.503–0.759. Our study revealed significant differentiation among populations (F_{ST}=0.162; p<0.001) and a lack of correlation between genetic and geographical distances; this outcome is in agreement with increased inbreeding in populations and implies limited gene flow among subcatchments. Our results also indicate that land-use type in the areas surrounding the river may have an effect on the genetic diversity pattern of *N. lutea* populations (Vyšniauskienė et al. *Aquat. Bot.* 2020, 131: 103173).

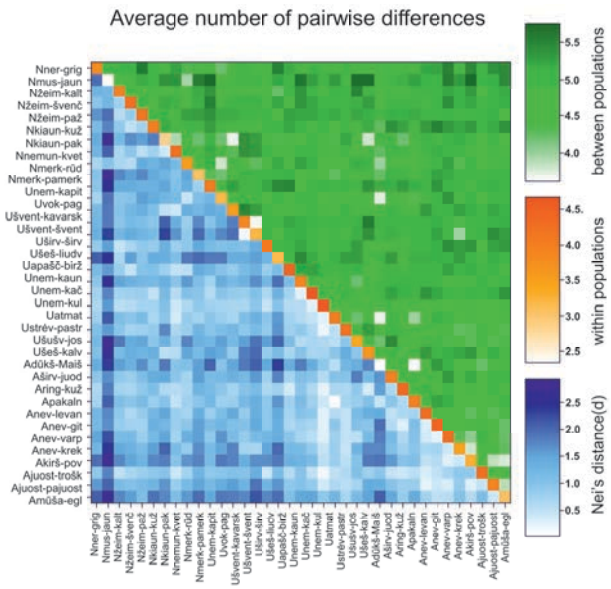

Fig. 2. Genetic differentiation between Lithuanian populations of *Nuphar lutea* based on nuclear microsatellite data and revealed using Arlequin v3.5.2.2.

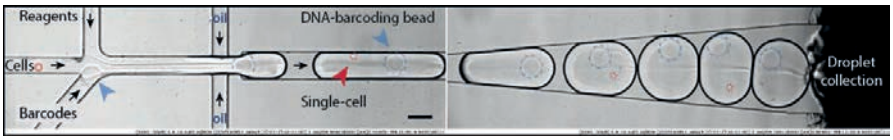

Fig. 1. The principle of *inDrops* technique. Digital micrographs of cell encapsulation together with hydrogel beads and reagents. Scale bars, 100 µm. Cell loading into droplets with hydrogel beads and assay reagents occurs at the flow-focusing junction. Hydrogel bead ferries ssDNA primers attached to hydrogel polymer mesh via UV light-sensitive bond.

LINAS MAŽUTIS
Research Professor
Research ID: A-5408-2012
Division of Microtechnologies
Institute of Biotechnology
Email: linas.mazutis@bti.vu.lt
Phone: +370 693 19333

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 (Dr A. Klein), we have studied the pluripotency of mouse embryonic cells [1], the T-cell activation in tumours [2] and the osteoblast role in lung adenocarcinoma [3], all single-cell level. In collaboration with Memorial Sloan Kettering Cancer Center and Columbia University (Dr. D. Pe'er), we have also shown that the T-cell exhibits a continuum of activated states to fight breast cancer [4] and, in a separate study, we have developed computational tools for recovering gene dropouts that are persistent in scRNA-Seq data [5].

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Screening and Isolation of B-cells Producing Therapeutic Antibodies

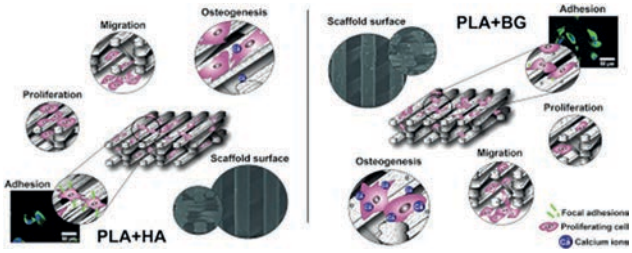
Monoclonal antibodies constitute important tools for scientific research and are the basis of numerous successful therapeutics. However, traditional approaches to generate monoclonal antibodies against a desired target, such as hybridoma-based techniques and display library methods, are laborious and, due to fusion inefficiency and display bias, respectively, are unable to sample the entire antibody repertoire. We are working on a new platform to rapidly generate recombinant monoclonal antibodies. We use droplet microfluidics and a bead-based binding assay to directly identify and isolate individual rare cells that secrete target-binding antibody from a primary cell population with high throughput, screening more than one million cells per day. We then perform RT-PCR on individual sorted cells to recover the correctly paired heavy- and light-chain antibody sequences. We verify target-specific binding using ELISA. Our platform can facilitate rapid screening of an animal's IgG-secreting cell repertoire to generate antigen specific recombinant antibodies and can also be adapted to isolate cells based on virtually any secreted product.

Transcriptional Profiling of Tumour

Tissue homeostasis is maintained by stem cells, whereas damaged tissues are repaired by facultative progenitors that are activated upon injury. The role of developmental plasticity in tumour progression and metastasis remains poorly understood and the extent to which tumour cells subvert regenerative processes during metastatic progression is unknown. In collaboration with biomedical and computational scientists, we applied *inDrops* technology to explore tumour cell heterogeneity through in lung cancer metastasis and to assess tumour cell plasticity. Using patient tumours as well as a mouse model of lung cancer metastasis, we identified the regenerative cell types and lineage promiscuity in untreated primary tumours and revealed a range of embryonic lung morphogenic states in metastases. We demonstrate an unexpected, developmental stage-specific differential sensitivity to natural killer cells that shapes the phenotypic landscape of latent metastasis-initiating cells.

Hydrogel Capsules for Single-Cell Multi-Step Processing

Droplet microfluidics technology provides a powerful approach to isolate and process millions of single cells simultaneously. However, multi-step reaction, including molecular biology and cell-based phenotypic screening assays, cannot be easily adapted to droplet format. To circumvent these limitations, we combined advantages offered by droplet-based and hydrogel-based systems to create capsules containing a thin, semi-permeable shell. The shell acts as a passive sieve retaining encapsulated, large molecular weight compounds while allowing smaller molecules (such as proteins) to diffuse through. We used an aqueous two-phase system (ATPS) composed of dextran and acrylate-modified polyethylene glycol to generate the biocompatible hydrogel particles and showcased a few examples of sequential reactions on encapsulated species. Specifically, we compared genome amplification reaction efficiency on Gram-negative and Gram-positive bacterial cells and found that the DNA amplification yields tend to be higher in the capsule-based system. Capsules readily sustained multiple pipetting steps when performing complex biochemical reactions and, in contrast to solid-hydrogel beads, retained a significantly larger fraction of encapsulated bacteria.



VIRGINIJA BUKELSKIENĖ
Senior Researcher
Head, Department of Biological Models
Institute of Biochemistry
Email: virginija.bukelskiene@bchi.vu.lt
Phone: +370 5 223 4408

DAIVA BALTRIUKIENĖ
Senior Researcher
Department of Biological Models
Institute of Biochemistry
Email: daiva.baltriukiene@bchi.vu.lt
Phone: +370 5 223 4379

Stem Cell Technologies for Bone Tissue Engineering

Tissue engineering or the fabrication of artificial tissue is a promising field of regenerative medicine, which meets a lot of scientific and technological challenges. Artificial tissues could be produced by using different biofabrication techniques, which depend on the specifics of the tissue being created.

Fabrication of an artificial biocompatible, osteoconductive and osteoinductive bone graft using special scaffold still remains a major issue in bone tissue engineering approaches. It is obvious that the newly produced bone graft should not only stimulate cells to regenerate damaged bone tissue, it also should mimic patient-specific bone defect morphology and should be low in price and high in production speed. One of the most important factors for constructing an artificial bone tissue is the morphology of the bone scaffold. It is known that the material and topography of the scaffolds have an impact on cell focal adhesions (FA) formation, which in turn modifies cell shape and morphology, leading to various signalling pathways activation and eventually influencing cell adhesion, proliferation, and differentiation.

We have previously demonstrated that low-cost 3D printed polylactic acid (PLA) macro structures (larger than cell diameter) without additional surface modifications could promote spontaneous stem cell osteogenic differentiation. Currently, we aimed to improve the osteoinductivity of these scaffolds and attenuate the negative effects of PLA degradation by creating PLA composites with 10% of hydroxyapatite (HA) or 10% of bioglass (BG) filaments, which were further used for scaffold production.

The tasks of our group are (1) to develop the best way of selected composite material microstructurization, (2) to compare the physical and osteoinductive properties of 3D printed PLA+ hydroxyapatite (HA), PLA+bioglass (BG), (3) to elucidate the fate of cells grown on these scaffolds and (4) to evaluate the influence of biodecoration effect on cell differentiation.

We have analysed various fused filament fabrication (FFF) 3D-printed PLA scaffold modifications' impact on the fate of rat dental pulp stem cells (DPSC). Primary rat DPSCs were selected as a model, which helped to understand the cellular response to different substrate modifications. PLA scaffolds were modified by altering their chemical composition (PLA composites with 10% of HA or PLA with 10% of BG), and surface coating with proteins to determine the impact of each substrate modification on cell function and properties, focusing on cell osteogenesis processes.

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Impact of Extracellular Environment on Cell Fate

Analysis of substrate macrophotography (>100 µm) impact on the fate of DPSCs revealed that for spontaneous cell osteogenesis induction, the precise structuring of the scaffold (nano- and micro-surface patterns) is not necessarily required. Nevertheless, we have found that PLA+HA filaments printed with FFF 3D printer produced equal or even better accuracy than scaffolds printed with pure PLA filaments (Gendviliene et al. 2020). After evaluating the impact of surface chemical composition on cell behaviour it was observed that PLA+HA composite was more suitable for DPSC attachment and proliferation, meanwhile the minimum number of focal adhesions were formed in the cells grown on PLA+BG scaffolds, which means that DPSCs adhesion strength on PLA+BG scaffolds was the weakest compared with PLA+HA ($p<0.05$) and pure PLA ($p<0.05$) (Fig.1). Despite this, PLA+BG composites promoted the earliest and strongest DPSC osteogenesis.

Further investigation has shown that PLA surface coated with DPSC-derived extracellular matrix (ECM) network significantly improved the osteoinductive properties of the surface. The obtained results of DPSC migration and proliferation showed that ECM proteins had a positive impact on both cell migration and proliferation (Fig. 2).

The *in vitro* assay of scaffolds (PLA, PLA+HA, PLA+BG, and PLA+ECM) and DPSCs ability to initiate angiogenesis revealed that DPSC-PLA+BG and DPSC-PLA+ECM constructs would be the most suitable candidates for bone engineering. According to our data, the combination of DPSCs, PLA+BG composite microstructured scaffold and DPSC-derived ECM network can be expected to reach successful bone tissue regeneration in vivo (Alksne et al. 2020).

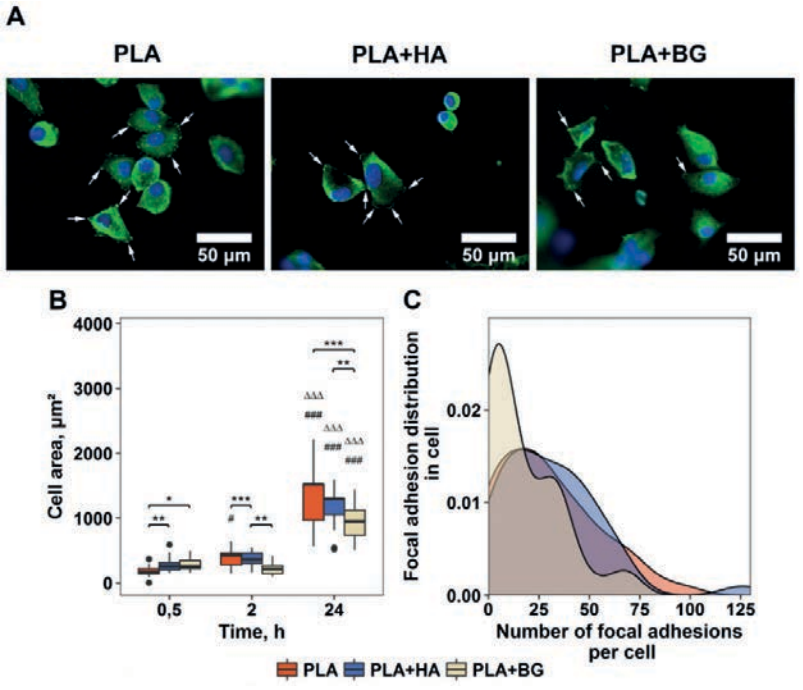


Fig. 1. DPSCs adhesion on 3D printed composite scaffolds. A – immunofluorescence staining of nucleus (DAPI, blue) and FA spots (vinculin, green) in DPSCs 24 h post-seeding; B – cell surface area after culturing for 0.5, 2 and 24 h on the scaffolds; C – quantitative FA evaluation within the cells after culturing for 24 h.

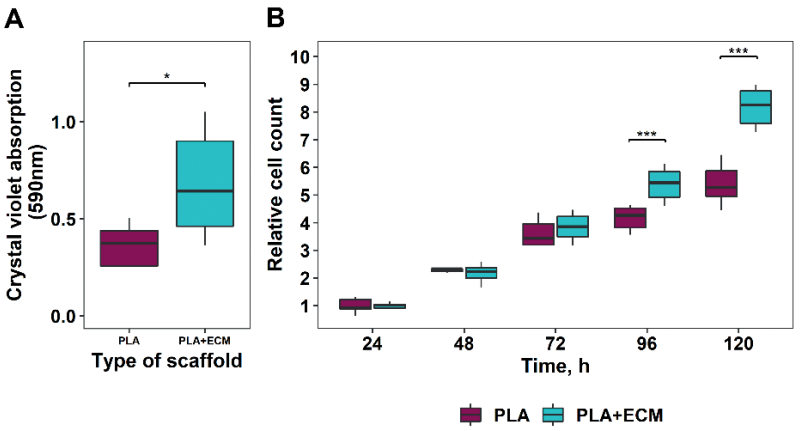
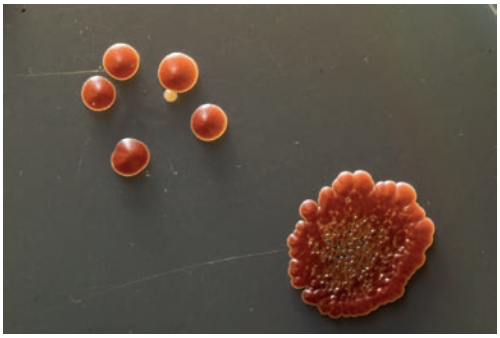


Fig. 2. DPSC migration and proliferation on ECM coated scaffolds. A – evaluation of vertical cell migration onto the scaffolds using crystal violet assay; B – relative DPSC proliferation rate.



EGLĖ LASTAUSKIENĖ
Associate Professor
Department of Microbiology and Biotechnology
Institute of Biosciences
Email: egle.lastauskiene@gf.vu.lt
Phone: +370 610 24191

RENATA GUDIUKAITĖ
Assistant Professor
Department of Microbiology and Biotechnology
Institute of Biosciences
Email: renata.gudiukaite@gf.vu.lt
Phone: +370 609 90189

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 [1]. RCCS is changing the pattern of the antibiotic resistance of *Pseudomonas aeruginosa* and *Staphylococcus aureus* as well. A combination of the novel chemical compounds with the pulsed electric field (PEF) and pulsed electromagnetic fields (PEMF) technologies allows us to perform a wide scale biocontrol of the drug resistant skin pathogens [2].

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. 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 lipolytic enzymes produced by *Geobacillus* bacteria. In our research, we predicted a few amino acids, which strongly affected the activity of these enzymes, constructed several fused lipolytic enzymes and developed new *Geobacillus* lipase variant with improved kinetics and physicochemical characteristics (4, 5).

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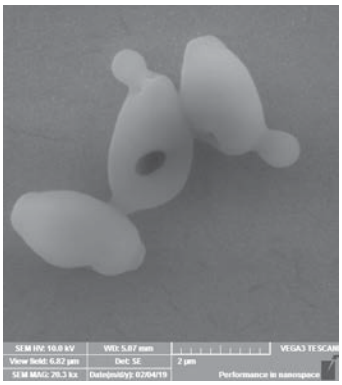
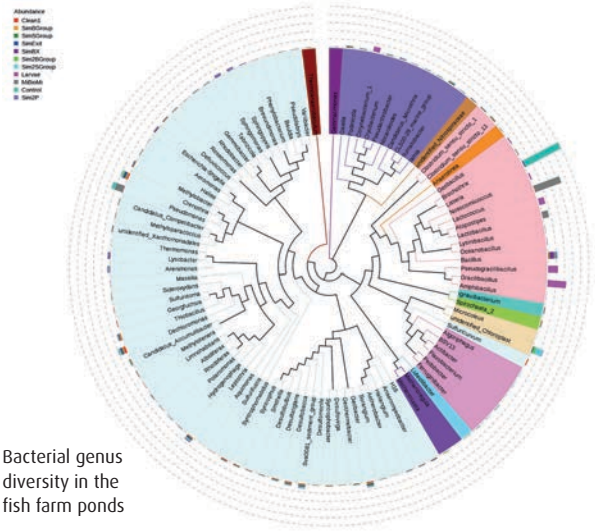
The *Candida genera* yeast-caused infections are frequent and difficult to treat, as the physiology and metabolisms of yeast are similar to the host [1]. Induction of the programmed cell death is of great medical relevance, since during apoptosis peptides, nucleotides, amino acids and other compounds are released to the surrounding media and can contribute to the regeneration of the human tissues. Discovery of the apoptogenic substances for the biocontrol of pathogenic yeasts as well as optimization of the apoptogenic conditions is the main task of our research group. PEF can be also successfully applied to inactivate bacterial pathogen colonizing human skin. Best results are achieved by combining PEF with the week organic acids [2, 3].



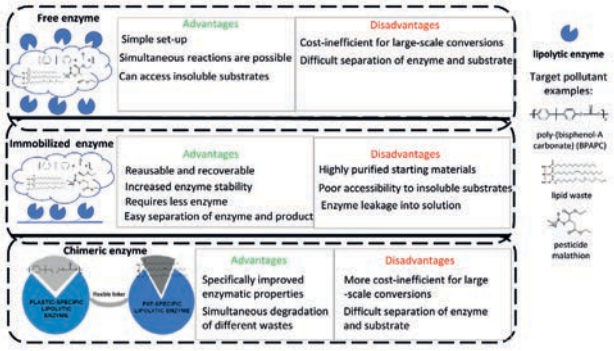
PEF treated yeast cells. Scanning electron microscopy

The biodiversity of fishery ponds is changed towards fulfilling the industrial needs, therefore reducing the microbial biodiversity and precautions should be taken to keep the system sustainable and protect the adjacent environment from possible damage. The metagenome analysis allows us to assess prokaryotic diversity in aquatic environment and evaluate the influence of intensive fish farming on the environment. We aim to evaluate the current situation in the fishery ponds, fresh water environments and adjacent aqua systems in order to understand the microbial population dynamics.



Bacterial genus diversity in the fish farm ponds

Microbial lipolytic enzymes have gained attention for the ability to catalyse biotransformation reactions of different esters-bond containing compounds. Conversion of the latter into high-energy products like biofuel and other value-added products (fatty acid esters, mono- and diacylglycerols, etc.) in an energy-efficient and ecologically-friendly way makes these biocatalysts an important tool for sustainable biotechnology. Protein engineering, immobilization and application of *Geobacillus* lipases and carboxylesterases is one of the major research fields of our group [4–6].



The main advantages and disadvantages of lipolytic microbial enzymes usage in immobilized, free and chimeric forms [5]

Microbially induced calcite precipitation (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.

Bioconsolidated sand particles obtained during MICP using *Staphylococcus* sp. H6



VIRGINIJA PODĖNIENĖ
Associate Professor
Head, Department of Zoology
Institute of Biosciences
Email: virginija.podeniene@gf.vu.lt
Phone: +370 5 239 4430

SIGITAS PODĖNAS
Professor
Department of Zoology
Institute of Biosciences
Email: sigitas.podenas@gf.vu.lt
Phone: +370 5 239 4430

RIMANTAS RAKAUSKAS
Professor
Department of Zoology
Institute of Biosciences
Email: rimantas.rakauskas@gf.vu.lt
Phone: +370 5 239 8264

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, 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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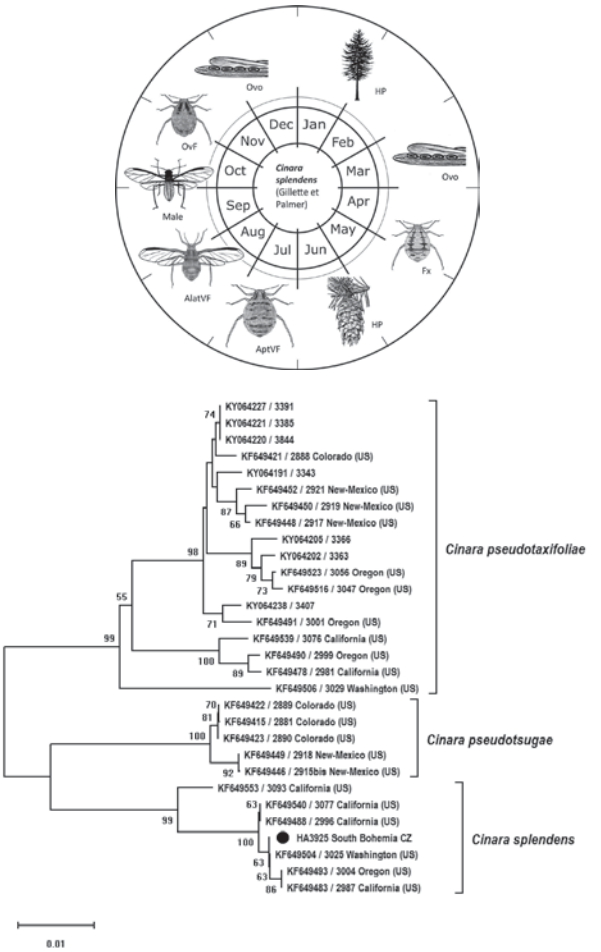
Cinara splendens (Hemiptera: Aphididae: Lachninae) - First Record in Palaearctic Region

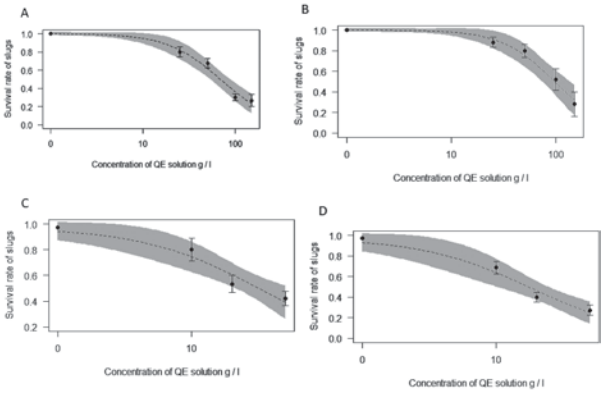
Nearctic aphid *Cinara splendens* (Gillette and Palmer, 1924) was collected on ornamental Douglas fir (*Pseudotsuga menziesii* (Mirb.) Franco) in South Bohemia in 2009. It was the first record of this species in the Palaearctic region. The aim of this research was to study the bionomy of this species in Central Europe and to make descriptions of all available morphs, as previous morphological descriptions of *C. splendens* appeared to be incomplete. Six monitoring sites of this species were established in South Bohemia and were then regularly attended in the period of 2009–2019. The colonies of *C. splendens* were observed; its natural enemies and honeydew users were also registered. Aphids were collected for the microscope slide preparation, followed by the evaluation of thirty of the basic quantitative and seven qualitative morphological characteristics. Partial sequences of mitochondrial COI and nuclear EF-1α were used to confirm morphology-based identification and to compare samples from the Czech Republic with those of North American origin. *Cinara splendens* survived successfully under new ecological conditions, but its population density remained quite low, except for 2009 and 2019, due to a synergistic effect of the dry weather and very high population density of the adelgid *Gilletteella coweni* (Gillette, 1907), which is a key pest of Douglas fir in the Czech Republic. The principle predators were coccinellid beetles, while the aphidophagous hover flies were less abundant. Together with a weak ability to migrate due to a low number of alate viviparous females in population, *C. splendens* cannot be a potential pest of *P. menziesii* in Central Europe.



Cinara splendens (Gillette and Palmer, 1924) life-cycle in Central Europe. (HP = host plant; Fx = fundatrix; AptVF = apterous viviparous female; AlatVF = Alate viviparous female; OvF = ovipara).
Neighbour-joining tree (p-distances, 1000 bootstrap replications) based on COI fragment (658 bp) of species of the genus *Cinara* collected from *Pseudotsuga* spp. Bootstrap values over 50% are shown next to branches. The sample from the Czech Republic is marked with a black circle.

Lethal Doses of Saponins from *Quillaja saponaria* for Invasive Slug *Arion vulgaris* and Non-Target Organism *Enchytraeus albidus* (Oligochaeta: Enchytraeidae)

We evaluated the effect of saponins extracts from the bark of the soap tree, *Quillaja saponaria*, against invasive slugs (of diferent age groups) and non-target decomposters, the white worms, by measuring the concentrations of toxins that kill 50% of individuals in the sample. In addition, we aimed to evaluate the impact at different temperatures.
The results of our studies showed that *Q. saponaria* saponins may be a successful slug control tool used during the colder time of the year, but its concentration should be selected according to the age group of *A. vulgaris*. However, the unexpected toxicity to model organisms (white worms) limits its use as an environmentally benign alternative means of slug control.
Dose–response curves for the toxicity of solution of *Quillaja saponaria* aqueous extract (QE) on: (A) *A. vulgaris* adults at 15°C; (B) *A. vulgaris* juveniles at 15°C; (C) *A. vulgaris* adults at 2°C; (D) *A. vulgaris* adults at 1°C. A grey band on the curve represents the standard error of the dose–response model. Black dots show the mean (with standard error bars) efect of the tested concentration of QE solution. Black dots represent tested concentrations of QE solution: 0; 25; 50; 100; 150 g/L (A and B) and 0; 10; 20; 50 g/L (C and D).



ERNESTAS KUTORGA
Professor
Department of Botany and Genetics
Institute of Biosciences
Email: ernestas.kutorga@gf.vu.lt
Phone: +370 5 239 8250

Biodiversity and Ecology of Plants, Algae and Fungi

Plants, algae and fungi are among the most important organisms, not only because of their vital roles in both natural and altered ecosystems, but also because of their influence on humans and human-related activities. Because of the diversity, abundance and vital roles of these organisms, they are included in considerations of biodiversity conservation, nature resource management and related subjects. These organisms encompass a great number of taxa, forms, life histories and ecology; however, only limited and incomplete information is available for most of the species. Moreover, changes in climate, environment and the traditional management of various habitats over the last decades have triggered changes in the composition and distribution of species, stimulated an introduction of alien species and increased interest in understanding the processes of biodiversity change and maintenance.

The herbarium and voucher specimens serve as a basis of scientific study; they are important for both current and future research. Therefore, the collection, study and preservation of plant, algal, lichen and fungal specimens in the Herbarium of Vilnius University (WI) is an essential task in providing research on the diversity and distribution of Lithuanian flora, algobiota and mycobiota.

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 conduct investigations of the historical herbarium collections, and study the history of botany in Lithuania.

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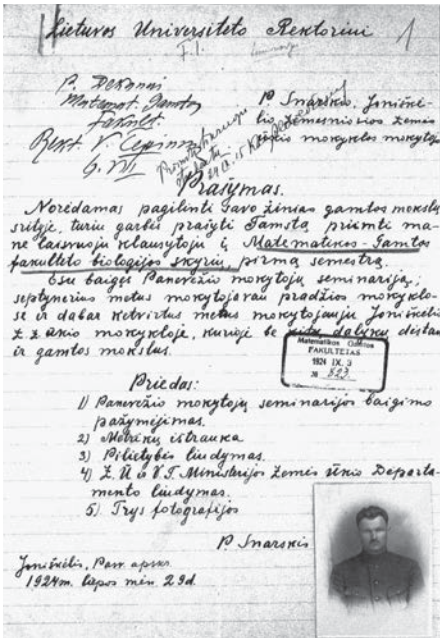
A Retrospective Study of Scientific Legacy of Professor Povilas Snarskis

"You have to prepare before going to nature. Similarly, like people did before going to the church in the early days: take a bath, get dressed well and have a good will" used to say Prof. P. Snarskis to his students.

The study presents a brief biography of Prof. P. Snarskis and discusses the importance of his work for Lithuania. P. Snarskis defended PhD thesis at Vytautas Magnus University, studied Lithuanian floristic diversity, taught botany at Vilnius University and Vilnius Pedagogical Institute, headed several departments and the Faculty of Natural Sciences. During his professional career, P. Snarskis published about 60 research and popular science works. He was a co-author of the edition on Lithuanian flora "Lietuvos TSR flora". His plant guidebooks still have their high value. More than 20 000 plant specimens collected by P. Snarskis were deposited at the herbaria of Vilnius University (WI) and the Institute of Botany of Nature Research Centre (BILAS) (Tupčiauskaitė & Rimgailė-Voicik. *Botanica*, 2020, 26(1): 101–108).

We studied the collection of the Poaceae specimens collected by P. Snarskis mainly from eastern Lithuania in 1943–1960. The collection is preserved in WI, and consists of 67 herbarium sheets representing 28 species. The collection provided new data on abnormal growth forms of common Lithuanian grasses and the distribution of rare and endangered Poaceae species in Lithuania. The discovered specimen of endangered species *Glyceria lithuanica* is the oldest of all known records of the species in Lithuania (Tupčiauskaitė et al. *Botanica*. 2020, 26(2): 138–149).


Lietuvos Universiteto Rektoriui

Prašymas.

Norėdamas pagilinti savo žinias gamtos mokslų srityje, turiu garbės prašyti Jamstą priimti mane laisvuoju klausytoju į Matematinės-Gamtos fakulteto biologijos skyrių pirmą semestrą.

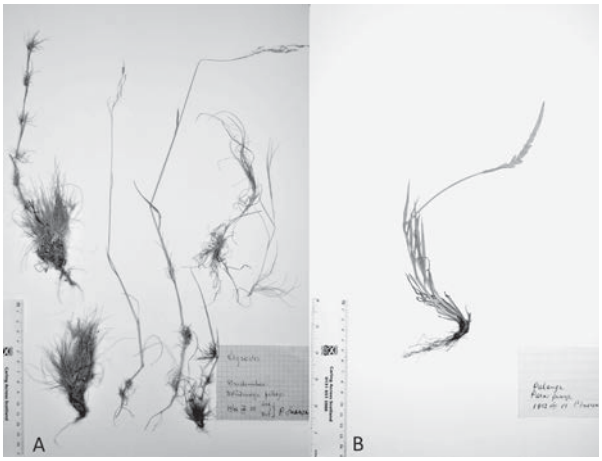
Esu baigęs Panevėžio mokytojų seminariją, septynerius metus mokytojavau pradžios mokyklose ir dabar ketvirtus metus mokytojauju Joniškėlio 2 m. mokyklose, kuriuos ir gamtos mokslus.

Priedai:
1) Panevėžio mokytojų seminarijos baigimo pažymėjimas.
2) Metrikų ištrauka.
3) Pilietybės liudymas.
4) Ž. Ū. ir V. S. Ministerijos Žemės ūkio Departamento liudymas.
5) Trys fotografijos.

P. Snarskis

Joniškelis, Pasv. apskr.
1924 m. lapkr. mėn. 29 d.

P. Snarskis' handwritten application to enter the University of Lithuania as a free listener, kept in the Office of the Chief Archivist of Lithuania (Vytautas Magnus University student files, LCSA F631/7/839/1)



Atypical Poaceae specimens collected by P. Snarskis: not fully identified (A) and unidentified specimens (B)

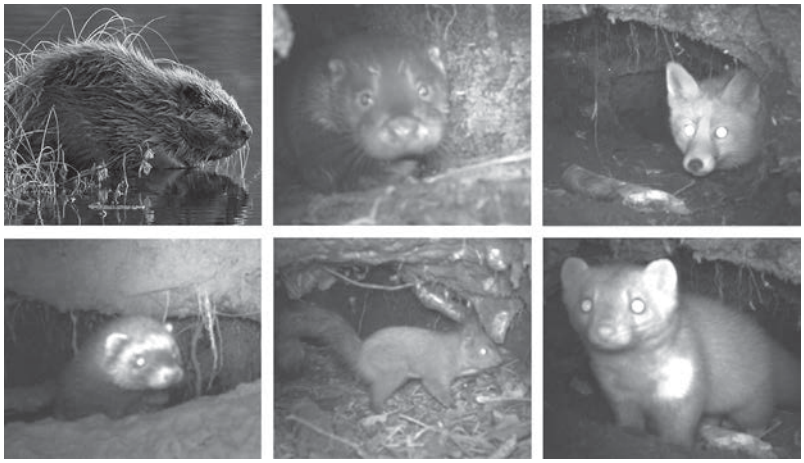
Species Diversity, Distribution and Ecology of Inoperculate Discomycetes in Lithuania

The monograph deals with the inoperculate discomycetes occurring in Lithuania, provides information about the species diversity, form and structure, ecology and distribution. In total, 381 species from 119 genera, 29 families, 8 orders (*Geoglossales*, *Helotiales*, *Leotiales*, *Marthamycetales*, *Orbiliales Phacidiales*, *Ostropales* and *Rhytismatales*) and 4 classes (*Geoglossomycetes*, *Lecanoromycetes*, *Leotiomycetes* and *Orbiliomycetes*) have been recorded in Lithuania. New nomenclatural combination, *Mollisia strobilicola* (Rehm) Kutorga, was proposed. The structures of the studied fungi are illustrated by line drawings and colour photographs. The vast majority of the fungi examined are saprotrophic on plant remnants and anthropogenic substrates. Some fungi function as biotrophs, cause diseases in plants, or damage fungal fruit-bodies (Kutorga, 2020).


Fruit-bodies of *Bulgaria inquinans* on oak branch


Fruit-bodies of *Leotia lubrica* on forest litter

ALIUS ULEVIČIUS
Professor
Head, Department of Ecology and Environment Research
Institute of Biosciences
Email: alius.ulevicius@gf.vu.lt
Phone: +370 699 89023



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 is 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. Anthropogenic pollution *sensu lato* also includes the introduction of alien biotic components and their impacts. 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. Introduction of alien species provokes new info chemical interactions and changes in the behaviour of organisms, which leads to reorganization of the functional groups within the impacted ecosystem.

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. landfill leachate, phytoplankton biomass of eutrophicated water bodies, wastewater, lake sediments) using luminescent bacteria test (ISO 11348-3:2007) was evaluated [1]. Hierarchical organisation of mixed-species groups of wintering birds was found important for winter survival in a not-disturbed forest environment [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. Our study documents the relationship between roe deer – vehicle collisions (RDVC) and season of the year with the highest RDVCs peaks occurring in late spring and late autumn [3]. In collaboration with the Natural Resources Institute, Finland (Luke), genetic structure of reintroduced Eurasian beaver *Castor fiber* was assessed. Results discover very different genetic structure and diversity among Scandinavian and other European beaver populations suggesting specific means of conservation [4]. Moreover, these populations demonstrate different ecological patterns of feeding behaviour [5].

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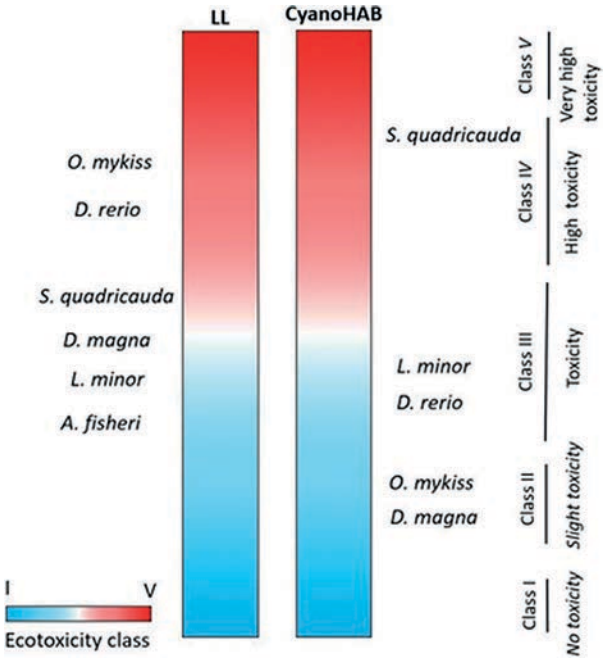
Ecotoxic Effects of Landfill Leachate and Cyanobacterial Biomass on Aquatic Organisms

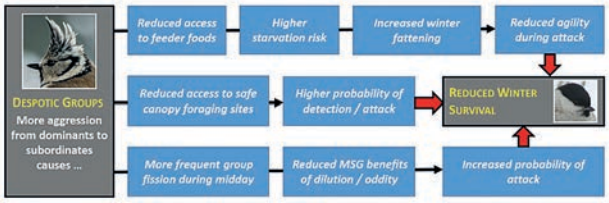
The effect of landfill leachate (LL) and cyanoHAB biomass on test organisms was concentration- and trophic-level-dependent, and in the case of fish, development stage-dependent. The secondary consumer *Oncorhynchus mykiss* and larvae of the *Danio rerio* proved to be most sensitive to LL additions, while *Scenedesmus quadricauda*, representing primary producers, to cyanoHAB exposure. The overall ecotoxic effect of both mixtures on the tested organisms varied from low (Class II) to high (Class IV). This study highlights complex and unambiguous effects of LL and cyanoHAB biomass on aquatic organisms [1].



Potential toxicity of LL and cyanoHAB biomass according to their effects on aquatic organisms. For LL (*A. fischeri* < *L. minor* < *D. magna* < *S. quadricauda* < larvae of *D. rerio* ≤ *O. mykiss* (larvae ≤ fry ≤ juveniles ≤ adults)) and for the biomass of cyanoHAB (*D. magna* = *O. mykiss* fry < *D. rerio* < *L. minor* < *S. quadricauda*)

Winter Survival of Subordinate Group Members of Egalitarian Mixed-Species Bird Groups in High-Quality Forests

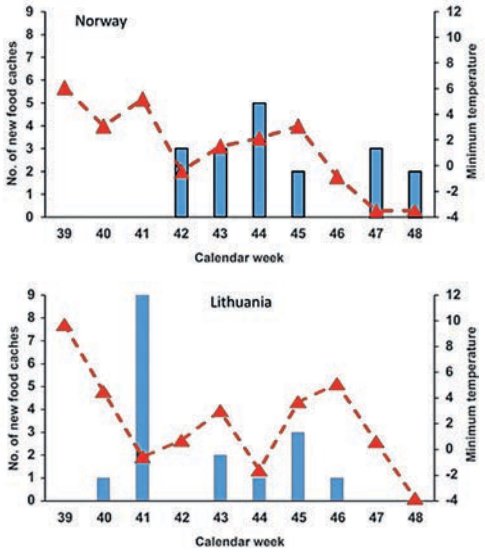
Birds often live in hierarchically organized mixed-species groups (MSGs), in which heterospecific individuals are considered to substitute for conspecifics as protection against predators at a significantly reduced competition cost. A comparison of individuals in the despotic MSGs of crested tits and willow tits revealed a strong negative correlation between subcutaneous fat stores and dominance rank in the interspecific dominance hierarchy. Egalitarian groups of crested tits and willow tits exhibited markedly less within-group aggression and improved winter survival in both tit species. However, winter survival of birds in egalitarian groups was impaired relative to despotic groups in forests recently affected by industrial forestry. This suggests that more egalitarian bird societies may be best adapted to less-disturbed environments [2].



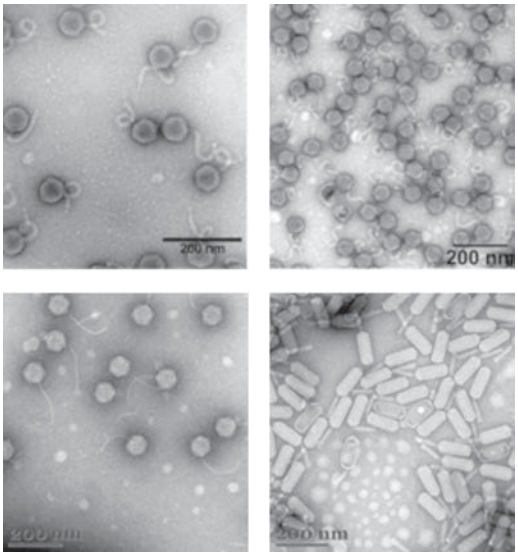
Mechanisms underlying lower subordinate survival in despotic mixed-species groups of crested and willow tits

Food Caching Behaviour of the Eurasian Beaver in Northern Europe

We studied the food caching behaviour of the Eurasian beaver in three northern European countries (Sweden, Norway, Lithuania). Construction of caches began as early as late September (week 39/40) in Sweden and Lithuania and by mid October (week 42) in Norway. We observed plasticity in timing of cache initiation. Declining air temperature and mean minimum temperatures of 0°C or below were associated with cache initiation. Caches in Lithuania were larger than in Sweden and Norway, which may be associated with colder winter temperatures [5].



Estimated and observed initiation dates of new food caches and mean weekly minimum temperatures at research sites



LAURA KALINIENĖ
Senior Researcher
Department of Molecular Microbiology and Biotechnology
Institute of Biochemistry
Email: laura.kaliniene@bchi.vu.lt
Phone: +370 687 92461

EUGENIJUS ŠIMOLIŪNAS
Researcher
Department of Molecular Microbiology and Biotechnology
Institute of Biochemistry
Email: eugenijus.simoliunas@bchi.vu.lt
Phone: +370 650 70467

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 ten different projects funded by Vilnius University (MSF-LMT-2) and the Research Council of Lithuania (MIP-002/2014, P-MIP-19-259, 01.2.2-LMT-K-718-03-0099, 01.2.2-LMT-K-718-01-0019). 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 low-temperature viruses to the mesophilic host. A number of unique *Pantoea*, *Arthrobacter*, and *Buttiauxella* phages have been isolated, characterised and published in the process [1–5]. In total, seventeen peer-reviewed scientific publications have been published by the research group during the last five-years.

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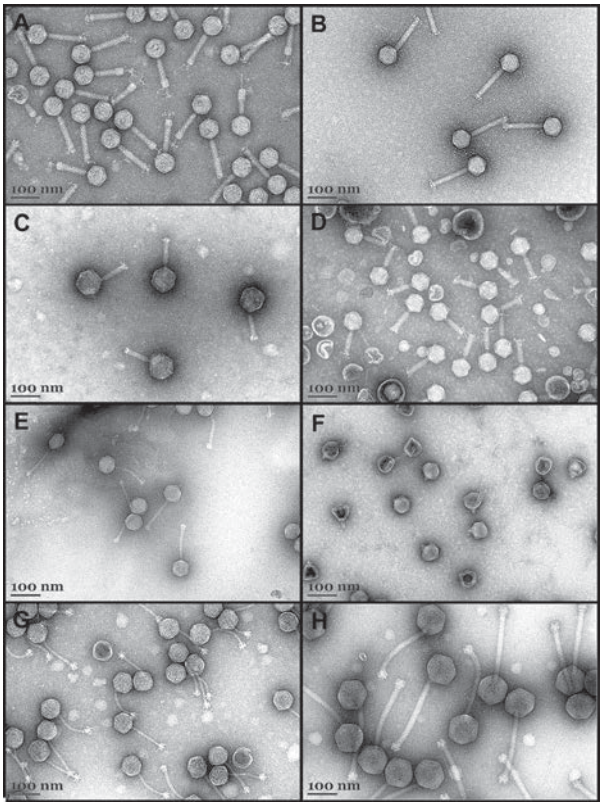
Ecogenomics and Potential Biogeochemical Impacts of Viruses in Gypsum Karst Lake Ecosystems

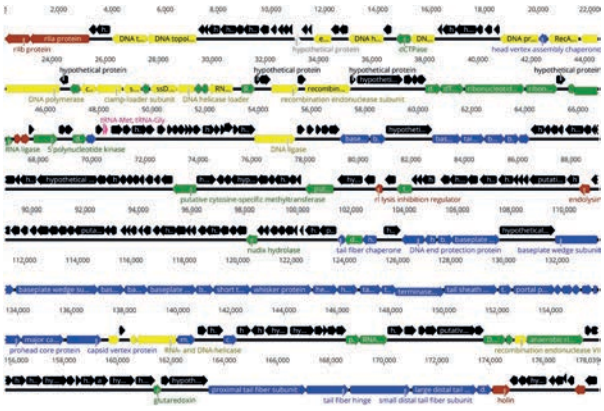
In this study, we present a number of bacteria and bacteriophages isolated from water samples of the unique sulfate-type gypsum karst lakes Kirkilai and Ramunėlis located near Biržai, Lithuania.
Nine bacteriophages were isolated using *Aeromonas, Bacillus, Paracoccus, Pseudomonas*, *Pararheinheimera* and *Pseudaeromonas* as the host for phage propagation and phage growth experiments. TEM analysis revealed that most of the phages are myoviruses, *Aeromonas* phage KLEA5, *Bacillus* phages KLEB27-1 and KLEB30-3S are siphoviruses, while *Paracoccus* phage KLEP18-1 is a podovirus. Most of aforementioned phages have isometric heads of about 60–65 nm in diameter with an exception of *Bacillus* phage KLEB27-3, which has a head about 108 nm in diameter and a contractile tail about 285 nm long. Phylogenetic analysis revealed that the vast majority of the phages have no close phylogenetic relatedness to other viruses published to date and potentially represent new genera within the order *Caudovirales*.
The data presented in this study will not only expand our knowledge of the diversity of bacteriophages but will also lead to a better understanding of almost unexplored communities of bacteria and viruses in the unique sulfate-type gypsum karst lakes (Grant Nr. MSF-LMT-2).



Transmission electron micrographs of phage particles isolated from the gypsum karst lakes Kirkilai and Ramunėlis: KLER1-1 (A), KLER1-2 (B), KLEA5 (C), KLEP7 (D), KLEP17-4 (E), KLEP18-1 (F), KLEB27-1 (G) and KLEB27-3 (H)

Complete Genome Sequence of *Buttiauxella* Phage vB_ButM_GuL6

Bacteriophage vB_ButM_GuL6 is the first virus isolated from *Buttiauxella*. Electron microscopy revealed that vB_ButM_GuL6 belongs to the family *Myoviridae*, order *Caudovirales*. The genome of vB_ButM_GuL6 is a linear, circularly permuted 178,039-bp dsDNA molecule with a GC content of 43.4%. It has been predicted to contain 282 protein-coding genes and two tRNA genes, tRNA-Met and tRNA-Gly. Using bioinformatics approaches, 99 (36%) of the vB_ButM_GuL6 genes were assigned a putative function. Genome-wide comparisons and phylogenetic analysis indicated that vB_ButM_GuL6 represents a new species of the subfamily *Tevenvirinae* and is most closely related to *Escherichia coli* virus RB43. These phages, together with *Cronobacter* phages Miller, CfP1, and IME-CF2, likely form a new genus within the subfamily *Tevenvirinae* (Noreika et al. *Arch Virol*. 2020, 165: 2685–2687).



Functional genome map of bacteriophage GuL6

The colour code is as follows: blue, structural proteins and those involved in virion morphogenesis; yellow, DNA replication, recombination, and repair; green, transcription, translation, and nucleotide metabolism; red, lysis/phage-host interactions; black, genes of unknown function; white, genes that encode unique proteins with no reliable homology to database entries; pink, tRNAs

LINAS MAŽUTIS
Research Professor
Research ID: A-5408-2012
Division of Microtechnologies
Institute of Biotechnology
Email: linas.mazutis@bti.vu.lt
Phone: +370 693 19333

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 2020, our group members have collaborated with Harvard University, ETH Zurich, Helsinki University and MSKCC to advance single-cell biology research in cancer, immunology and beyond.

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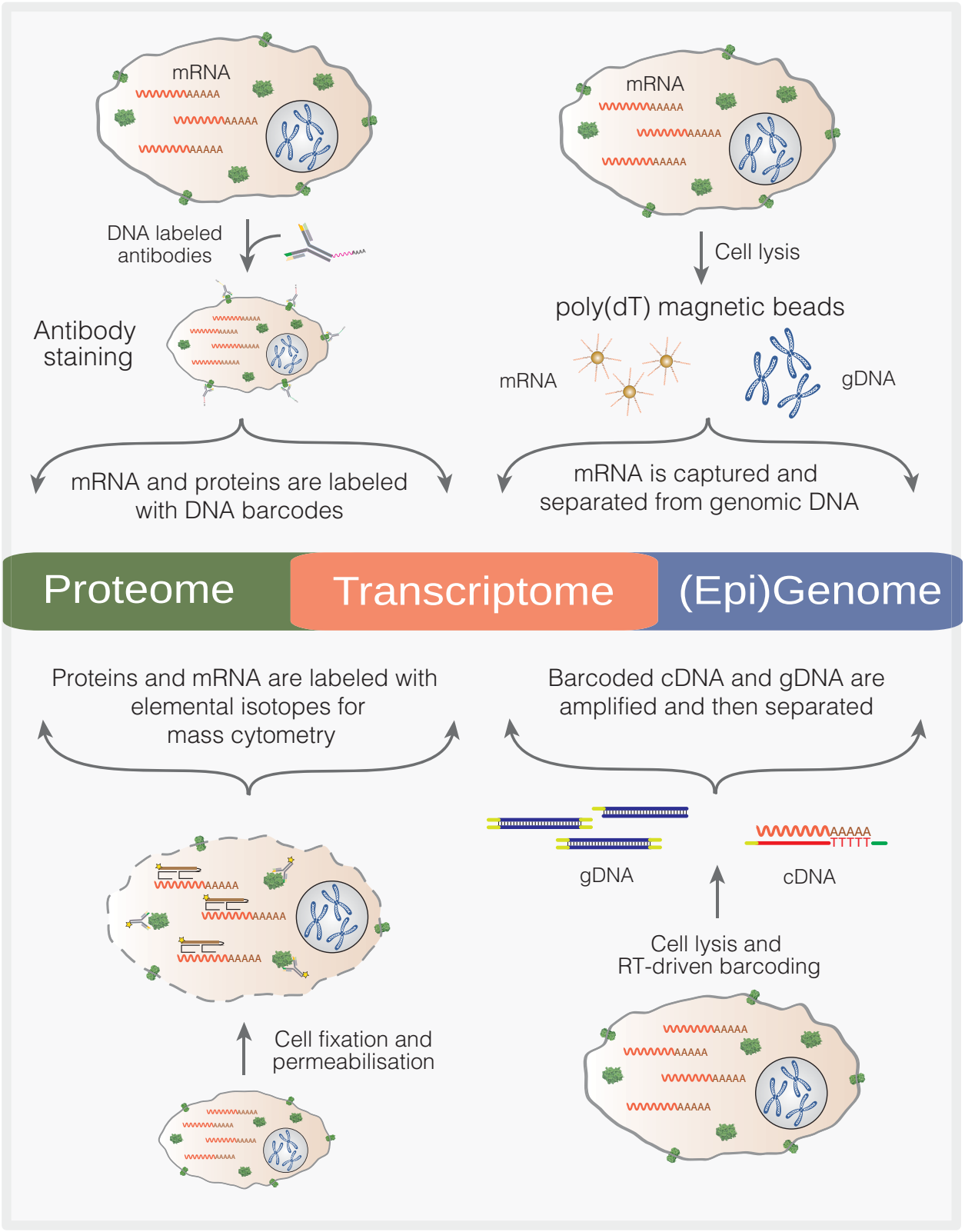
5. Patents: EP3299469B1, US10596541B2, EP2941642B1, US10710073B2, EP3402594B1.

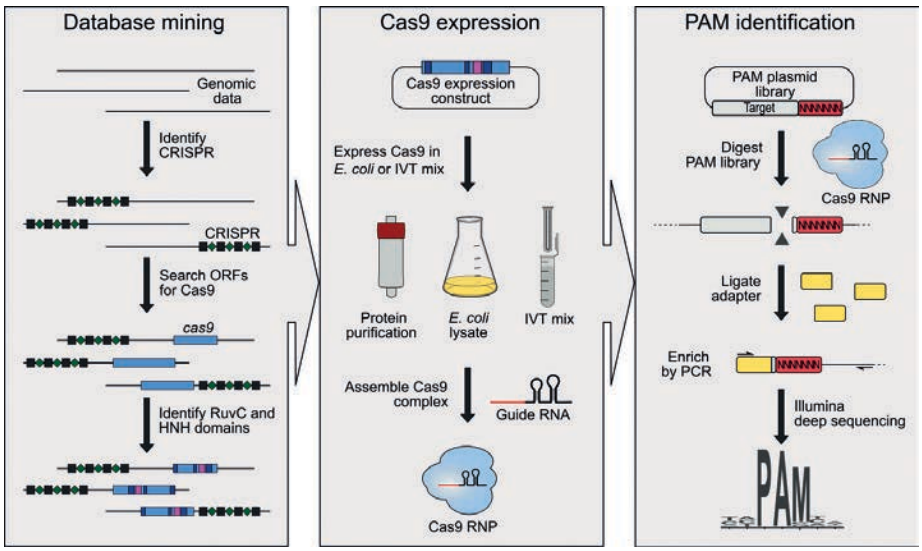

mRNA
DNA labeled antibodies
Antibody staining
mRNA and proteins are labeled with DNA barcodes
mRNA
Cell lysis
poly(dT) magnetic beads
mRNA
gDNA
mRNA is captured and separated from genomic DNA
Proteome
Transcriptome
(Epi)Genome
Proteins and mRNA are labeled with elemental isotopes for mass cytometry
Cell fixation and permeabilisation
Barcoded cDNA and gDNA are amplified and then separated
gDNA
cDNA
Cell lysis and RT-driven barcoding

VIRGINIJUS ŠIKŠNYS
Distinguished Professor
Head, Department of Protein-Nucleic Acids Interactions
Institute of Biotechnology
Email: virginijus.siksnys@bti.vu.lt
Phone: +370 5 223 4354

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 search string. Using this system, DNA sequences within the endogenous genome and their functional outputs are now easily edited or modulated in virtually any organism of choice.

Cas9 specificity is dictated by base pairing of the guide RNA to the complementary DNA strand, however to initiate hybridization, a short protospacer adjacent motif (PAM) sequence is required in the vicinity of the target sequence. The PAM is recognized by the Cas9 protein and varies among Cas9s. To characterize the PAM recognition diversity provided by Cas9 orthologs, we developed a phylogeny-guided bioinformatics approach and streamlined our experimental procedures for Cas9 expression and RNP complex assembly using cell lysates and *in vitro* translation mixtures. This approach 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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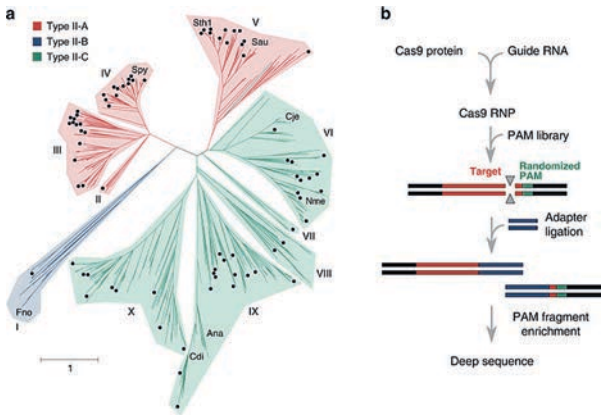
Catalogue of Biochemically Diverse CRISPR-Cas9 Orthologues

Bacterial Cas9 nucleases from type II CRISPR-Cas antiviral defence systems are very diverse. We combined a bioinformatic analysis and biochemical screen to explore this largely uncharacterized diversity. We applied cell-free biochemical screens to assess the PAM and guide RNA requirements of 79 Cas9 proteins, thus identifying at least 7 distinct gRNA classes and 50 different PAM sequence requirements. PAM recognition spans the entire spectrum of T-, A-, C-, and G-rich nucleotides, from single nucleotide recognition to sequence strings longer than 4 nucleotides. Characterization of a subset of Cas9 orthologues using purified components reveals additional biochemical diversity, including both narrow and broad ranges of temperature dependence, staggered-end DNA target cleavage, and a requirement for long stretches of homology between gRNA and DNA target. Our results expand the available toolset of RNA-programmable CRISPR-associated nucleases (Gasiunas et al. *Nat Commun*. 2020, 11: 5512; Wang et al. CRISPR J. 2020, 3(6): 427–430).

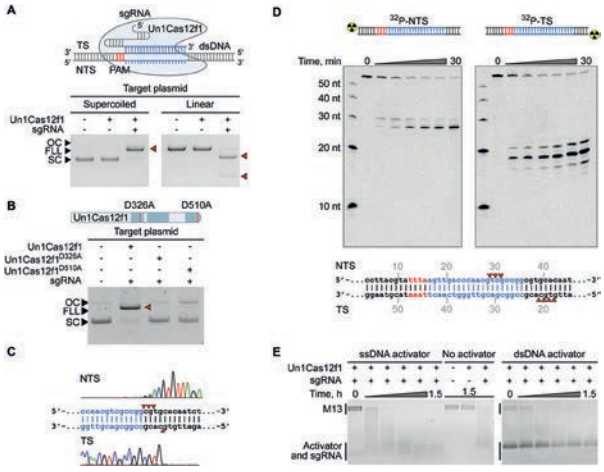


Characterization of Cas9 diversity

Characterisation of Compact CRISPR-Cas12f Nuclease

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. Recently, small CRISPR-associated effector proteins (Cas12f) belonging to the type V-F subtype have been identified through the mining of sequence databases. The majority of Cas12f proteins are nearly half the size of the smallest Cas9 or Cas12 nucleases.

We selected a collection of 10 exceptionally compact (422-603 amino acids) CRISPR-Cas12f nucleases that recognize and cleave dsDNA in a PAM dependent manner. Categorized as class 2 type V-F, they originate from the previously identified Cas14 family and distantly related type V-U3 Cas proteins found in bacteria. Using biochemical methods, we demonstrate that a 5′ T- or C-rich PAM sequence triggers dsDNA target cleavage. Based on this discovery, we evaluated whether they can protect against invading dsDNA in *Escherichia coli* and find that some but not all can. Altogether, our findings show that miniature Cas12f nucleases can protect against invading dsDNA like much larger class 2 CRISPR effectors and have the potential to be harnessed as programmable nucleases for genome editing (Karvelis et al. *Nucleic Acids Res*. 2020, 48(9): 5016–5023).



Un1Cas12f1 (Cas14a1) RNP complex is a PAM-dependent dsDNA endonuclease

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 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.

EUROPEAN JOINT PROGRAMME RARE DISEASES

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

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

GET-READY

RASA PETRAITYTĖ-BURNEIKIENĖ
Senior Researcher
Head, Department of Eukaryote Gene Engineering
Institute of Biotechnology
Email: rasa.burneikiene@bti.vu.lt
Phone: +370 5 223 4421

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.

This year 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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Investigation of *K. lactis* Mutations Conferring Enhanced Secretion Phenotype and Generation of Yeast Strains for Supersecretion of Recombinant Proteins

As the yield of secreted recombinant proteins in yeast is usually far from optimal and needs optimization, the aim of this study was identification and characterization of gene and its mutations conferring the enhanced secretion phenotype of *K. lactis* mutant stain MD2/1-9 and application of acquired knowledge for generation of new yeast super-secretion strains. The mutated gene *KlSEC59* encoding dolichol kinase (DK) was identified after sequencing of genomes of both parental and mutated *K. lactis* strains using the next-generation sequencing technology. It was confirmed that the double mutant strain with G405S and I419S mutations in DK sequence displayed improved secretion of recombinant proteins despite some glycosylation defects. The introduction of analogical mutations in DK of S. cerevisiae also improved secretion of recombinant proteins but the partial glycosylation deficiency in the double *sec 59* mutant strain had stronger impact on yeast cell wall integrity and survival of yeast cells at high temperature compared with the *K. lactis Klsec59* mutant.

Identification of New Polyomaviruses and Investigation of Their Evolutionary History

Polyomaviruses (PyVs) are widespread, non-enveloped double stranded DNA viruses that infect mammals, birds, and fish but polyomavirus-like sequences have also been recovered from arthropods which points to very ancient association of polyomaviruses with animals. Despite the discovery of numerous PyVs within the last 10 years, still only a very limited number of species have been assessed for the presence and diversity of polyomaviruses (in total, probably fewer than 100) when there are about 5000 mammalian species belonging to 29 orders. Therefore, it is evident that there is a need to identify more PyVs from other host species for a better understanding of overall polyomavirus diversity and evolution, of the long-term dynamics of the coevolution with their hosts or explanation of variations in their toxicity and cross-species transmission.

Molecular Mechanisms of Heart Regeneration

Cardiovascular disease is one of the leading causes of death in the Western world. If we are 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. We are using the zebrafish *Danio rerio* to decipher the transcriptional program required for cardiac regeneration to occur. Our primary focus is on two highly conserved transcription factors: *Tbx5a* (Grajevskaja et al. 2018, *PMID:* 29933372) in the myocardium and *Tcf21* in the epicardium. 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.

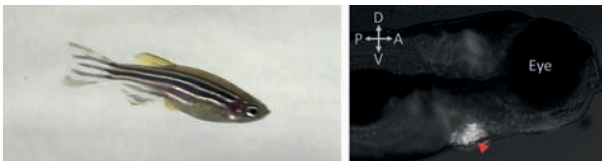


Fig. 1. Adult wild type zebrafish (left) and live transgenic 5-day-old zebrafish (right) expressing red fluorescent protein in the heart (red arrow) under the control of an evolutionarily conserved enhancer of the zebrafish tbx5a.

New Technologies for Development of Recombinant Allergens

The main goal of this project is 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 obtained results show that the platform is well applicable for bacterial expression systems (more than 40 allergens tested). 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 allergy diseases. Two new food allergens have been identified from the Lithuanian carp species. Beta-enolase allergen from carp named as Cyp c 2 has been included into WHO/IUIS allergen nomenclature data base (http://www.allergen.org/index.php). Constructed allergen expression plasmids have been deposited in Belgium collection of microorganisms (BCCM; https://bccm.belspo.be/catalogues) for the open-access and public use.

Screening for New Methods for Treatment of Neurodegenerative Disorders

In this project, a range of recombinant human endoplasmic reticulum (ER) chaperones, which potentially could inhibit aggregation of proteins involved in progression of neurodegenerative disorders (ND), are to be tested using both *in vitro* and *in vivo* models for these diseases. Inhibition of aggregation will be 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. The most efficient ways to deliver recombinant ER chaperones through a blood brain barrier (BBB), and their ability to cross the BBB, will be investigated.

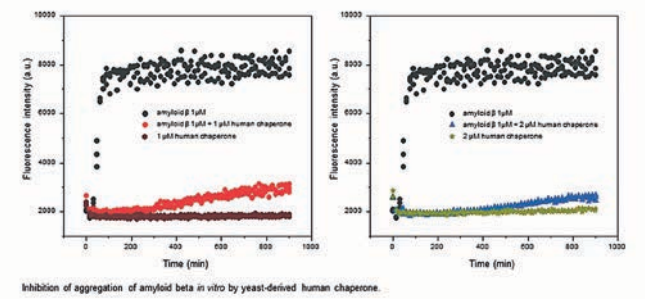
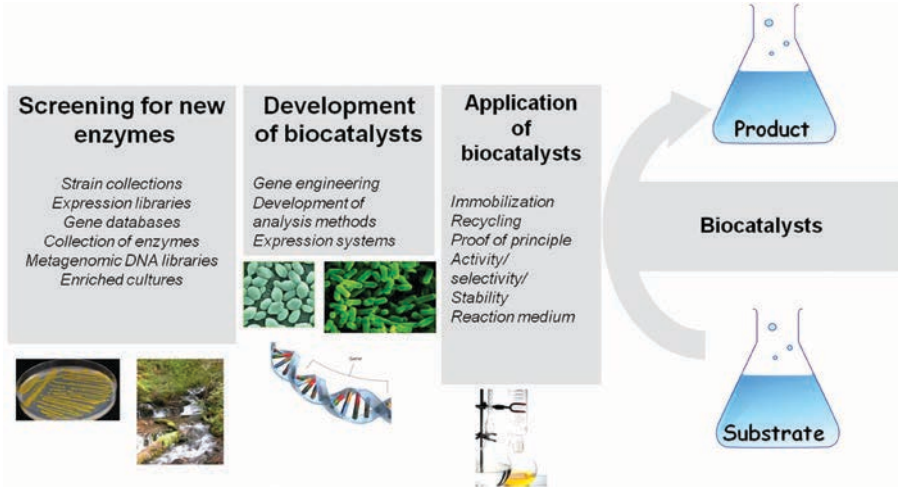


Fig. 2. Inhibition of aggregation of amyloid beta *in vitro* by yeast derived human chaperone.



INGA MATIJOŠYTĖ
Senior Researcher
Head, Division of Applied Biocatalysis
Institute of Biotechnology
Email: inga.matijosyte@bti.vu.lt
Phone: +370 5 223 4371

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 180 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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Synthetic Polymer Biodegradation

One of the sector's topics of interest is biodegradation of various synthetic polymers. It is a relatively new field of research focusing on finding possible ways to degrade synthetic polymers that comprise a major industrial and environmental problem – plastic containing waste. Such waste has a significant ecological impact and is being increasingly regulated on the European Union level. For example, the EU proposed a ban of burying recyclable waste in landfills, and a goal of ensuring that all plastic packaging is reusable or recyclable by 2030. Synthetic polymers are used in various sectors, hence their recyclability is different and depends on their chemical structure. One of the hardiest synthetic polymers are 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 organisms that are capable to degrade it. For this purpose, various environmental samples have been screened to identify PU degrading organisms. In total, bacteria belonging to eleven different genera have been isolated as potential PU degraders. One of the isolated bacteria, *Lysinibacillus* sp., contained a gene coding an enzyme that has urethanase activity (ID: TBS 101 urethanase). This enzyme could potentially be used to hydrolyse urethane bond in polyurethane.

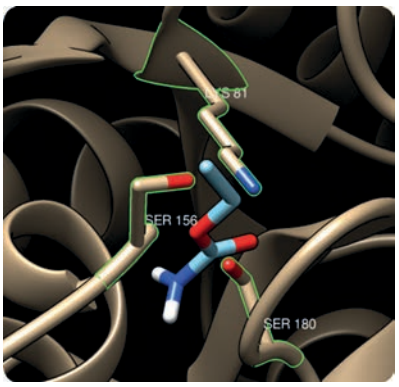


Fig. 1. Molecular docking of urethanase using urethane as a substrate.

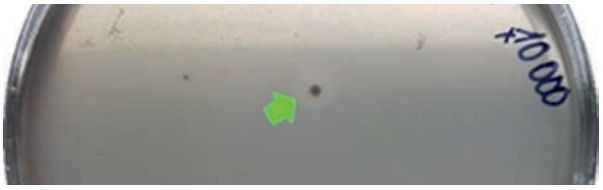


Fig. 2. Hydrolysis zone formed by *Rhodococcus* sp. on a selective growth media containing polyether polyurethane. Bacteria was isolated from Lithuanian soil.

Synthesis of Substrates for Functional Analysis

Laccase is an oxidoreductase used for various applications such as bioremediation, biofuel production, textile pre-treatment, paper pulp manufacturing, food processing, improvement of household chemicals, organic synthesis and many more. However, this enzyme shows 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 a few factors. One of these factors is a lack of substrate variety for the enzyme functional analysis. Therefore, in the current PhD project, we are creating and investigating specifically designed polyamine compounds for laccase spectrophotometric activity assay, well-plate and agar-plate based screening methods. Recent results have shown that one of the agar-plate methods can selectively detect laccase activity as low as 0.1 U/ml (1 U/ml = 1 µmol

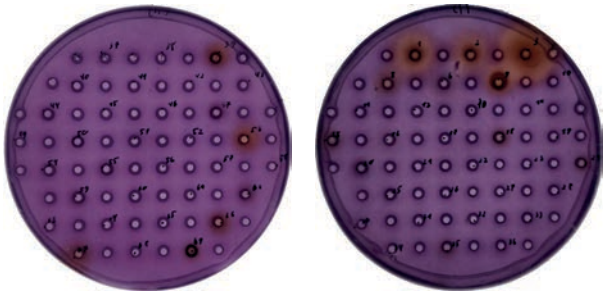


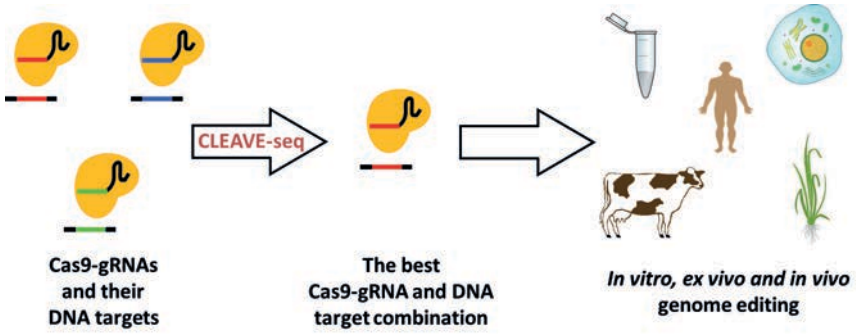
Fig. 3. Visual presentation of the developed agar-plate screening method.

metol catalysed in a minute at pH 5.0 and room temperature by Novozym® 51003 laccase). The collection of wild fungi protein extracts provided by Jožef Stefan Institute, Slovenia, was screened using this method.

European Transdisciplinary Networking Platform for Marine Biotechnology

Marine organisms produce a vast diversity of various enzymes, proteins, metabolites etc. Marine biomass itself can serve as the source material for the production of various bulk commodities (e.g., biofuels, bioplastics, biomaterials). The sustainable exploitation of marine bio-resources and the development of biomolecules and polymers are also known as the growing field of marine biotechnology. Up to now, over 35 000 natural products have been identified 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, inclusive, sustainable, 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), an Action within the European Cooperation in Science and Technology (COST), which connects stakeholders with an interest in marine biotechnology in Europe and beyond. The scientific collaboration agreement with the University of Torino was signed in 2020, another agreement with Jožef Stefan Institute, Slovenia, will be launched in 2021. Close collaboration with Marine Research Institute of Klaipėda University, Lithuania, resulted in a joint publication and a thesis for scientific conferences.

Ocean4Biotech
COST Action CA18238



MINDAUGAS ZAREMBA
Research Professor
Department of Protein-Nucleic Acids Interactions
Institute of Biotechnology
Email: mindaugas.zaremba@bti.vu.lt
Phone: +370 5 223 4357

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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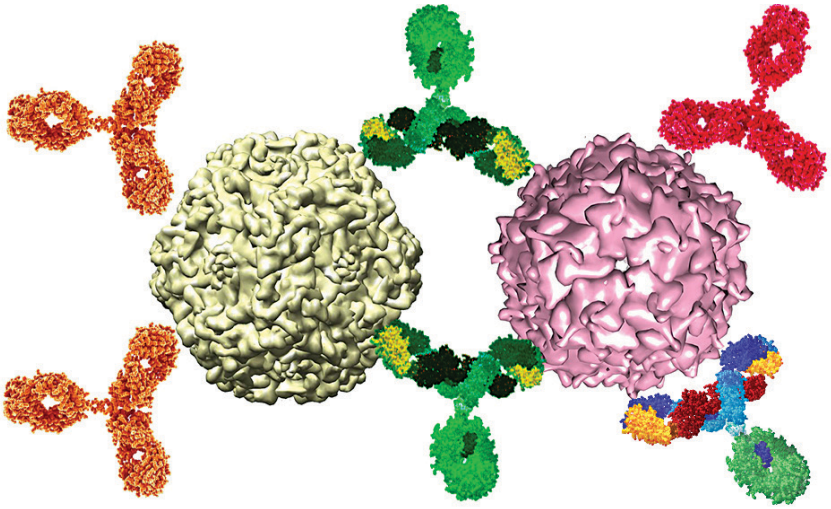

AURELIJA ŽVIRBLIENĖ
Research Professor
Head, Department of Immunology and Cell Biology
Institute of Biotechnology
Email: aurelija.zvirbliene@bti.vu.lt
Phone: +370 5 223 4360

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 [1], cellular proteins [2], 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 [3] 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. During COVID-19 pandemic, our team contributed to developing microarray-based serologic assays for SARS-CoV-2 infection in collaboration with Lithuanian biotech companies *UAB Baltymas* and *UAB Imunodiagnostika*.

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 [4]. 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.

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/.

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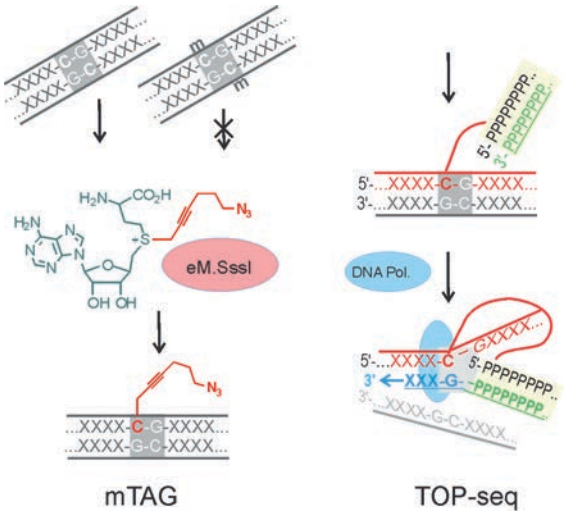
SAULIUS KLIMAŠAUSKAS
Distinguished Professor
Head, Department of Biological DNA Modification
Institute of Biotechnology
Email: saulius.klimasauskas@bti.vu.lt
Phone: +370 5 223 4350

EDITA KRIUKIENĖ
Senior Researcher
Department of Biological DNA Modification
Institute of Biotechnology
Email: edita.kriukiene@bti.vu.lt
Phone: +370 5 2234351



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 bioorthogonal reporter tags. Among the variety of enzymes involved in nucleic acids metabolism, AdoMet-dependent methyltransferases (MTases) uniquely combine two features required for targeted labelling: recognition of a specific target and its covalent modification. To unlock the technological potential of these enzymes we seek to repurpose them for the transfer of pre-derivatized (extended) versions of the methyl group. A series of synthetic analogs of the AdoMet cofactor were developed that allowed MTases to tag DNA and RNA with extended moieties [1], inspiring the appearance of the mTAG technology (methyltransferase-directed Transfer of Activated Groups) for targeted covalent derivatization and labelling of DNA and RNA [2,3]. 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 [4]. 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 [5]. 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.

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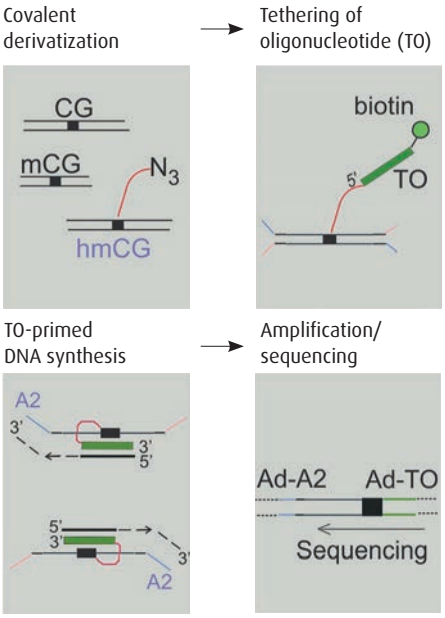
Related Patents: EP2414528 (B1), US8822146 (B2), US8889352 (B2), US9505797 (B2), EP2414527 (B1), LT5706 (B), US9988673 (B2).

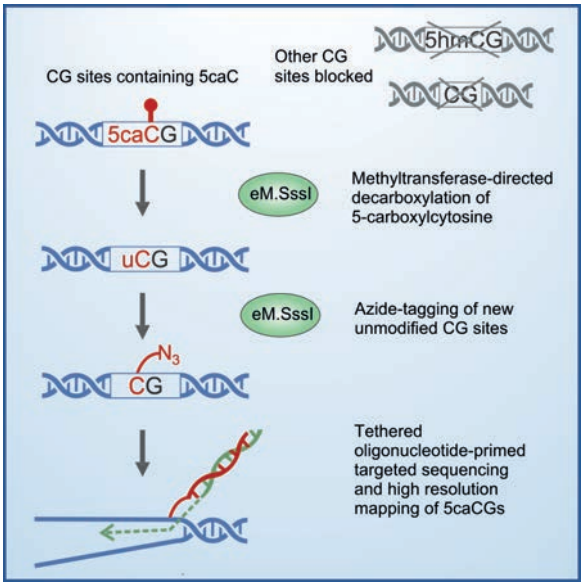
Precise Genomic Mapping of 5-Hydroxymethylcytosine via Covalent Tether-Directed Sequencing

5-hydroxymethylcytosine (5hmC) is the most prevalent oxidized form of 5mC and is implicated in DNA demethylation and transcriptional regulation in different biological settings. Profiling of this relatively scarce genomic modification in clinical samples requires cost-effective high-resolution techniques that avoid harsh chemical treatment. We developed a bisulfite-free approach for 5hmC profiling at single-nucleotide resolution, named hmTOP-seq, which is based on direct sequence readout primed at covalently labelled 5hmC sites from an *in situ* tethered DNA oligonucleotide. The developed procedure was used to construct 5hmC maps in mouse embryonic stem cells, which revealed subtle differences in 5hmC distribution at various genomic elements, intron-exon junctions and both strands of protein-coding genes. Collectively, hmTOP-seq provides a new valuable tool for cost-effective and precise identification of 5hmC in characterizing its biological role and epigenetic changes associated with human disease (Gibas et al. *PLOS Biol.* 2020, 18(4): e3000684).



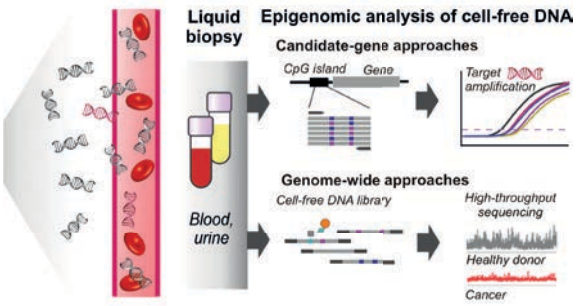
Bisulfite-Free Approach for Base-Resolution Analysis of Genomic 5-Carboxylcytosine

Due to an extreme rarity of 5-carboxylcytosine (5caC) in the mammalian genome, investigation of its role presents a considerable challenge. Methods based on bisulfite sequencing have been proposed for genome-wide 5caC analysis which demand significant experimental and computational resources. We developed a bisulfite-free approach, caCLEAR, for high-resolution mapping of 5caCGs. The method uses an atypical activity of the methyltransferase eM.SssI to remove the 5-carboxyl group from 5caC, generating unmodified CGs [5], which are then mapped by uTOP-seq sequencing [6]. Following validation on model DNA systems, we constructed genomic 5caCG profiles of naive and primed for development pluripotent mouse embryonic stem cells to show their distinct demethylation dynamics and association of 5caC with gene expression and cell state. Our studies thus demonstrate that caCLEAR is a robust economical approach suitable for gaining deeper insights into biological roles of 5caC (Ličytė et al. *Cell Rep.* 2020, 32: 108155).



Identification of Foetal Unmodified and 5-Hydroxymethylated CG Sites in Maternal Cell-Free DNA for Non-Invasive Prenatal Testing

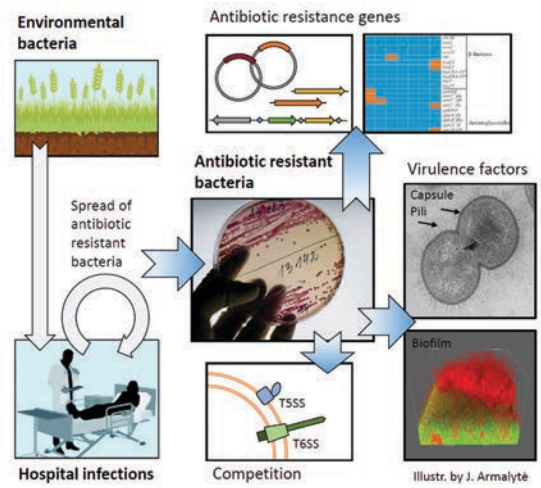
Cell-free DNA (cfDNA) present in various body fluids proved valuable for noninvasive detection of cancer and other diseases or states. In particular, sequencing of maternal cfDNA is widely used to detect foetal genetic abnormalities in non-invasive prenatal testing. We examined if targeting of unmodified or 5-hydroxymethylated CG sites could specifically enrich the foetal genetic material and reduce the numbers of required sequencing reads, thereby decreasing overal analytical costs. Using our previously developed the uTOP-seq [6] and hmTOP-seq [Gibas et al. 2020] techniques we constructed uCG and 5hmCG maps of maternal cfDNA samples which demonstrated that, in contrast to conventional whole genome sequencing, such epigenomic analysis specifically enriches foetal DNA fragments from maternal cfDNA. While both techniques yielded 100% accuracy in detecting Down syndrome



in foetuses, hmTOP-seq maintained a 100% detection accuracy even at ultra-low sequencing depths. Altogether, we show that robust covalent derivatization followed by targeted analysis of foetal cfDNA by sequencing or qPCR presents an attractive strategy for achieving superior sensitivity and specificity in prenatal diagnostics (Gordevičius et al. *Clin. Epigenetics.* 2020, 12: 153).

EDITA SUŽIEDĖLIENĖ
Professor
Department of Biochemistry
and Molecular Biology
Institute of Biosciences
Email: edita.suziedeliene@gf.vu.lt
Phone: +370 5 239 8226

JULIJA ARMALYTĖ
Senior Researcher
Department of Biochemistry
and Molecular Biology
Institute of Biosciences
Email: julija.armalyte@gf.vu.lt
Phone: +370 5 239 8230



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. 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 opportunistic pathogen *Acinetobacter baumannii* causes a variety of difficult-to-treat nosocomial infections to critically ill patients. The characteristic features of *A. baumannii* 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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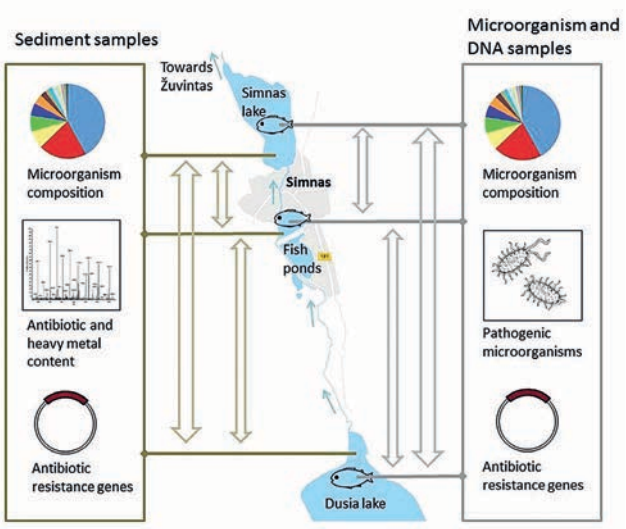
The Impact of Fish Farming on Microbiota and Antibiotic Resistance of the Ecosystem

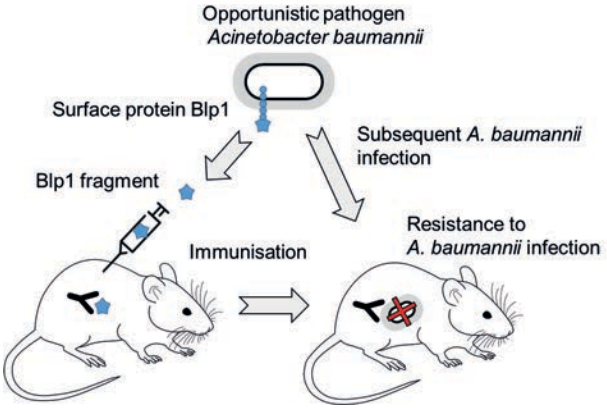
Aquaculture is a fast growing animal food sector. As fish are susceptible to infectious diseases, antibiotics are used in aquaculture production systems. The constant exposure to antimicrobials could contribute to antibiotic resistance selection in aquaculture products and the ecosystem, with a possibility of dissemination.
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.
The project is carried out together with the Lithuanian University of Health Sciences.



Project "The influence of intensive fish farming on aquatic microbiome and resistome" of the National Research Programme Sustainability of *Agro-, Forest and Water Ecosystems*

Acinetobacter baumannii Surface Proteins as Vaccine Targets

A large surface Blp1 protein from opportunistic pathogen *A. baumannii* is present in all the clinically relevant strains examined. A C-terminal fragment of Blp1, which is located on the surface of the cell, elicits an efficient protection to a lethal subsequent *A. baumannii* infection in a murine model.
This indicates that *A. baumannii* Blp1 protein could be considered as a new vaccine candidate. In our recent project "Development of virus-like particles-based vaccine against *Acinetobacter baumannii*", we are exploring the use of smaller Blp1 fragments as vaccination antigens. To increase the immunogenic properties of protein fragments we will display them on virus-like particles.



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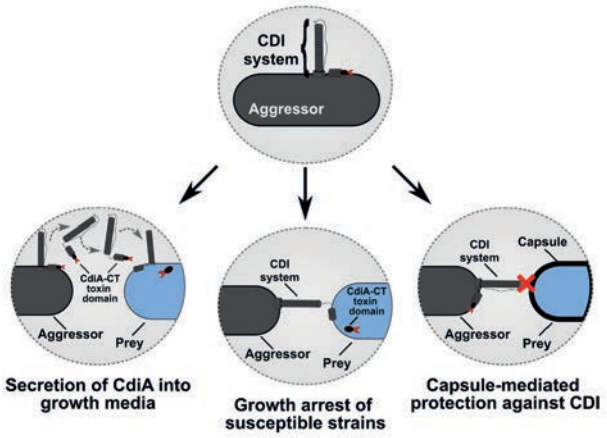
Acinetobacter baumannii Capsule Protects from Contact-Dependent Growth Inhibition

Clinically important pathogens express contact-dependent growth inhibition (CDI) phenomenon, which modulates cell-cell and cell-environment interactions. These systems generally are used for the inhibition of the growth of genetically different individuals within the same species. In this study, we show that *A. baumannii* capsule is the main feature protecting bacterium from CDI-mediated inhibition. We also demonstrate that the CDI component, an effector protein CdiA, can be secreted into the growth media and retains functional activity by causing the growth arrest of the susceptible *A. baumannii* cells.



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ARTŪRAS PETRONIS
Research Professor
Head, Laboratory of Epigenomic Studies
Institute of Biotechnology
Email: art.petronis@camh.ca
Phone: +370 5 210 9730

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 2020, 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 have established collaboration with clinical scientists and started 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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MINDAUGAS VALIUS
Research Professor
Head, Proteomics Center
Institute of Biochemistry
Email: mindaugas.valius@bchi.vu.lt
Phone: +370 5 223 4410

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 match the effective first and second-line therapy to successful treatment.

Our research focus encompasses the novel as well as conventional pathways of cancer cell signalling. In collaboration with the R. Prekeris Lab (University of Colorado 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 [1,4]. 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]. We also applied multiparametric proteomic analysis to reveal the enhanced SCF receptor c-KIT signalling in multidrug-resistant breast cancer cells. The treatment with c-KIT targeted inhibitor was shown to be a promising second-line therapy in the current case of acquired chemotherapy resistance [2].

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SONATA JARMALAITĖ
Professor
Department of Botany and Genetics
Institute of Biosciences
Email: sonata.jarmalaite@gf.vu.lt
Phone: +370 5 219 3242

Genetic and Epigenetic Mechanisms of Cancer Development and Progression

The incidence of cancer is continuing to rise. The current COVID-19 pandemic reduced cancer screening activities, restricted timely access to clinical service and negatively impacted early cancer detection. Thus, in 2021 the European Union is planning to start an implementation of new Cancer Plan with the main goal – to minimise cancer mortality.

During the last decade, the increased understanding of genetic alterations in 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. Genomic data are used for the identification of new drug targets of personalized cancer therapy. Future progress in oncology is feasible through further development and implementation of personalised medicine means.

Using a variety of genome-wide and target-oriented methodologies [1], 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 [2–4]. Focusing on altered DNA methylation, we have recently proposed biomarker panels for prostate cancer detection and more accurate characterization by using liquid biopsy samples [3]. Genetic and epigenetic analyses were used to identify novel players of renal carcinogenesis. Some of metallothionein genes were shown as specifically hypermethylated in renal cell carcinomas and showed significant associations with clinical variables of the disease [2]. In collaboration with the Institute of Biochemistry and National Cancer Institute of Lithuania, we continued the investigation of molecular mechanisms of combined action of dichloroacetate and salinomycin for the development of novel solutions in cancer therapy [4].

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Predictive Value of Germline DNA Damage Repair Gene Mutations in Metastatic Castration-Resistant Prostate Cancer

Prostate cancer (PCa) is a heterogeneous and dynamic disease, which usually progresses to metastatic castration resistant PCa (mCRPC) – the highly lethal form of PCa. Recent data suggest that mCRPC may carry the germline or sporadic mutations in DNA damage response (DDR) pathway genes *BRCA1*, *BRCA2*, *CHEK2*, *ATM*, *NBN* etc. To determine the relationship between DDR mutation status and clinical response to therapy as well as survival outcomes, 149 leukocyte samples from patients with mCRPC were evaluated. Our results demonstrated that pathogenic germline mutations were detected in 15.4% of the patients (Fig. 1) and correlated with inferior responses to conventional treatment. NGS based-studies can uncover the complex genomic landscape of mCRPC and identify novel targets for efficient anticancer therapies.

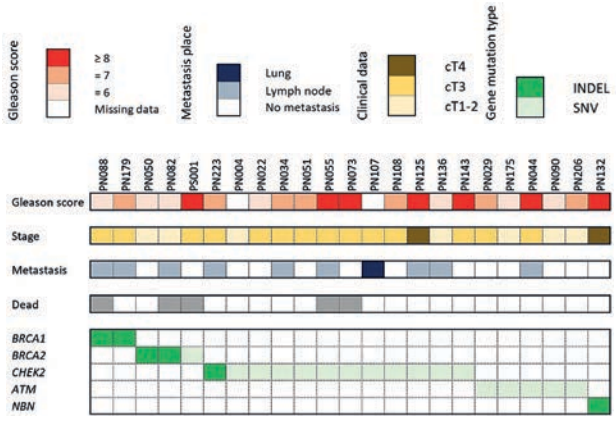


Fig. 1. Combined gene mutation analysis data from 23 patients. Gleason score, metastasis place, tumour stage and gene mutation type is shown.

Androgen Receptor Variant Analysis for Predicting Response to Abiraterone Acetate in Patients with Castration Resistant Prostate Cancer

Androgen receptor (AR-FL) and AR variants (AR-V1, -V3, and -V7) were evaluated in 185 serial blood samples, prospectively collected from 102 patients with castration resistant prostate cancer (CRPC) before and during abiraterone acetate (AA) therapy via reverse transcription quantitative PCR. AR-FL was present in all the samples, while AR-V1, AR-V3, AR-V7 were less frequent, and at least 1 of them was detected in 17%, 55%, 65% and 81% of CRPC patients' blood samples, respectively. The highest amount of AR-V1 was found in the blood of patients whose response time was short and medium in comparison to extended (Fig. 2). Patients with a higher level of AR-FL and/or AR-V1 had the shortest progression-free survival and overall survival ($p<0.0001$). Blood circulating AR-FL or AR-V1 can serve as blood based biomarkers for identification of the primary resistance to AA and the tool to monitor *de novo* resistance development during AA treatment.

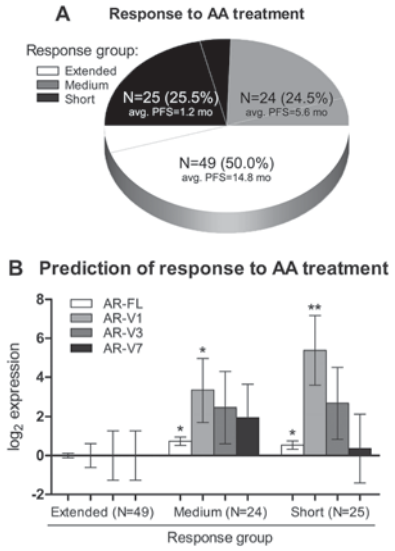


Fig. 2. Prediction of response to AA treatment by level of AR transcripts at baseline and variation of AR transcript level in CRPC blood during treatment. **A** – PFS of patients treated with AA grouped by response time. **B** – Prediction of response to AA therapy from AR-FL and AR-Vs, detected in blood samples collected before treatment initiation. * – $p<0.050$, ** – $p<0.001$

Frequent DNA Methylation Changes in Cancerous and Noncancerous Lung Tissues of Smokers with Non-Small Cell Lung Cancer

Lung cancer (LCa) is the leading cause of cancer-related death. Smoking is one of the major LCa risk factors, and tobacco-related carcinogens are potent (epi)mutagens. The present study evaluated DNA methylation status of five tumour suppressor genes in paired LCa and noncancerous lung tissues (NLT) from 104 patients, 90 of whom were smokers or ex-smokers. Methylation of >1 gene was detected in 59% of LCa samples and in 39% of NLT (Fig. 3). More frequent methylation of >1 gene was observed in LCa samples of ever smokers (63%) as compared with never smokers (36%). To sum up, DNA methylation changes are widespread in cancerous and NLT from smokers creating an epigenetically altered field for cancer development and further spread.

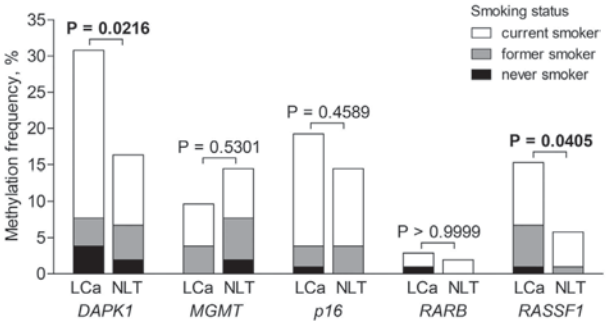
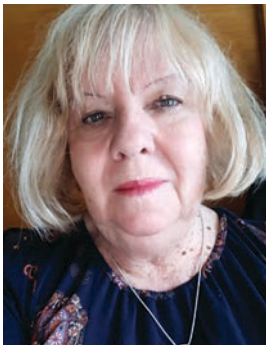


Fig. 3. DNA methylation frequencies of tumour suppressor genes in cancerous (LCa) and noncancerous lung tissues (NLT) according to patients' smoking status.



VERONIKA BORUTINSKAITĖ
Senior Researcher
Department of Molecular Cell Biology
Institute of Biochemistry
Email: veronika.borutinskaite@bchi.vu.lt
Phone: +370 5 223 4406

AUDRONĖ V. KALVELYTĖ
Senior Researcher
Department of Molecular Cell Biology
Institute of Biochemistry
Email: audrone.kalvelyte@bchi.vu.lt
Phone: +370 5 223 4407

Molecular Mechanisms of Cancer Cell Treatment

Cancer is the disease caused by alterations in genes coding cell proliferation, apoptosis and differentiation of controlling proteins. 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, heterogeneous group of malignancies with different clinical behaviours and different responses to therapy. AML karyotypes are most commonly classified into three prognostic categories with differing median survivals as follows: 1) favourable risk, 7.6 years; 2) intermediate risk, 1.3 years; 3) poor risk, 0.5 years. The 5-year relative survival of adults diagnosed with AML was less than 10%. Given the poor prognosis, patients are encouraged to participate in clinical trials and/or pursue aggressive therapy. For many types of cancer, identifying the cancer early makes it easier to treat. There are a few screening tests on the market for an early detection of certain cancers in people without any symptoms. However, at this time, there are no special tests recommended to find acute myeloid leukaemia (AML) early. Identifying prognostic molecular markers and understanding their biology are the first steps toward developing novel diagnostic tools or/and therapies for patients with AML. Our team has over 30 years of experience in the cancer research field: human leukaemia differentiation and epithelial cancer growth inhibition/death molecular mechanisms.

Signalling in Cancer Treatment. Understanding cell death signalling networks concerning anticancer drug treatment is essential for identifying new drug targets and biomarkers in cancer therapy. Manipulating chemotherapeutic drug-induced signalling provides a promising strategy for targeted cancer treatment. The sequence of events and relations among signalling molecules, leading cancer cells to apoptosis or protecting normal cells during anticancer drug treatments are studied by the scientists of our team (A. Kalvelytė, A. Imbrasaitė, N. Krestnikova and A. Stulpinas). The induction or down-regulation of different and opposite cell signalling pathways, which may counteract one another, were found in lung cancer and muscle-derived stem cells as well as their differentiated progenies during chemotherapeutic treatments current tumour cell models and combination therapies directed to signalling molecules were rewieved (Kalvelyte et al. 2013, 2015; Abdelwahid et al. 2015, 2017; Stulpinas et al. 2012; 2016; Krestnikova et al. 2015), [4, 5].

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Epigenetic Treatment 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.
Today, our research is focused on the following:

- on the pharmacological manipulation of chromatin remodelling that might develop into a potent and specific strategy for the treatment of leukaemia;

We found that the combination treatment we used had a slightly greater effect on inhibiting cell proliferation and a stronger effect on the induction of apoptosis than conventional treatment.

- on the establishment of novel, potentially prognostic biomarkers useful for the diagnostics of leukaemia or disease outcome predictions.

Our results suggested that detailed analysis of bone marrow samples for c-Myc gene expression of APL patients can predict success in treatment. WT1 gene expression can predict relapse cases earlier than changes in peripheral blood parameters or than bone marrow blast count (data not published).

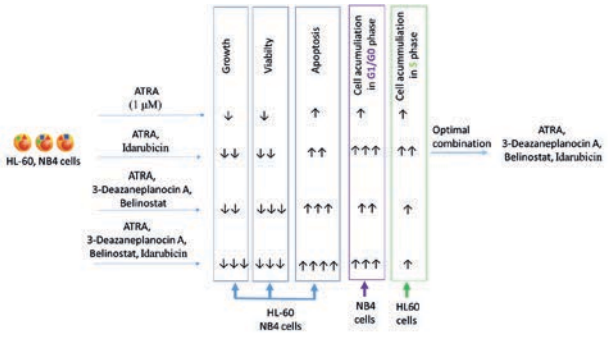


Fig. 1. Potential of a histone deacetylase (HDAC) inhibitor and a histone methyltransferase (HMT) inhibitor to enhance conventional therapy *in vitro*. NB4 and HL60 cell lines were used as an *in vitro* model. Cell samples were exposed to Belinostat (HDAC inhibitor) and 3-Deazaneplanocin A (HMT inhibitor) alone or in combination with standard chemical agents (Retinoic acid (ATRA) and Idarubicin) used in the treatment.

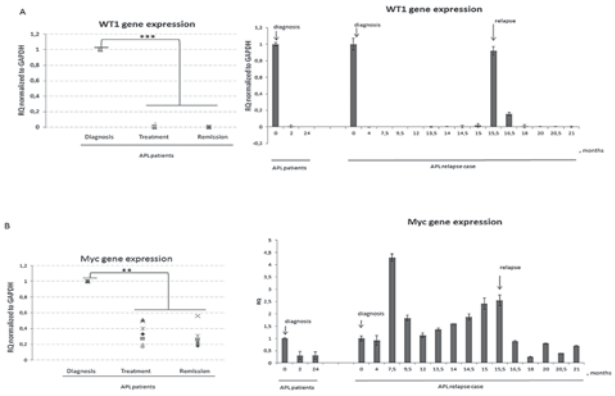
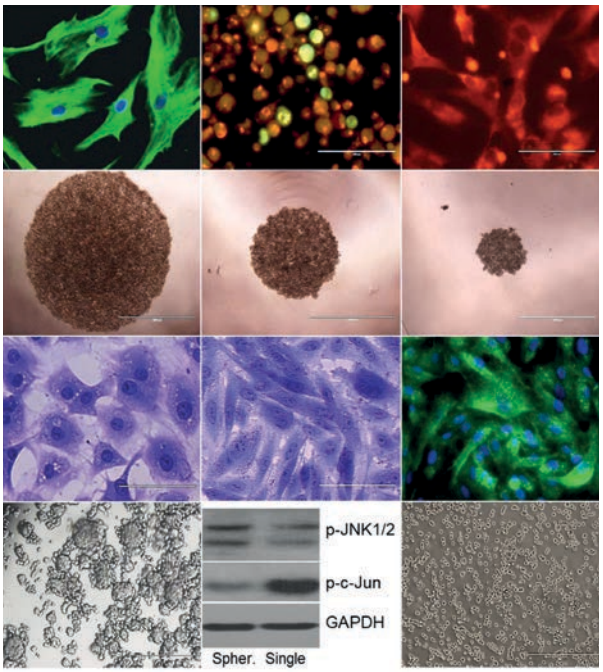
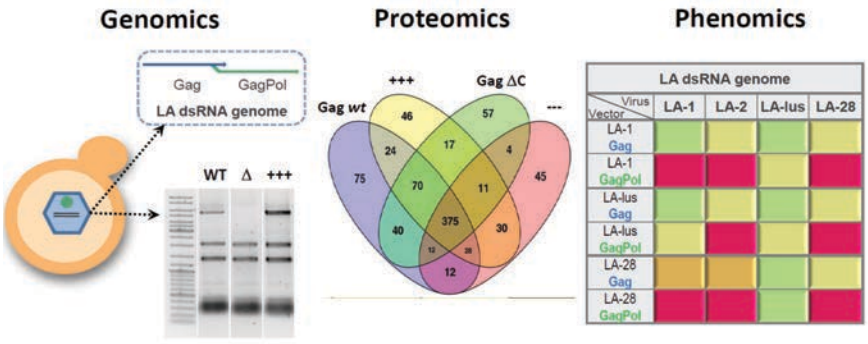


Fig. 2. Prognostic biomarkers useful for the diagnostics of leukaemia or disease outcome predictions. WT1 gene (A) and c-Myc gene (B) expression was determined by RT-qPCR method.

Signalling in Lung Cancer Cell Fate Regulation

During the implementation of the SMART project in 2020, by using a panel of phenotypically and genotypically different lung cancer and stem cell lines, we have studied dependency of the intracellular cell fate-determining signalling molecules on extracellular contacts. Modelling different cellular states, adherent, single-cell suspension, and aggregated cells were compared in respect of cellular signalling. The studies revealed cell state-dependent changes in the phosphorylation of protein kinases studied, pointed out differences in ERK1/2 activation between the cell lines under detachment conditions, highlighted the different dependency of molecules of the same signalling pathway, JNK and c-Jun, on cell-cell contacts.
Within the frame of the project, as a chapter of the book, we have published an overview of current *ex vivo* tumour cell models and clinically relevant platforms to functionally test drug combinations for cancer treatment outcome prediction in individual patients, encompassing the phenotypic cell heterogeneity and solving the problem of cancer resistance.





SAULIUS SERVA
Professor
Department of Biochemistry and Molecular Biology
Institute of Biosciences
Email: saulius.serva@gf.vu.lt
Phone: +370 5 239 8244

Molecular Virology: Mechanisms, Evolution, Antivirals

The *Totiviridae* family dsRNA viruses from the *Saccharomycetaceae* family yeast are ubiquitous yet poorly understood benign inhabitants of the host. In our lab, they are being investigated by means of molecular biology techniques. The impact of the dsRNA viruses uncovered by genomic, transcriptomic, proteomic and phenomic analysis is interpreted as a model framework for establishing the universal mechanisms behind any virus of interest, in such a way creating a paradigm network for virus-host interactions. We aim at the understanding of intra- and extracellular relations of yeast dsRNA viruses in order to elucidate the evolutionary pathways of these viruses and reveal the ultimate principles of distribution within an ecosystem [1].

Nucleoside and nucleotide-based antivirals constitute the essence of modern high-efficacy antiretroviral HIV treatment. Once a revolutionary approach upon discovery, nowadays it suffers from an emerging resistance and multiple side effects due to life-long administration. 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], constituent genes of a virus genome were re-introduced into model hosts to manipulate the phenotype conferred by the virus. 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 a 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, in such a way ensuring selectivity among a variety of cellular polymerases.

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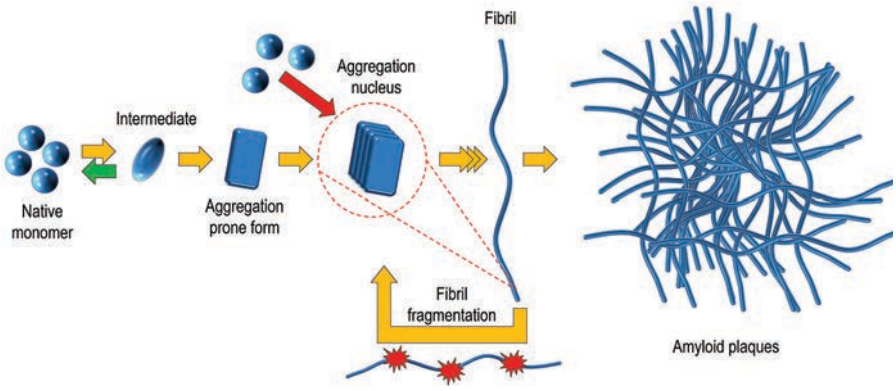
KRISTINA DANIŪNAITĖ
Associate Professor
Department of Botany and Genetics
Institute of Biosciences
Email: kristina.daniunaite@gf.vu.lt
Phone: +370-5-2234441

Strategies in Fighting the 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 late 2019, the pandemic has spread rapidly worldwide, which resulted in incapacitated healthcare systems and disrupted socioeconomic activities. Despite stringent control measures (quarantine, social distancing and work from home policies, etc.) deployed by the governments, the high transmission rate of SARS-CoV-2 has led to outbreaks in public gathering places as well as workplaces either due to their poor implementation or limited efficiency. Currently, our group focuses on developing new strategies for SARS-CoV-2 detection and improved diagnostic testing.

Pooled-Sample Testing. The emergence of COVID-19 has introduced an acute increase in the diagnostic demand that requires optimization of laboratory workflows. Pooled-sample testing is a promising strategy to screen large populations rapidly with limited resources. Using nasopharyngeal swab samples from a local biobank, we have estimated that the optimal pool size of five samples would allow to significantly reduce the testing costs retaining the diagnostic sensitivity at >86%. As the economic benefit of sample pooling depends on the prevalence in a population, we have estimated that implementation of this pooling strategy would be most effective and would allow to reduce the testing costs as well as time and manpower at least twice if applied to a group of individuals with the expected positivity rate of <6.5%.

Environmental Surface Testing. One of the reasons why this pandemic has been difficult to contain is the inability to identify presymptomatic and asymptomatic SARS-CoV-2 carriers as some of these individuals can be highly contagious when they have mild or no symptoms. Such individuals can shed a high viral load in their workplace and expose co-workers to constant fomite spread. We assayed over 200 samples of environmental surfaces in the Life Sciences Center of Vilnius University, which led to the identification of several pre-/ asymptomatic carriers among the community. The surfaces tested included door handles of the entrances and corridors, elevators' keypads, light switches, control panels of appliances, etc. Most of the samples were collected from the offices, which are typically shared by 2–3 employees. Surprisingly, the amount of viral RNA in some of the surface samples was as high as commonly observed in nasopharyngeal swab samples from symptomatic patients. This allowed not only to identify potentially infected individuals but also to estimate the spread of the fomite in the facility. Moreover, trace amounts of viral RNA found in two laboratories were associated with the individuals working there who tested positive roughly a week after the surface samples had been collected. The surface testing has been performed at four other institutions, including one hospital (>250 samples in total), and showed promising results for further validation steps of the methodology.



VYTAUTAS SMIRNOVAS
Research Professor
Department of Biothermodynamics and Drug Design
Institute of Biotechnology
Email: vytautas.smirnovas@bti.vu.lt
Phone: +370 670 69929

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, sup35NM domain and beta-microglobulin. The main methods used to follow amyloid formation include UV, visible and fluorescence spectrometry (a Thioflavin T fluorescence assay as the main method to follow kinetics), Fourier transform infrared spectrometry and atomic force microscopy.

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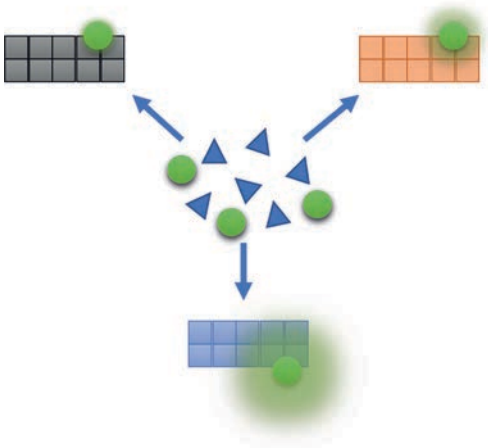
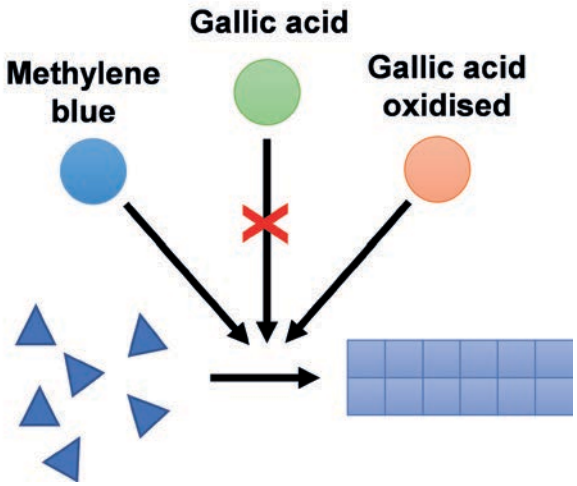
Polymorphism of Amyloid Fibrils

Thioflavin T (ThT) fluorescence assay is one of the main methods used to detect amyloid fibrils and follow aggregation kinetics. We have demonstrated that deeper studies of ThT binding and fluorescence characteristics makes it possible to differentiate between structurally distinct amyloid aggregates (Ziaunys et. al. *Biomacromolecules.* 2020) without employing more complex techniques. Using this knowledge, we were able to perform analysis of many samples in a short time and found out that distinct conformations of prion protein amyloid fibrils can form even in the identical conditions (Ziaunys et. al. *Scientific Reports*. 2020).



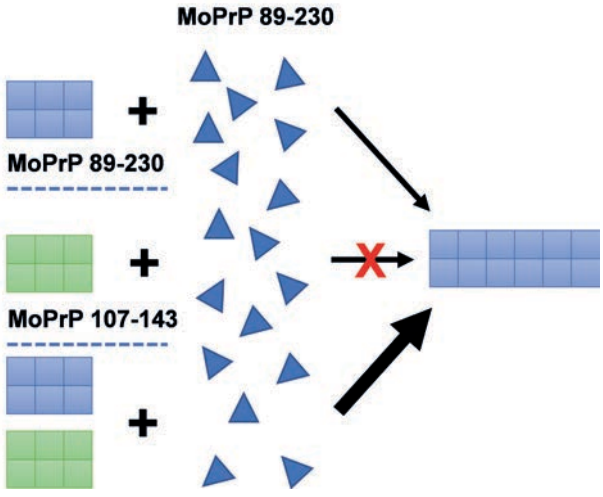
Inhibition of Amyloid Formation

Amyloid protein aggregation is related to tens of diseases, including some widely spread ones, which makes the search for potential anti-aggregation drugs vitally important. We have demonstrated that Methylene blue can inhibit both the spontaneous amyloid aggregation of superoxide dismutase 1 (related to amyotrophic lateral sclerosis) as well as elongation of preformed fibrils (Musteikyte et al. *PeerJ*. 2020). Also, we have continued our work on polyphenolic compounds and found out that gallic acid greatly enhances its anti-amyloid potency upon oxidation (Sakalauskas et al. *Scientific Reports*. 2020). Since 2020, we participate in projects for screening sulfonamide compounds and testing recombinant endoplasmic reticulum chaperones as inhibitors of amyloid aggregation.



Cross-Interactions in Amyloid Aggregation

Self-replication is one of the key hallmarks of amyloid fibrils. Typically, aggregates composed of a certain type of monomer tend to elongate by incorporating proteins pertaining to the same amino acid sequence. In recent years, it has been shown that amyloid fibrils, generated from one type of protein, can either elongate using a different kind of protein or affect their rate of aggregation. Interestingly, aggregates of a prion protein fragment (107-143) do not elongate in the presence of 89-230 monomers, however, they have a synergistic effect when in the presence of both 89-230 fibrils and monomers. These 107-143 fragment aggregates greatly enhance the self-replication properties of 89-230 fibrils, without incorporating any of the native protein into its own structure (Sneideris et al. *International Journal of Molecular Sciences*. 2020). In addition, we have started a project "Cross-interactions in amyloid fibril formation: from mechanisms to inhibition" funded by the Research Council of Lithuania and helped our colleagues in the study of interactions between S100A9 and Amyloid beta (Pansieri et al. *Chemical Science*. 2020).



INGA GRIŠKOVA-BULANOVA
Research Professor
Department of Neurobiology and Biophysics
Institute of Biosciences
Email: inga.griskova-bulanova@gf.vu.lt
Phone: +370 671 10954

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 [2, 3]) and pathological states (i.e. prolonged disorders of consciousness, schizophrenia and association to clinical symptoms [1, 4, 5]).

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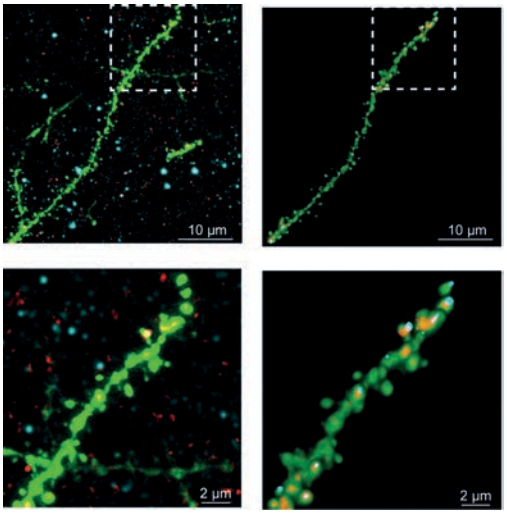
URTĖ NENIŠKYTĖ
Researcher
Email: urte.neniskyte@gf.vu.lt
Phone: +370 5 233 4437



Molecular Signalling Pathways for Synaptic Pruning

The development of the mammalian nervous system is associated with the generation of excess neuronal synapses, which is followed by their removal – a process known as synaptic pruning. Depending on the area of the brain, up to 70% of pre-formed synapses are lost during developmental circuitry refinement. Appropriate synaptic pruning appears to be required for the strengthening of remaining synapses and is critical for normal brain development. Under-pruning or over-pruning may lead to neuropsychiatric conditions such as autism spectrum disorders, schizophrenia or epilepsy. Recent studies have revealed that unnecessary synapses may be eliminated by resident immune cells – microglia. 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.

Our current projects are:

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) The Role of Maternal Metabolic Status for the Neurodevelopment of the Offspring. Vilnius University Science Promotion Fund (2020);
4) GLIOGLY: The Investigation of the Glycobiology ofDdissected Glioblastoma Human Brain Tissue. The Baltic-German University Liaison Office, the German Academic Exchange Service (DAAD) in collaboration with the Albert Ludwig University of Freiburg (2020).

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OSVALDAS RUKŠĖNAS
Professor
Head, Department of Neurobiology and Biophysics
Institute of Biosciences
Email: osvaldas.ruksenas@gf.vu.lt
Phone: +370 687 45546

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.

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, electrophysiological and neurovascular levels.

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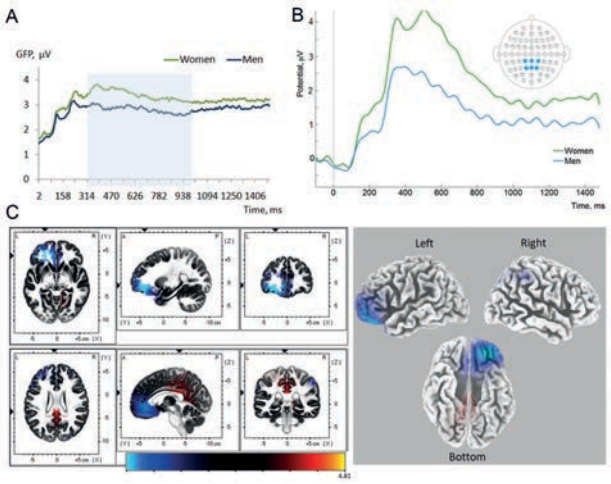
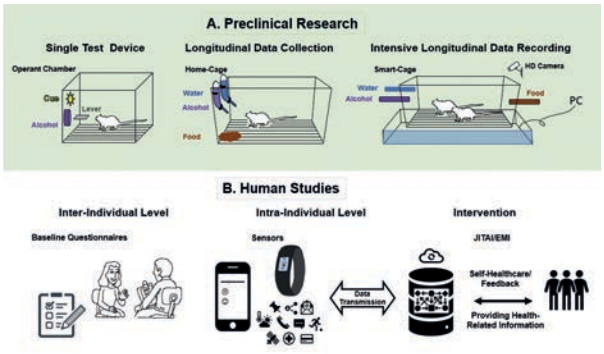
Mental Rotation of Sequentially Presented 3D Figures: Sex and Sex Hormones-Related Differences in Behavioural and ERP Measures

Mental rotation of 3D objects demonstrates one of the largest sex differences. We investigated sex and sex hormones-related differences in behavior and event related potentials (ERP) using a modified Shepard and Metzler task composed of sequentially presented 3D figures in 29 men and 32 women. We demonstrated a significant increase in response time and decrease in both accuracy and positivity of the parietal ERP with increasing angular disparity between the figures. Men performed the task more accurately than women. Performance accuracy in women tended to be negatively related to estradiol while the response time tended to increase with increasing progesterone.



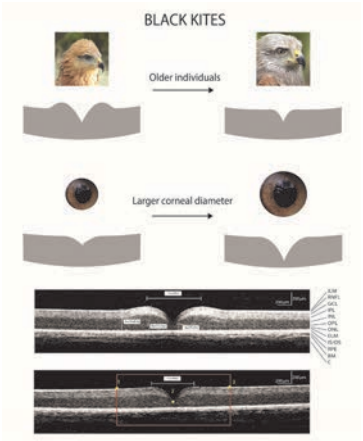
Translational Approach to Understanding Momentary Factors Associated with Alcohol Consumption

Multiple inter- and intra-individual factors underlie variability in drinking motives, challenging clinical translatability of animal research and limiting treatment success of substance-use related problems. Intra-individual variability refers to time-dependent continuous and discrete changes within the individual, and in substance-use research is studied as momentary variation in the internal states (craving, stressed, anxious, impulsive, tired) and response to external triggers (stressors, drug-associated environmental cues, social encounters). These momentary stimuli have a direct impact on behavioural decisions, and may be triggers and predictors of substance consumption; they also present potential targets for real-time behavioural and pharmacological interventions.



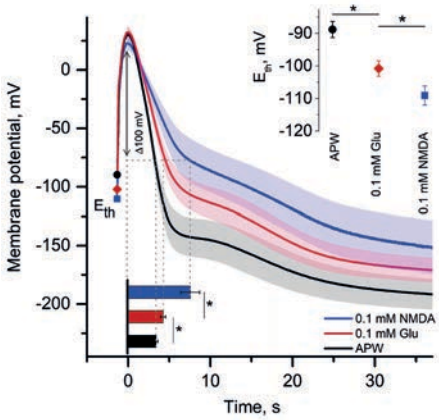
Inter-individual Differences in Foveal Shape in a Scaventing Raptor, the Black Kite *Milvus migrans*

A fovea is a retinal region where photoreceptor densities are highest and other retinal layers are displaced. Some primates have a shallow fovea whereas many birds possess shallow or deep fovea. Although the function of the foveal shape remains unknown so far and intraspecific variation largely unstudied, we examined the eyes of 47 black kites *Milvus migrans* using spectral domain optical coherence tomography. We found that younger individuals had a wider fovea with a more pronounced rim and the fovea was deeper in larger eyes. No relationship between foveal shape and genetic proximity was found, suggesting that foveal shape is not a hereditary trait.



Glutamate and NMDA Affect Cell Excitability and Action Potential Dynamics of Single Cell of Macrophyte *Nitellopsis obtuse*

Alterations of action potentials (AP) and excitation current transients upon glutamate and NMDA was studied in a single intact macrophyte *Nitellopsis obtusa* (Characeaen) internodal cell. Investigation of the neurotransmitters indicate that exposure to Glutamate (Glu, 0.1-1 mM) and NMDA (0.01-1 mM) directly alters the excitability of plant cell: increases electrically induced AP amplitude by hyperpolarizing excitation threshold potential (Eth) and prolongs AP fast repolarization phase.



AURELIJUS BUROKAS
Senior Researcher
Department of Biological Models
Institute of Biochemistry
Email: aurelijus.burokas@gmc.vu.lt
Phone: +370 5 223 4382

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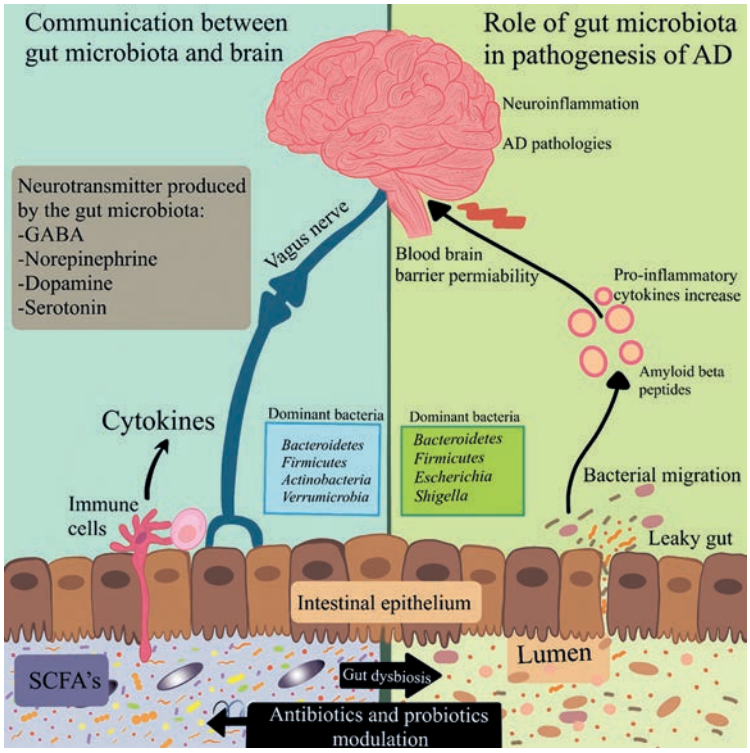
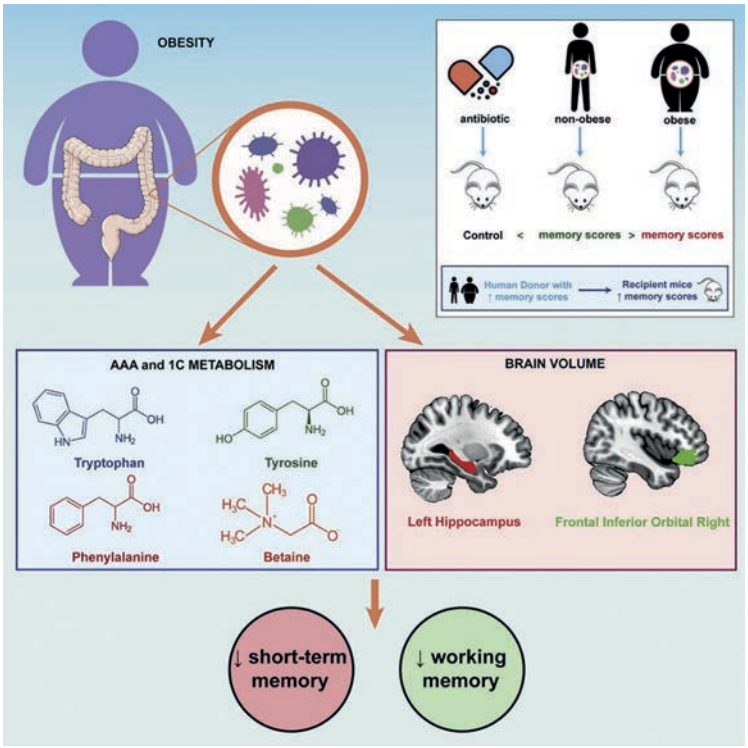
Modulation of the Microbiota–Gut–Brain Axis by Probiotics, Prebiotics, and Antibiotics

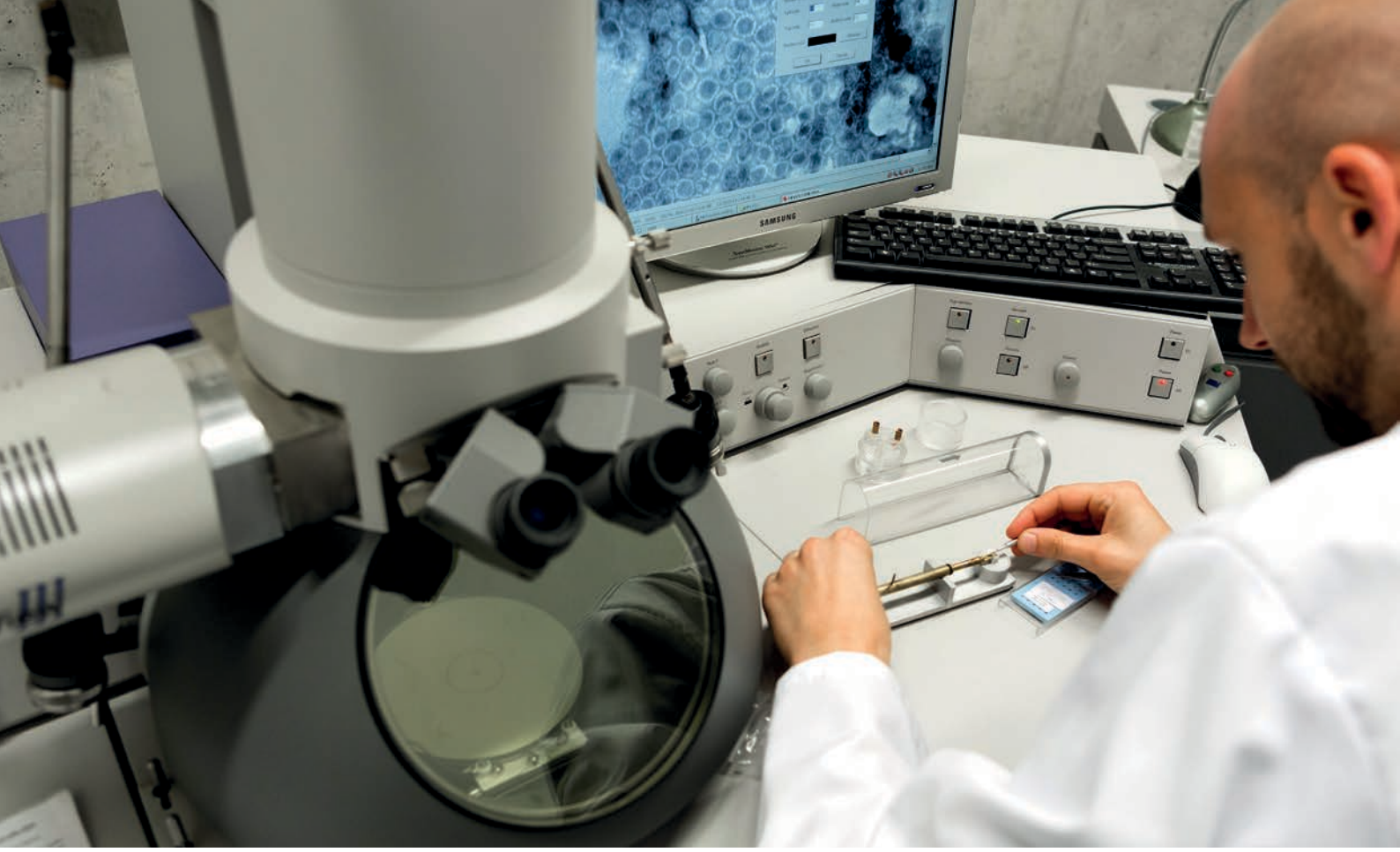
The communication between the gut microbiota and the brain includes neuronal, immune-mediated, and metabolite-mediated pathways. Gut dysbiosis leads to activation of the immune response and alters the production of neurotransmitters as well as bacterial metabolites. These may have a contribution to abnormal 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 have the potential to normalize the gut microbiota in microbiota–gut–brain processes (Megur et al. *Nutrients*. 2021).



Modulation of the Microbiota–Gut–Brain axis by Microbiota Transplantation

Mice are one of the most popular animal models used in biomedical research and have been adopted as one of the primary animal models used in microbiota research. On the contrary to probiotics studies, here it involves transferring a complex gut microbiota. Thus, microbiota transplantation from humans to mice is an important tool to study the relationship between disease and the intestinal microbiota and its role on host physiology. Interestingly, learning and memory have recently been associated with microorganisms and their metabolites. We with our partners in Spain have revealed a unique microbiota profile associated with memory through pathways involving aromatic amino acid and one-carbon metabolism. Importantly, these relationships are modulated by obesity; faecal microbiota transplantation from human subjects with obesity decreases the memory score of recipient mice (Arnoriaga-Rodríguez et al. *Cell Metabolism*. 2020).





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.

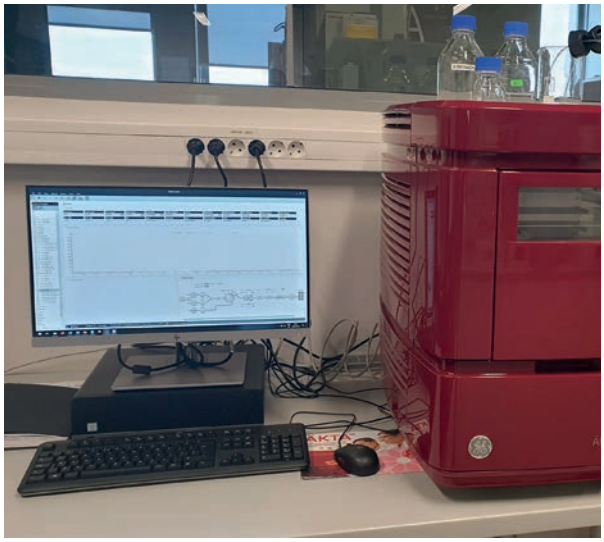
CONTACT INFORMATION

VIRGINIJA BUKELSKIENĖ
Email: virginija.bukelskiene@gmc.vu.lt
Phone: +370 5 223 4408

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.

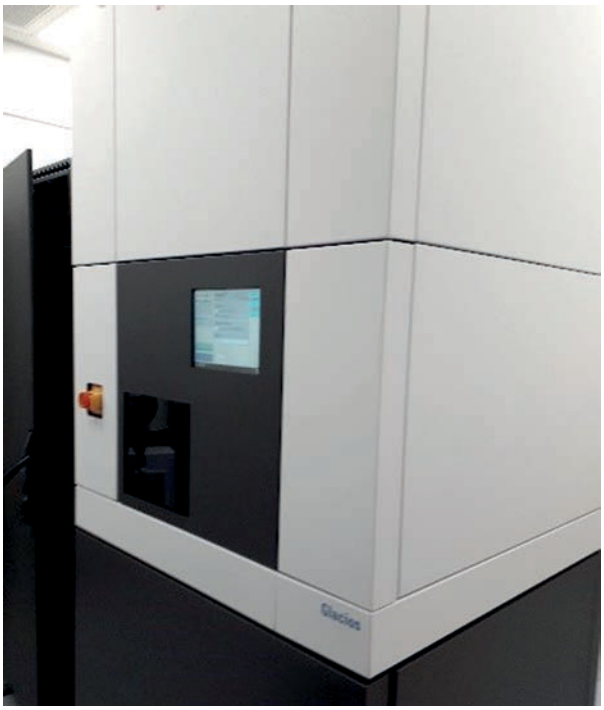


CONTACT INFORMATION

ARŪNAS ŠILANSKAS
Email: arunas.silanskas@bti.vu.lt

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).



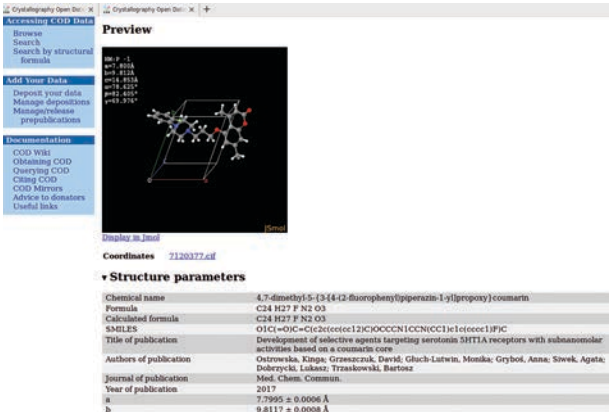
CONTACT INFORMATION

GIEDRĖ TAMULAITIENĖ
Email: giedre.tamulaitiene@bti.vu.lt
Phone: +370 5 223 4357

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, including the community of Vilnius University, 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 of the COD and the curation of its data collection is carried out at Vilnius University with the help of an international advisory board.

The Crystallography Open Database is used by scientists world-wide. The two seminal papers [1,2] were cited together over 1000 times by various media according to Google Scholar. The Dimensions citation service (https://dimensions.ai/) reports about the [2] that "45% of its citations have been received in the past two years, which is higher than you might expect, suggesting that it is currently receiving a lot of interest. Compared to other publications in the same field, this publication is extremely highly cited".



Below, we provide five publications of most recent years citing the COD papers from the list returned by the Dimensions service, indicating the fields that benefit from the Crystallography Open Database [3–7]:

1. Gražulis, S.; Chateigner, D.; Downs, R. T.; Yokochi, A. F. T.; Quirós, M.; Lutterotti, L.; Manakova, E.; Butkus, J.; Moeck, P. & Le Bail, A. Crystallography Open Database – an open-access collection of crystal structures. *Journal of Applied Crystallography*. 2009, 42: 726–729, DOI: 10.1107/S0021889809016690.
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7. Aguiar, J. A.; Gong, M. L. & Tasdizen, T. Crystallographic prediction from diffraction and chemistry data for higher throughput classification using machine learning. *Computational Materials Science*, Elsevier BV. 2020, 173: 109409, DOI: 10.1016/j.commatsci.2019.109409.

CONTACT INFORMATION

SAULIUS GRAŽULIS
Email: saulius.grazulis@bti.vu.lt
Phone: +370 5 223 4353

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).

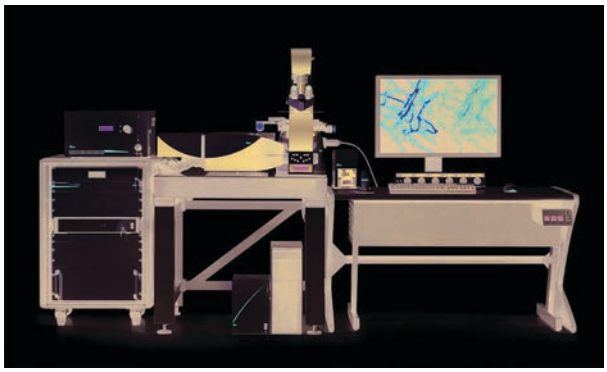
This High-Speed AFM can observe real-time imaging as a movie; it allows a dynamic visualization of the nano-scale world.

CONTACT INFORMATION

MARIJA JANKUNEC
Email: marija.jankunec@gmc.vu.lt
Phone: +370 5 223 4400

Super-Resolution Imaging Facility

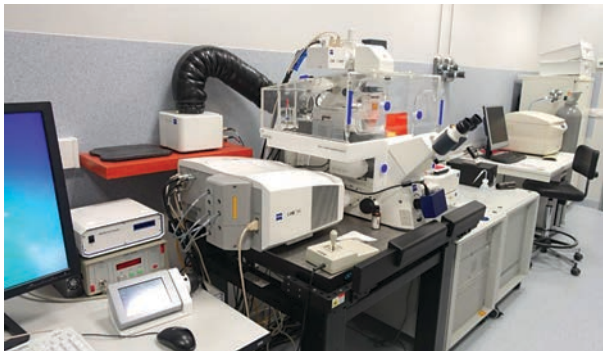
Super-resolution imaging facility has recently acquired a Leica TCS SP8 confocal scanning microscope, equipped with motorized xy-stage 12 kHz tandem scanner, field of view scanner, gated HyD™ detection system, white light laser as well as LIGHTNING, FALCON and STED 3x modules. The white light laser source (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).

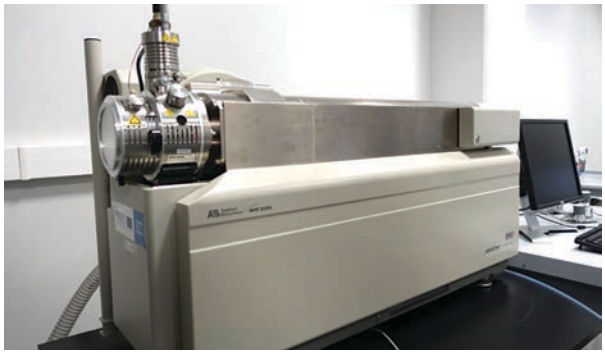


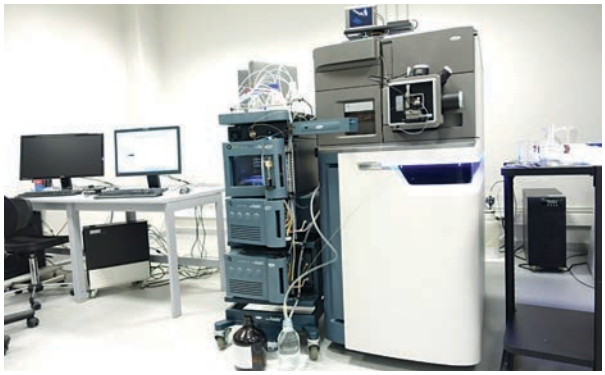
CONTACT INFORMATION

URTĖ NENIŠKYTĖ
Email: urte.neniskyte@gf.vu.lt

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. This allows us to offer the following services to our users: 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. This whole spectrum of capabilities allows us to be involved in biomarker discoveries and validations including the search for biomarkers for the chemotherapeutic resistance of colon cancer chemotherapy (in collaboration with A. Laurinavičius, the National Centre of Pathology, Vilnius, Lithuania) and the early diagnostic markers of pancreatic cancer (in collaboration with L. M. Graves, UNC School of Medicine, Chapel Hill, US and K. Strupas, Vilnius University Hospital *Santaros Klinikos*, Vilnius, Lithuania). It also allows us to perform a proteomic analysis of cell midbodies (in collaboration with R. Prekeris, CU, Denver, the USA and A. Skeberdis, Lithuanian University of Health Sciences, Kaunas, Lithuania).

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.

CONTACT INFORMATION

MINDAUGAS VALIUS
Email: mindaugas.valius@bchi.vu.lt
Phone: +370 5 223 4410

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 Kα 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.

The X-ray diffractometer is mostly used by in-house users of the Life Science Center. The five most recent papers reporting the result obtained using the facility are provided below:

1. Czapinska, H.; Kowalska, M.; Zagorskaite, E.; Manakova, E.; Slyvka, A.; Xu, S-Y.; Siksnys, V.; Sasnauskas, G. & Bochtler, M. Activity and structure of EcoKMcrA. *Nucleic Acids Res*. 2018, 46(18): 9829–9841.
2. Toliusis, P.; Tamulaitiene, G.; Grigaitis, R.; Tuminauskaite, D.; Silanskas, A.; Manakova, E.; Venclovas, C.; Szczelkun, M. D.; Siksnys, V. & Zaremba, M. The H-subunit of the restriction endonuclease CglI contains a prototype DEAD-Z1 helicase-like motor. *Nucleic Acids Res*. Epub 2018 Feb 20. doi: 10.1093/nar/gky107. PubMed PMID: 29471489.
3. Tamulaitiene, G.; Manakova, E.; Jovaisaite, V.; Tamulaitis, G.; Grazulis, S.; Bochtler, M. & Siksnys, V. Unique mechanism of target recognition by PfoI restriction endonuclease of the CCGG-family. *Nucleic Acids Res*. 2019 Jan 25, 47(2): 997–1010. doi: 10.1093/nar/gky1137. PMID: 30445642.
4. Kazokaitė, J.; Kairys, V.; Smirnovienė, J.; Smirnov, A.; Manakova, E.; Tolvanen, M.; Parkkila, S. & Matulis, D. Engineered carbonic anhydrase VI-mimic enzyme switched the structure and affinities of inhibitors. *Scientific Reports*. 2019, 9: 12710, doi.org/10.1038/s41598-019-49094-0, PMCID: PMC6722136, PMID: 31481705.
5. Zakšauskas, A.; Čapkauskaitė, E.; Jezepčikas, L.; Linkuvienė, V.; Paketurytė, V.; Smirnov, A.; Leitans, J.; Kazaks, A.; Dvinskis, E.; Manakova, E.; Gražulis, S.; Tars, K.; Matulis, D. Halogenated and di-substituted benzenesulfonamides as selective inhibitors of carbonic anhydrase isoforms. *Eur J Med Chem*. 2020 Jan 1, 185: 111825, doi: 10.1016/j.ejmech.2019.111825. Epub 2019 Oct 31. PMID: 31740053.

CONTACT INFORMATION

SAULIUS GRAŽULIS
Email: saulius.grazulis@bti.vu.lt
Phone: +370 5 223 4353

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).

CONTACT INFORMATION
REGINA JANČIENĖ
Email: regina.janciene@bchi.vu.lt
Phone: +370 5 272 9058

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).

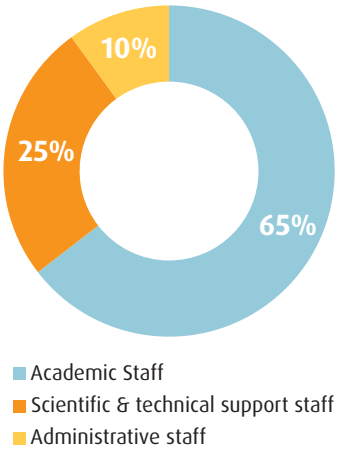
CONTACT INFORMATION
EGLĖ RUDOKIENĖ
Email: egru@ibt.lt; eglute.rudokiene@bti.vu.lt
Phone: + 370 5 223 4370

FACTS & FIGURES

Staff and Students

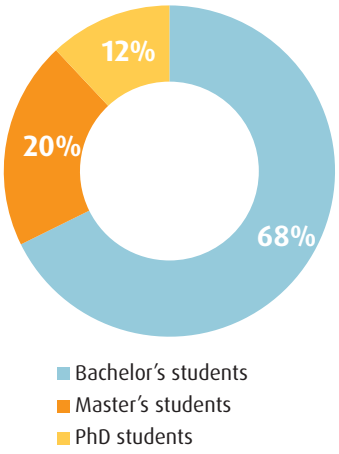
Staff

Academic staff	264
Scientific & technical support staff	104
Administrative staff	41
Total staff	**409**



Students

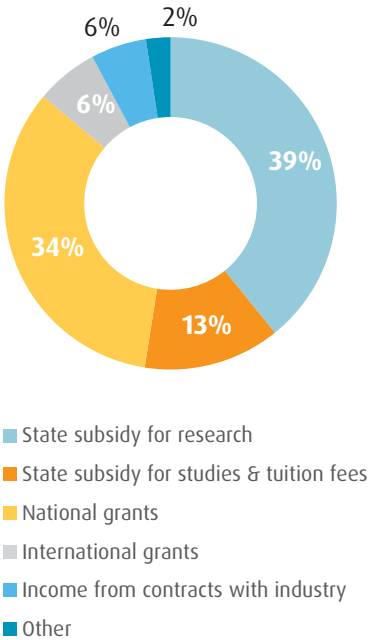
Bachelor's students	700
Master's students	210
PhD students	124
Total students	**1034**



Financing Sources

Total income – 14.6 m EUR
Income increase 16%

Source of funding	*2020*
State subsidy for research	5 711 709 €
State subsidy for studies & tuition fees	1 937 950 €
National grants	4 899 067 €
International grants	885 124 €
Income from contracts with industry	789 996 €
Other	347 254 €
Total	**14 571 100 €**



International Advisory Council

International Advisory Council of the VU Life Sciences Center was established at the end of 2017 with the aim to get the insight, high quality guidance and advice of the outstanding scientists, industrial leaders and administrative experts that could contribute to further development and growth of the VU Life Science Center into one of the leading research and education centres in Europe.

HEINRICH LEONHARDT
Professor
Dean of the Faculty of Biology
Biozentrum
Ludwig Maximilians University
Munich, Germany

CHRIS LOWE
Professor
Director
Cambridge Academy of
Therapeutic Sciences, UK

BARBARA MAZUR
Strategic advisor
Pontifax Global Food and
Agriculture Technology Fund

ANDRES METSPALU
Professor
Tartu University, Estonia

TOMMY NYLANDER
Professor
Lund University, Sweden

TAINA PIHLAJANIEMI
The Vice Rector for
Research
Oulu University, Finland

SILKE SCHUMACHER
Director International
Relations
EMBL, Germany

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	2017–2023
Eat me microglia: lipid scrambling as a signal for synaptic pruning MSCA-IF-2015-EF: 705452	U. Neniškytė A. Alaburda	2016–2021
SINGLY: Glycobiology of synaptic pruning in developing brain, Marie Skłodowska Curie Actions Individual Fellowship	A. Parimisetty U. Neniškytė	2020–2022

Title	*Lead scientist*	*Duration*
Sonic drilling coupled with automated mineralogy and chemistry On-Line-On-Mine-Real-Time (SOLSA) SC5-11d-2015: 689868	S. Gražulis	2016–2020
Directed EVOlution in DROPS (EVOdrops) MSCA-ITN-2018: 813786	L. Mažutis	2018–2022

Baltic Research Programme

Title	*Lead scientist*	*Duration*
Novel high-performance polymers from lignocellulosic feedstock (EMP426)	I. Matijošytė	2020–2024

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

European Joint Programme on Rare Diseases

Title	*Lead scientist*	*Duration*
Unveiling the role of glutamate in dopamine transporter deficiency syndrome (URGENT)	J. Razumienė	2020–2023

Lithuanian-French Programme *Gilibert*

Title	*Lead scientist*	*Duration*
Characterization of antiplasmodial agent plasmodione and its metabolites (P-LZ-19-10)	N. Čėnas	2019–2020
New methods for modelling protein-protein and protein-ligand interactions (P-LZ-19-11)	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

Lithuanian-Latvian-Taiwanese Tripartite Cooperation Programme

Title	*Head of the project*	*Duration*
Brain-computer music interfacing for embodied musical interaction (P-LLT-19-12)	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 (P-LL-18-47)	V. Kisnerienė	2018–2021
Genomic insights into the mechanisms of drug resistance, virulence, and transmission of *Mycobacterium tuberculosis* strains from Lithuania and Poland	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*
Cdk5 confers tumour resistance to adaptive immunity by PDL1 upregulation (St. Baldrick's Foundation grant)	A. Petrošiūtė	2020–2021
The processing of highly salient and biologically relevant emotional stimuli: gender differences and relationship to sex steroids. DAAD	R. Grikšienė	2020
The investigation of the glycobiology of dissected glioblastoma human brain tissue. GlioGly. DAAD	U. Kuliešiūtė	2020
Mycorrhizal partners of Diphasiastrum spp. - are fungi involved in a nutrient network. Mycorrhizal. DAAD	R. Rimgailė-Voicik	2020
IBRO-PERC InEurope Short Stay Grants	U. Kuliešiūtė	2020–2021
IBRO Return Home Fellowship	R. Guzulaitis	2020–2022
European Space Agency (ESA). Fifth call under the Plan for European Cooperating States (PECS) in Lithuania Antimicrobial Photoinactivation Approach Based on Natural Agents for Control of Bacteria Biofilms in Spacecraft. Feasibility Study (LT5_1)	L. Kalėdienė A. Gricajeva	2020–2021

COST

Title	*Management Committee members*	*Duration*
In vitro 3-D total cell guidance and fitness (CA16119)	D. Baltriukienė V. Bukelskienė	2016–2021
European Network of Multidisciplinary Research and Translation of Autophagy Knowledge (TRANSAUTOPHAGY) (CA15138)	V. Borutinskaitė R. Navakauskienė	2015–2020
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
Evaluation of antifungal activity of silver nanocomposites against various clinically relevant yeast and fungi. ECOST-STSM-Request-CA15114-45747	V. Kalcienė	2020
European transdisciplinary networking platform for marine biotechnology (CA18238)	I. Matijošytė R. Šiekštelė	2019–2023
Between atom and cell: integrating molecular biophysics approaches for biology and healthcare (MOBIEU) (CA15126)	D. Matulis A. Zubrienė	2015–2020
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	2018–2020
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
Multi-target paradigm for innovative ligand identification in the drug discovery process (Mu TaLig) (CA15135)	A. Zubrienė L. Baranauskienė	2015–2020

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-20-256)	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. P-SEN-20-35)	A. Burokas	2020–2021
Self-assembling phage proteins for targeted nanomedicine (No. P-SEN-20-34)	V. Časaitė	2020–2021
Redox chemistry, biochemistry and cytotoxicity of aromatic nitrocompounds and N-oxides: new insights (No. DOTSUT-34/09.33-LMT-K712-01-0058)	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
Investigation of K.Lactis mutations conferring enhanced secretion phenotype and generation of yeast strains for supersecretion of recombinant proteins (No. S-MIP-17-88)	A. Gedvilaitė	2017–2020
Determinants of quality of life in Lithuanian students: problematic usage of the Internet and neuropsychological profile (No. S-GEV-20-5).	I. Griškova-Bulanova	2020–2022
Modern technologies to resolve a complex structure of tumour (No. S-MIP-17/54)	S. Jarmalaitė	2017–2020
Molecular mechanisms of adaptation of low-temperature phages to the mesophilic host (No. P-MIP-19-259)	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. Kalvelytė	2018–2022
Hypoxia as cell stress in mRNA diversity and aging(No. S-SEN-20-17)	A. Kanopka	2020–2021
Handles for selective manipulations of biosynthetic proteins (No. S-MIP-17-57)	S. Klimašauskas	2017–2020
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
A technology for single-cell analysis of genomic DNA modification. Neuroblastoma epigenetic heterogeneity (No. S-MIP-17-58)	E. Kriukienė	2017–2020

Analysis of immunological, genetic, epigenetic factors in the etiopathogenesis of autoimmune arthritis (No. S-MIP-17-12)	R. Kubiliūtė	2017–2020
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
Surface nano-structures for mechanistic studies of DNA - protein interaction at the single-molecule level (No. S-MIP-17-59)	E. Manakova	2017–2020
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
The mechanism of inhibitor recognition by carbonic anhydrases - towards anticancer therapy (No. S-MIP-17-87)	D. Matulis	2017–2020
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
Prodrug-Enzyme selection system (No. 31V-59/(1.78)SU-1687)	R. Meškys	2019–2020
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. P-SEN-20-14)	R. Navakauskienė	2020–2021
The role of epigenetic oscillations in predicting biological age (No. S- MIP-19-192)	A. Petronis	2019–2022
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
The prevalence and distribution of virulence factors among subgroups of vaginal bacteria *Gardnerella Vaginalis* (No. S-MIP-17-49)	M. Plečkaitytė	2017–2020
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
Biodiversity and ecological peculiarities of aphid species (Hemiptera: Adelgidae, Lachnidae) inhabiting coniferous host plants in Central Europe (No. P-MIP-17-365)	R. Rakauskas	2017–2020
Adaptation mechanism in Class 2 CRISPR-Cas systems (No. S-MIP-19-305)	G. Sasnauskas	2019–2022
Cross-interactions in amyloid fibril formation: from mechanisms to inhibition (No. S-SEN-20-3)	V. Smirnovas	2020–2021
The role of atmospheric nitrogen fixation in the largest eutrophicated European lagoon (No. P-MIP-17-126)	R. Stanislauskienė	2017–2020
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-20-329)	E. Šimoliūnas	2020–2022
Search of Anti-CRISPR proteins and research of their action (No. S-MIP-20-39)	T. Šinkūnas	2020–2022
Research and practical applications of a type I-F CRISPR-Cas system (No. S-MIP-17-47)	G. Tamulaitienė	2017–2020
Studies of genome editing tools at the single-molecule level (No. S-MIP-20-55)	M. Tutkus	2020–2022
Quantitative detection of phospholipid membrane damage by pore-forming toxin (No. P-MIP-19-394)	G. Valinčius	2019–2020
Development of novel proteomics-based drug selection approach for pancreatic cancer individualized therapy (No. P-SEN-20-39)	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
Analysis and prediction of structural features of proteins and protein complexes using interatomic contact areas and evolutionary information (No. S-MIP-17-60)	Č. Venclovas	2017–2020
Analysis of 5`-capped RNAs and its modulating proteins in *E. coli* and probiotic lactic acid bacteria (No. S- MIP-19-217)	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 prokaryotic Argonaute proteins (No. S-MIP-17-61)	M. Zaremba	2017–2020
Structural and functional studies of split prokaryotic Argonaute proteins (No. S-MIP-20-37)	M. Zaremba	2020–2022
Development of labelled antibodies for *in vitro* diagnosis of allergies (No. TPP-03-031)	A. Žvirblienė	2019–2020
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

Partnership with the European Molecular Biology Laboratory

In June 2019, Lithuania became 27th member state of the European Molecular Biology Laboratory (EMBL). To intensify institutional synergies, on September 8th, 2020, Edith Heard, EMBL Director General, Gintaras Valinčius, Director of the Life Sciences Center and Rimvydas Petrauskas, Rector of Vilnius University, signed an agreement with Vilnius University on the establishment of a VU LSC–EMBL Partnership for Genome Editing Technologies at the VU Life Sciences Center.

The Partnership builds on scientific and technological complementarity in the field of targeted genome modification. It will include the establishment of a VU LSC-EMBL partner unit based on the successful EMBL operational model, which will employ 6 high-level groups of international researchers whose scientific focus will be the development and application of novel genome editing tools and technologies to improve understanding of fundamental biological processes and disease mechanisms.

The Partnership will intensify existing links, inspire scientific collaborations, facilitate knowledge exchange and enable the sharing of scientific services and scientific training. It will open even more opportunities for Lithuania to implement competitive research and innovations on the international arena.

Instruct-ERIC

On the 1st of January 2020, Lithuania became a new member of Instruct-ERIC, which is a research infrastructure, providing open access to cutting edge structural biology, specifically supporting research that uses integrated approaches and technologies. This membership will significantly expand the instrumental and technological capabilities of the Lithuanian researchers and will allow the synergy of structural biology activities with cellular, molecular and neurobiology. The VU LSC scientists have got access to the unique infrastructure and are now eligible to apply for funding to use structural biology services at all Instruct Centres, as well as training courses, internships, and R&D awards.

Arqus Alliance

ARQUS – 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.

At the end of 2020, Arūnas Samas from the Institute of Biosciences and Andrius Merkys from the Institute of Biotechnology participated in the committee planning one of ARQUS activities for the spring semester of 2021 named “Climate Risks”, an international elective course for all ARQUS alliance students based on the principles and methodology of Problem-Based Learning analysing a current hot topic of the climate change. Both scientists will be leading two international students groups at Vilnius University, an international course unit called “Climate Risks”, moderated by the ARQUS Problem-Based Learning committee.

Graduate School

DAIVA BALTRIUKIENĖ
Director of VU LSC Graduate School

Over 120 graduate students perform original research at the Life Sciences Center in the fields of biology, biophysics, ecology and environmental sciences, zoology, biochemistry, and biotechnology. The innovative graduate education is promoted by the Graduate School, which offers centralized services for PhD students, their mentors and PhD Committees at the Life Sciences Center. The Graduate School also focuses on increasing the visibility and attractiveness of PhD programs world-wide to reach those undergraduates, who consider doing a PhD in the life sciences field.

Graduate student community is an integral part of the administration and strategic management of the Life Sciences Center. Student-elect representatives at the Council and Boards of Life Sciences Center propose and follow the Center's strategy to attract and maintain young scientists and create a pleasant environment to conduct their research. Senior graduate students suggest improvements to PhD programs, welcome new students and consult fellow colleagues individually. The graduate 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 http://www.gmc.vu.lt/en/graduate-school

Contact details: 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. A joint (double) degree will be awarded and each doctoral degree will contain a reference to the fact that the two institutions administered the doctoral procedure jointly.

124	40	24	8
PhD students	New PhD students	Defended doctoral theses	International students

PhD Students' Impressions

My first impression was that the building was very new, the lab well equipped and my co-workers friendly.
LORENZO CAMISI, PhD student in Biochemistry

Everyone in the group, right from senior PhD students to postdocs, helped me to get familiar with the technology and the experiments that were regularly conducted in our Lab. Apart from research, the Doctoral Students' Association also helped me with my journey to make myself familiar with potential opportunities and tackle some of the issues regarding my doctoral studies.
SHIVABALAN ARUN PRABHA, PhD student in Biochemistry

PhD studies at the LSC have helped me to grow both personally and professionally. I am more than happy to be studying here and proud to wear LSC on my sleeve as an international student.
AKSHAY KUMAR, PhD student in Biochemistry

My studies fully met my expectations, mostly because I had a knowledgeable and responsible supervisor. I would recommend the VU LSC to others who want to pursue a PhD as it provides high-quality education and plenty of international communication, so they could acquire valuable knowledge and have international perspective.
WENMING LIANG, PhD student in Biophysics

I enjoy my studies as they provide a great opportunity to test my abilities to be a scientist. I am happy with the conditions here as rules of assessments, communication between administration and students and financing for research and scientific trips have been improved during the last years. I recommend PhD studies at the VU LSC for students who wish to do research in life sciences.
MANTAS ADOMAITIS, former PhD student in Zoology

I have performed all my scientific work at LSC and started to collaborate with scientists from different departments. Here I can see many collaboration possibilities with scientists from different fields.
ALIONA AVIŽINIENĖ, former PhD student in Biotechnology

Doctoral Theses 2020

Name	*Topic*	*Supervisors*
M. Alksnė	Substrate-dependent fate of stem cells: insights into artificial bone fabrication	V. Bukelskienė
E. Balčiūnas	Development of bioactive scaffolds for tissue engineering applications	D. Baltriukienė J. G. Hardy
L. Baliulytė	Quantum chemical investigations of the fragmentation of amino acids by low energy electrons	J. Tamulienė
R. Bandariavičiūtė	Gene expression research in prostate adenocarcinoma tissues	S. Jarmalaitė
N. Dreižė	Elucidation of the complexity of molecular mechanisms of cancer cell drug resistance to enhance treatment efficiency	M. Valius
R. Dulinskas	Effect of acute and chronic alcohol consumption and withdrawal on rat visual evoked potentials: focus on ON/OFF responses	O. Rukšėnas
S. Gasiulė	Functional analysis of canonical and mirtronic miRNAs in thoracic ascending aortic aneurysm tissues and digestive system tumours	G. Vilkaitis
A. Kamarauskaitė	The habitat preferences of Common Buzzard *(Buteo buteo)* and interaction with other birds of prey	R. Treinys R. Rakauskas
R. Krasauskas	The role of *Acinetobacter baumannii* BfmRS system in environmental fitness and inter-bacterial competition	E. Sužiedelienė
I. Lapeikaitė	Effect of amino acids and NMDA on electrical signalling parameters of macrophyte Nitellopsis obtuse	O. Rukšėnas
A. Laurynėnas	Investigation and modelling of complex biocatalytic and bioelectrocatalytic processes	J. Kulys
R. Monèiunskaitė	The influence of oral contraceptives on female cognitive functions and affective processing	K. Dapšys R. Grikšienė
J. Nainys	High-throughput single-cell sequencing and analysis	L. Mažutis
I. Pečiulienė	Pre-mRNA splicing associated with oncological diseases: a study of splicing factors and hypoxic microenvironment	A. Kanopka
R. Petkauskaitė	Screening, characterization and functionality analysis of heteropolysaccharide degrading enzymes	N. Kuisienė
E. Pipinis	Evaluation of electrical brain responses to linear chirp-modulated tones: effect of task and changes in neuropsychiatric disorders	I. Griškova-Bulanova
M. Sadauskas	Indigo-producing enzymes: selection and application	R. Meškys
A. Skeberdytė	Salinomycin and dichloroacetate synergistically inhibit cancer cells in vitro and *in vivo*	S. Jarmalaitė
K. Stuopelytė	Detection of miRNAs in urine of prostate cancer patients	S. Jarmalaitė
T. Šneideris	Towards understanding amyloid fibril formation and self-replication	V. Smirnovas
M. Talaikis	Vibrational spectroscopy study of membrane anchoring monolayers and adsorbed biomolecules	G. Niaura
N. Urbelienė	Methods for the selection of hydrolases by applying *E. coli* uridine auxotrophic strain and synthetic nucleosides	R. Meškys
B. Valiauga	Investigation of the mechanism of quinone- and nitroreductase reactions by flavoenzymes dehydrogenases-transhydrogenases	N. Čėnas
A. Vitkevičienė	Investigation of molecular mechanisms in human myeloid leukaemia cells using new epigenetic and metabolic regulators	R. Navakaukienė

Bachelor and Master Studies

INGRIDA PRIGODINA LUKOŠIENĖ
Deputy Director for Studies

Studies in numbers

910 Total number of bachelor & master students

700 BSc students

210 MSc students

277 New BSc students

105 New MSc students

28 ERASMUS students

9 International MSc students

115 Graduations BSc studies

98 Graduations MSc studies

Bachelor study programmes

Title	*Study language*	*Number of students in 2020*
Biology	Lithuanian	109
Genetics	Lithuanian	155
Microbiology and Biotechnology	Lithuanian	139
Molecular Biology	Lithuanian	143
Neurobiophysics	Lithuanian	96
Environmental Science and Protection *New in 2020*	Lithuanian	24
Molecular Biotechnology *New in 2020*	Lithuanian	34

Master study programmes

Title	*Study language*	*Number of students in 2020*
Environmental Studies and Management	Lithuanian	20
Biochemistry	English/ Lithuanian	25
Biodiversity	Lithuanian	15
Biophysics	English/ Lithuanian	13
Genetics	English/ Lithuanian	25
Microbiology	Lithuanian	34
Molecular Biology	English/ Lithuanian	35
Molecular Biotechnology *New in 2020*	English	12
Neurobiology	English/ Lithuanian	30

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/56-studies/studies/4593-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/56-studies/studies/4594-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/56-studies/studies/4595-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/mokslas/56-studies/studies/4596-molecular-biology

Academic contact: Ass. 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/studijos/studentams/studiju-baigimas/56-studies/studies/4598-neurobiology

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

Molecular Biotechnology. Technological Sciences, master, 2 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 (mentoring).

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.

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 2020, the VU LSC scholarships were awarded to 14 BSc and MSc students:

- Austėja Balevičiūtė (MSc, Molecular Biology), Denis Baronas (BSc, Biochemistry), Valentas Brasas (BSc, Molecular Biology), Gytis Druteika (MSc, Microbiology), Evelina Jankaitytė (MSc, Biophysics), Jurga Jasinskaitė (BSc, Biophysics), Ieva Juškaitė (BSc, Molecular Biology), Julius Martinkus (MSc, Molecular Biology), Rūta Matulevičiūtė (BSc, Genetics), Danguolė Norkūnaitė (MSc, Biochemistry), Roberta Statkevičiūtė (MSc, Microbiology), Aistė Savickaitė (BSc, Microbiology), Aivaras Vilutis (BSc, Neurobiophysics)
  and 7 doctoral students:
- Greta Jarockytė (Biophysics), Vaida Paketurytė (Biochemistry), Andrius Sakalauskas (Biotechnology), Joana Smirnovienė (Biotechnology), Aistė Zentelytė (Biochemistry), Eglė Žalytė (Biochemistry), Mantas Žiaunys (Biotechnology).

President Kazys Grinius' Scholarship

On 6 July 2020, in commemorating the coronation of King Mindaugas and the National Anthem, state awards were handed out 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. Eleven Vilnius University students were given these scholarships. President Kazys Grinius' scholarship for excellent academic results was awarded to Gytis Druteika, a student of the Life Sciences Center (MSc, Microbiology).

Gytis Druteika

Internship at NASA

In 2020, the Lithuanian Agency for Science, Innovation and Technology (MITA) selected five students to participate in the international internship programme at NASA Ames Research Center. Students left for NASA Ames, located in the USA, California, and on the first days of September started their autumn internship programme together with other international students from more than 12 countries. One of the winners of the internship at NASA was a student from the VU LSC Aivaras Vilutis (BSc, Neurobiophysics).

Aivaras Vilutis

Contribution to Overcome COVID-19 Pandemic

In 2020, humanity faced the novel coronavirus SARS-CoV-2. It caused the COVID-19 pandemic that has heavily affected all spheres of life from public health to economy, education and environment. Researchers all over the world joined the front lines responding to this global challenge and aiming to find solutions and lessen the impact of the pandemic on society.

In response to the COVID-19 challenges in Lithuania, VU Life Sciences Center immediately provided its research infrastructure, personnel resources and knowledge and contributed to the national effort to get through the crisis.

Temporary Molecular Diagnostics Laboratory for COVID-19 Testing

During the first outbreak of the pandemic in Lithuania, the VU Life Sciences Center (VU LSC) joined the COVID-19 fighting forces aiming to increase the diagnostic throughput in the country. In March 2020, responding to the state-wide shortage of both technical and human resources, the LSC scientists established the Temporary Molecular Diagnostics Laboratory for diagnostic testing of COVID-19 samples. Having all the necessary infrastructure and equipment *in situ* as well as high expertise in the field of molecular biology and genetics, the start of operation of the newly formed unit was greatly anticipated by the government. A number of the LSC laboratories were transformed into the controlled-access area, complying to all the operational and safety requirements for the biological safety level-2 laboratories. Kristina Daniūnaitė became the head of the diagnostics, Eglė Lastauskienė, the head of the management, and Rokas Abraitis, the head of the technical readiness. Twelve volunteers joined the COVID-19 diagnostics team and were assigned to the sample registry, sample preparation, viral RNA detection and result management groups.

During the first wave of the pandemic, in less than 2 months of active operation, 8689 nasopharyngeal samples were tested for SARS-CoV-2 infection in the laboratory. In mid-April, the laboratory reported 52 positive cases in a 217-sample batch, which was a record number of new cases per day at that time and remained so until mid-September. As all the positive-testing individuals were employees of one particular factory, the fact served as the basis for the urgent closedown of a small suburban town near Vilnius attempting to stop the spread of the infection, which later turned out to be a very timely decision.

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Since the numbers of the COVID-19 positive tests decreased to only a few cases per day in the whole country, at the end of June 2020, the laboratory's testing operation was discontinued.

In autumn 2020, the diagnostic testing was resumed as Lithuania entered the second wave of the pandemic, with dramatically increasing prevalence rate day by day. By the end of November 2020, the number of volunteers reached 40 members, including both LSC personnel and students. Furthermore, the laboratory acquired new equipment (The KingFisher Flex Purification System) in order to increase the testing throughput. By the end of 2020, Lithuania reached the terrifying numbers of the positive COVID-19 cases, with almost 1 of 3 samples testing positive for SARS-CoV-2. By the end of 2020, 20535 clinical samples

were analysed in the laboratory, of which 2900 tested positive for SARS-CoV-2 infection, and testing is continued in 2021.

Volunteers of Temporary Molecular Diagnostics Laboratory:

Rokas Abraitis, Kristina Daniūnaitė, Eglė Lastauskienė, Lauryna Abraitytė, Julija Armalytė, Gabrielė Dzimitravičiūtė, Shamish Ganpule, Alisa Gricajeva, Kristina Grigaitytė, Povilas Jučinskas, Mindaugas Juozapaitis, Rugilė Jurevičiūtė, Bernadeta Kaminskaitė, Rūta Kananavičiūtė, Ignė Kanopaitė, Karina Kasperovičiūtė, Arnoldas Kaunietis, Austėja Kvedaraitė, Karolina Limanovskaja, Inga Liužinaitė, Vaidas Mačiulskis, Rūta Maleckaitė, Agnė Mikelevičiūtė, Irmantas Mogila, Rasa Mončiunskaitė, Daina Pamedytytė, Ugnė Peldžiūtė, Shivabalan Arun Prabha, Emilija Radlinskaitė, Mikas Sadauskas, Raminta Saulėnaitė, Pavel Semaško, Aistė Sipavičiūtė, Roberta Statkevičiūtė, Daugvydas Stepanovas, Diana Šapaitė, Daniel Šematovič, Kristina Šveistytė, Edvardas Timoščenka, Jurga Turčinavičienė, Silvija Urnikytė, Akshay Kumar Vijaya, Aušra Želvytė.

Expert Groups:

Vilnius University Expert Group

IIn response to the government call to contribute to the national effort against the outbreak, the expert group was set up initiated by the VU Life Science Center (VU LSC) on a voluntary basis. The work of the expert group was led by the LSC director G. Valinčius. Experts prepared an overview of the latest research and biotechnology developments related to the COVID-19 pandemic, the possible further spread of the new coronavirus and its control options. This document was forwarded to the Government of the Republic of Lithuania and helped to better understand of what was happening on a global scale and contributed towards selection of the best actions.

Experts from the VU LSC were N. Čėnas, J. Dapkūnas, G. Gasiūnas, V. Kairys, T. Karvelis, K. Krikštopaitis, L. Mažutis, R. Navakauskienė, R. Petraitytė Burneikienė, M. Simanavičius, J. Šarlauskas, M. Tomkuvienė, A. Vitkevičienė, A. Zentelytė, A. Žvirblienė; experts from Center for Physical Sciences and Technology: J. Kavaliauskaitė, R. Pauliukaitė, A. Stirkė; expert from VU Faculty of Medicine: E. Pranckevičienė; experts from foreign universities: M. Gabrielaitė (University of Copenhagen), I. Olendraitė (University of Cambridge and L. Tamošaitis (University of Kent).

Council of Health Experts

In November, the President of the Republic of Lithuania Gitanas Nausėda initiated forming a group of COVID-19 pandemic management experts – the Council of Health Experts - to analyse, evaluate and provide recommendations to the responsible authorities on stopping the spread of the coronavirus. The activities of the Council are organized into 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 Pro-Rector for Research E. Sužiedelienė and Head of the Department of Immunology and Cell Biology A. Žvirblienė. E. Sužiedelienė is leading the Public Health Measures Group.

Expert Group of the Ministry of Health

The Ministry of Health of the Republic of Lithuania set up a special expert group in charge of vaccination issues preparing expert conclusions on vaccines. Aurelija Žvirblienė, Head of the Department of Immunology and Cell Biology at the VU LSC, is a member of this expert group.

Research Related to COVID-19

The Nasopharyngeal Sample Pooling Methodology

With significant demand for virus testing, LSC scientists are developing the nasopharyngeal sample pooling methodology. Pooled-sample testing is a promising strategy to screen large populations rapidly with limited resources. More information on page 77.

Environmental Surface Sample Testing for SARS-CoV-2 Traces

One of the reasons why this pandemic has been difficult to contain is the inability to identify presymptomatic and asymptomatic SARS-CoV-2 carriers as some of these individuals can be highly contagious when they have mild or no symptoms. Such individuals can shed a high viral load in their workplace and expose co-workers to constant fomite spread. We assayed over 200 samples of environmental surfaces in the Life Sciences Center of Vilnius University, which led to the identification of several pre-/ asymptomatic carriers among the community. More information on page 77.

Serologic Testing of COVID-19

Serologic tests detect past SARS-CoV-2 infection by measuring virus-specific antibodies in the blood. Antibodies are indicators of the humoral immune response to the virus. Therefore, serologic tests are important for monitoring COVID-19 pandemic. The scientists of the Department of Immunology and Cell Biology (DICB) of the Institute of Biotechnology contributed to the development and validation of COVID-19 serologic assays and employed them for testing of clinical samples. In May 2020, a group of volunteers – Indrė Kučinskaitė-Kodzė, Martynas Simanavičius, Aistė Imbrasaitė (head – Aurelija Žvirblienė) – validated commercial rapid serologic tests for detection of SARS-CoV-2-specific IgM and IgG antibodies. This study was performed in collaboration with the Biobank of Vilnius University Hospital *Santaros Klinikos*. The Biobank provided blood serum samples from patients with confirmed SARS-CoV-2 infection (n=60) and healthy individuals (n=160). The performance of rapid serologic tests was evaluated by comparing them with quantitative ELISA tests. Rapid serologic tests demonstrated high specificity (99.6%) and sensitivity (96.5%) for detection of virus-specific IgG antibodies in serum samples collected >10 days after infection. Validation data were reported to the State Health Care Accreditation Agency to provide recommendations for serologic testing. The validated rapid serologic tests were employed for a sero-epidemiologic study performed by Vilnius University and the Lithuanian University of Health Sciences.

To investigate the persistence of humoral immune response, the research group of the DICB performed serologic testing in the outbreak zone at different time points: 2 months and 6 months after the outbreak. In total, blood samples of a 100 individuals with known SARS-CoV-2 status (positive/negative PCR test) were analysed for the presence of virus-specific IgG antibodies. This study demonstrated that IgG antibodies are raised both after a symptomatic and asymptomatic SARS-CoV-2 infection and persist for at least 6 months.

The research group of the DICB collaborates with industrial partners UAB *Baltymas* that produces recombinant SARS-CoV-2 antigens and UAB *Imunodiagnostika* developing quantitative microarray-based COVID-19 serologic tests. The researchers of DICB contributed to the antigenicity studies of recombinant SARS-CoV-2 antigens and their evaluation as components for serology.

Full Genome Analysis of SARS-CoV-2 Isolates

Sequencing of whole genome of SARS-CoV-2 is of great importance to understand virus evolution and identify mutations having a potential impact on virus transmissibility and pathogenicity. New SARS-CoV-2 mutations may also have possible implications on effectiveness of vaccines and diagnostic testing. Many countries started sequencing of SARS-CoV-2 variants since the beginning of the pandemic and reporting the sequences to the open-access database Global Initiative on Sharing All Influenza Data (GISAID). The scientists of the Department of Eukaryote Gene Engineering (Alma Gedvilaitė) and the Department of Bioinformatics (Albertas Timinskas) of the Institute of Biotechnology in collaboration with *Thermo Fisher Scientific Baltics* analysed full genomes of 15 SARS-CoV-2 isolates collected by the Biobank of *Santaros Klinikos* during the first outbreak and submitted these data to the GISAID database. Later VU Science Promotion Fund supported analysis of more SARS-COV-2 genomes isolated from Lithuanian patients collected by Biobank of *Santaros Klinikos* during the second outbreak. The sequences of 35 SARS-CoV-2 isolates of full genomes were obtained in the Department of Eukaryote Gene Engineering by Emilija Vasiliūnaitė and Milda Norkienė and submitted to the open-access database GISAID. The sequencing of full genomes of SARS-COV-2 circulating in Lithuania allowed the identification of unique mutations of virus variants and provided epidemiological data.

International Awards

Top Performance in CAPRI (Critical Assessment of PRedicted Interactions) and CASP (Critical Assessment of Protein Structure Prediction) Competitions

A team of bioinformaticians from the VU Life Sciences Center - Česlovas Venclovas (team leader), Justas Dapkūnas and Kliment Olechnovič – participated in CASP14 and CAPRI competitions running in parallel in summer of 2020. The team shared the first place jointly with two other groups in CAPRI and were ranked second in the category of protein complex structure modelling in CASP. The same team was the first in both CASP and CAPRI competitions in 2018.

CASP is a biennial worldwide experiment for protein three-dimensional structure prediction from its amino acid sequence taking place since 1994. The aim of CASP is to help advance the methods of computational protein structure modelling. More than a 100 research groups from all over the world participate in CASP and objectively test the accuracy of protein structure prediction methods developed by the research groups. CAPRI is another community-wide initiative inspired by CASP and running since 2001. CAPRI is focusing entirely on prediction of structures of protein complexes.

More about the experiments:

CAPRI https://www.ebi.ac.uk/pdbe/complex-pred/capri/

CASP14 https://predictioncenter.org/casp14/index.cgi

Česlovas Venclovas

Kliment Olechnovič

Justas Dapkūnas

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

In 2020, we greeted with pride two young scientists, Rima Budvytytė and Dominyka Dapkutė, who were given fellowships of the L'Oreal Baltic program *For Women in Science*.

In 16 years' time, since the L´Oréal Baltic *For Women in Science* fellowship programme has been established, 60 talented women scientists in the Baltic countries were awarded. The aim of this program is to encourage young talented scientists to continue their research and to honour their contribution to social progress as well as to inspire more young women to choose a career in science.

Rima Budvytytė, a senior researcher at VU Life Sciences Center, was recognized for her research that focuses on investigation of biochemical and biophysical processes triggering neurodegenerative diseases such as Alzheimer's and Parkinson's on molecular level. Her project aims to investigate the interaction between disordered proteins and phospholipid membranes that may lead to the understanding of the causes of neurodegeneration and the development of new therapies and new diagnostic methods to diagnose them at the earliest possible stage of the disease.

Dominyka Dapkutė, a doctoral student in biophysics at the VU Life Sciences Center and a junior researcher at the Laboratory of Biomedical Physics of the National Cancer Institute, was recognized for her research into the ways of introducing nanotechnology-based teranostatic measures (combining diagnostics and therapy) into the cancerous tissue by using unique cells of the human body, mesenchymal stem cells. These interdisciplinary studies address the current gaps in cancer diagnostics and treatment and can make a significant contribution to the ongoing fight against cancer, as well as provide innovative insights at the fundamental level.

Rima Budvytytė

Dominyka Dapkutė

The Grand Prize in iGEM Competition

The Vilnius-Lithuania iGEM team won the grand prize and became the best iGEM team in the largest and the most prestigious International Genetically Engineered Machine Competition (iGEM) in 2020. In addition to the Grand Prize of the competition, VU students won gold medals and were nominated in 8 additional prize categories, becoming the winners also of the Best Environment Project and Best Measurement.

The Vilnius-Lithuania iGEM outdid more than 250 teams from universities around the world, including teams of the Massachusetts Institute of Technology (MIT), Harvard University as well as William & Mary University, Virginia and many other prestigious universities.

In this year's competition Vilnius-Lithuania iGEM team presented the FlavoFlow project dedicated to the topic of exogenous fish infections. It is estimated that infections caused by *Flavobacterium genus* bacteria can kill about 70% of fish in fish farms in just 72 hours. For this reason, it is essential to identify the pathogen as accurately and quickly as possible. To solve this problem the team developed a rapid detection tool for early diagnosis of diseases in fish farms. At the same time, seeking to lay the foundations for various solutions to the problem, the Vilnius-Lithuania iGEM team focused on the development of platforms for the treatment and prevention of exogenous fish infections.

Lithuanian representatives have been participating in the iGEM competition since 2015. In 2015, 2016, 2018 and 2019 Vilnius-Lithuania iGEM team won gold medals, in 2018 also a bronze medal by undergraduate team and in 2017, like this year, the Grand Prize of the competition.

2020 iGEM team students: Eglė Vitkūnaitė, Auksė Kazlauskaitė, Austėja Sungailaitė, Edvinas Jurgelaitis, Emilija Radlinskaitė, Emilis Gaidauskas, Liepa Šiupšinskaitė, Kamilė Liucija Vainiūtė, Paulius Sasnauskas, Monika Gineitytė, Barbora Vasiliauskaitė.

Team leader – Ieva Lingytė
Primary PI – Rolandas Meškys
Instructors – Denis Baronas, Povilas Šėporaitis
Advisor – Paulius Toliušis

National Awards

Medals of the Order of the Lithuanian Grand Duke of Lithuania Gediminas

On 6 July 2020, on commemorating the Coronation of King Mindaugas and the National Anthem in Lithuania, Gitanas Nausėda, the President of the Republic of Lithuania, presented deserving persons with state awards. It is most gratifying that even three representatives of our community – Kristina Daniūnaitė, Eglė Lastauskienė and Rokas Abraitis – were awarded for their contribution to the fight against the new coronavirus pandemic.

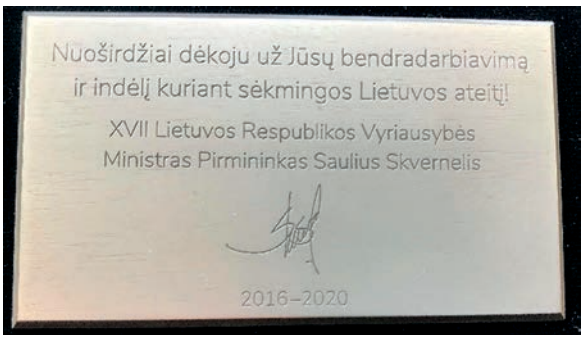
Rokas Abraitis, Gitanas Nausėda, President of the Republic of Lithuania

Eglė Lastauskienė, Gitanas Nausėda, President of the Republic of Lithuania

Kristina Daniūnaitė, Gitanas Nausėda, President of the Republic of Lithuania

Medal of the Prime Minister of the 17th Government of the Republic of Lithuania

Four members of the Life Science Center community – Marius Dagys, Kastis Krikštopaitis, Gintaras Valinčius, Aurelija Žvirblienė – received medals from the Prime Minister of the 17th Government of the Republic of Lithuania Saulius Skvernelis *For Joint Work for Lithuania*.



Letter of Thanks from the Prime Minister of the Republic of Lithuania S. Skvernelis

The Life Science Center community showed initiative during the pandemic by contributing to the testing of COVID-19 samples for coronavirus SARS-CoV-2 infection and providing advice to decision makers and public. Marius Dagys, Kastis Krikštopaitis, Miglė Tomkuvienė, Gintaras Valinčius, Aurelija Žvirbliene received Letters of Thanks from the Prime Minister of the Republic of Lithuania Saulius Skvernelis.

Medals of the Minister of Health *For a Significant Contribution to the Health of the Lithuanian People*

The medals *For a Significant Contribution to the Health of the Lithuanian People* created especially for this occasion were awarded to the management of the Life Sciences Center (LSC) - director Gintaras Valinčius and deputy director Rokas Abraitis - and the scientists Eglė Lastauskienė, Kristina Daniūnaitė, Aurelija Žvirblienė. In awarding the medals, the Minister of Health Aurelijus Veryga thanked for the great efforts and teamwork, done by specialists and volunteers from various institutions working towards one goal to prevent the further spread of the coronavirus COVID 19.

Medal *For a Significant Contribution to the Health of the Lithuanian People*

Winners of the Competition *The Best Doctoral Thesis in 2019*

In November 2020, the Lithuanian Union of Young Scientists announced winners of the competition *The Best Thesis in 2019*. The event is sponsored by the President of the Republic of Lithuania Gitanas Nausėda.

39 scientific works on natural, technological, medical and health and agricultural sciences were submitted to the competition. Eleven authors of the best works became laureates, including two GMC scientists:

Aleksandr Osipenko, Development of Methods for Labelling of Small Non-Coding RNAs (Physics), VU;

Marijonas Tutkus, Fluorescence Microscopy of Single Molecules for Protein Dynamics Studies (Physics), who in his doctoral thesis was actively collaborating with the scientists from the Center for Physical Sciences and Technology.

Marijonas Tutkus

Aleksandr Osipenko

Vilnius University Awards

Vilnius University Grant *Rewriting the Code of Life*

The Royal Swedish Academy of Sciences awarded the Nobel Prize in Chemistry 2020 to Emmanuelle Charpentier and Jennifer A. Doudna for developing the precise genome-editing technology - CRISPR/Cas9 technology that has a revolutionary impact on the life sciences and enabled researchers to change the DNA code of living organisms with extremely high precision and over the course of a few weeks.

Vilnius University marked the Nobel Prize week by granting Virginijus Šikšnys, a scientist who was eligible for the Nobel Prize in Chemistry, and his team a sizeable grant *Rewriting the Code of Life* for major contribution to world-class scientific discoveries and the promotion of Vilnius University in the world.

Back in 2011, V. Šikšnys and his team were the first in the world to show that the CRIPSR-Cas9 system can be transferred from a bacterium, having such a protection system, to bacteria that do not have such a system, and it can function perfectly in a new environment.

Virginijus Šikšnys, Rimvydas Petrauskas, Rector of Vilnius University

Virginijus Šikšnys' contribution in the development of CRISPR-Cas9 technology was recognized by such high-profile awards as Warren Alpert Foundation Prize in 2016, Novozymes Prize in 2017 and Kavli prize in 2018, the latter shared with Emmanuelle Charpentier and Jennifer A. Doudna.

Rector's Awards for Efforts during First COVID Outbreak

The *Finis Anni Academici* celebration at the end of the academic year became a special opportunity to celebrate the merits of the Life Sciences Center (LSC) community to the university and Lithuania. For the first time, the Rector of the Vilnius University and the Senate expressed special thanks to three faculties, the Life Sciences Center, the Faculty of Medicine and the Faculty of Mathematics and Informatics. This gratitude is dedicated to the professionalism, personal initiatives and responsibility of the community members, demonstrated at a crucial time for Lithuania and the world, contributing to the successful management of the first wave of the COVID-19 pandemic.

Personal Rector's thanks went to four members of the LSC community: Rokas Abraitis, Kristina Daniūnaitė, Eglė Lastauskienė and Aurelija Žvirblienė. The Rector also thanked the volunteers, who contributed to the fight against the new coronavirus pandemic.

Rector's Award for Science

Three researchers of the Life Sciences Center – Darius Kazlauskas, Edita Kriukienė and Virginijus Šikšnys – received Rector's Award for Science for their exceptional scientific achievements, contribution to the development of science at the University and in Lithuania, and raising the prestige of university science in the world in 2020.

Edita Kriukienė

Darius Kazlauskas

Virginijus Šikšnys

Scientific Events

4th *Vita Scientia* International Life Sciences Conference

The 4th *Vita Scientia* conference took place at VU LSC on 3 January 2020.

Vita Scientia is an international conference, which aims to facilitate expansion of life sciences by developing international networks between Lithuanian and foreign researchers. The conference is designed to bring people together from diverse international communities and spark international collaboration.

In 2020, conference *Vita Scientia* brought together professionals from life sciences both working in Lithuania and abroad. More than 150 professionals from more than 30 research and business institutions joined the event.

The COINS 2020

The COINS conference, organized by Vilnius University students since 2004, was held for the 15th time last year. In the past three years, as many as six Nobel Prize winners attended the conference, also having an opportunity to get acquainted with the science perspectives of Lithuania. Last year, lectures were given by well-known lecturers, for instance, Nobel Prize Laureates in Chemistry Aaron Ciechanover and Jean-Marie Lehn as well as by other professionals in their fields such as Warren Alpert Foundation Prize winner Peter Hegemann.

In addition to the topics of biochemistry, molecular biology, microbiology or genetics, the lectures also focused on chemistry and neuroscience. More importantly, the COINS 2020 allowed students to present their research to a large audience and to get constructive feedback during the poster and oral sessions.

For the first time, the conference provided an opportunity for the general public to take part in the public engagement programme that offered workshops and laboratory works. The conference brought together the academic community and the society, who could learn about world-class research and challenge themselves.

Science Day by Thermo Fisher Scientific

The traditional event of the year, *Science Day* organized by the Life Sciences Center's industrial partner Thermo Fisher Scientific, took place on 11–12 November 2020.

The event taking place for the 10th time invited researchers and students from various Lithuanian research and study institutions to a virtual meeting. The 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.

Conference Dedicated to the 120th Birth Anniversary of Professor Antanas Minkevičius

On 10 December 2020, a conference dedicated to the 120th birth anniversary of Professor Antanas Minkevičius was held at the Life Sciences Center (VU LSC).

Prof. Antanas Minkevičius was a Lithuanian botanist, mycologist and phytopathologist, habilitated doctor of biomedical sciences, corresponding member of the Lithuanian Academy of Sciences, a distinguished Lithuanian scientist. He was a researcher, lecturer and head of departments of Vytautas Magnus and Vilnius Universities, a researcher and head of departments at the Institutes of Biology and Botany of the Lithuanian Academy of Sciences.

Professor is the author and co-author of a great number of scientific and popular articles, books and textbooks on plant and fungal diversity, biology and ecology.

Prof. Antanas Minkevičius (1900–1998)

2nd Baltic Biophysics Conference – Open Lectures

On 15 October, the 2nd Baltic Biophysics Conference - Open Lectures were organized by the Lithuanian Society of Biophysicists. The event aims to encourage and further develop cooperation between scientific teams as well as joint research with partners in Lithuania and abroad. This conference attracted famous lecturers from Lithuania and around the world: Gražvydas Lukinavičius (Max Plank Institute for Biophysical Chemistry, Germany), Ago Rinken (Tartu University, Estonia), Virginijus Barzda (University of Toronto, Canada), Rytis Prekeris (University of Colorado, USA). Two laureates of L'Oréal Baltic program *For Women in Science* 2020, Rima Budvytytė and Dominyka Dapkutė, participated in this conference.

12th Conference of the Lithuanian Neuroscience Association

On 6 November 2020, the Lithuanian Neuroscience Association (LNA) organized the 12th conference of the LNA virtually. It attracted over 200 registered participants from Lithuania, Latvia, Estonia, Germany, Poland, UK, Switzerland, Australia and Russia. The conference consisted of two parts: 12 invited oral presentations and a poster session (35 posters).

During the poster session, competition for the best poster was organized and winners were awarded valuable prizes established by the sponsors, *Linea Libera* and *Expertus Vilnensis*.

Guests

Visit of the President of the Republic of Lithuania

On 21 October 2020, the President of the Republic of Lithuania Gitanas Nausėda visited the VU LSC. During the visit, the President inspected the top-level research laboratories, met with the LSC scientists and discussed the problems of financing Lithuanian science, the development of research infrastructure and the significance of science in society. The President stressed that Lithuanian scientists had achieved a lot in their fields and they were making impressive progress with their research.

From the left: V. Šikšnys, the Chairman of the LSC Board, G. Nausėda, the President of the Republic of Lithuania, R. Petrauskas, the Rector of the University and G. Valinčius, the LSC director

Visit of the Prime Minister of the Republic of Lithuania

On 21 February 2020, the Prime Minister Saulius Skvernelis together with the Minister of Education, Science and Sport A. Monkevičius visited the VU LSC. "LSC stands out by the added value that it creates. Thanks to the innovations of the Center, Lithuania, being such a small country, becomes a leader in the field of intellectual property in the world", the Prime Minister acknowledged after visiting the labs of VU LSC.

From the left: A. Monkevičius, S. Klimašauskas, R. Abraitis, G. Valinčius, S. Skvernelis, V. Šikšnys, V. Razumas

Visit of NordForsk's Director and Director of the Nordic Council of Ministers

On 19 February 2020, Arne Flåøyen, Director of NordForsk, Helén Nilsson, Director of the Nordic Council of Ministers' Office in Lithuania and Brigita Urmanaitė, Adviser to the Office, visited the VU LSC. NordForsk is an organisation under the Nordic Council of Ministers that provides funding for and facilitates Nordic cooperation on research and research infrastructure. Guests were acquainted with the most important achievements of LSC scientists and discussed cooperation possibilities.

Meeting of Foreign Ambassadors Accredited to Lithuania

On 17 February 2020, a meeting of foreign ambassadors accredited to Lithuania took place at the VU LSC. This event is traditionally organized to mark the Statehood Day. G. Valinčius acquainted the guests with the development of life sciences in Lithuania, introduced new study programs, presented scientific achievements and researchers working at the Center. Discussions also included presentations by representatives of companies working in the life sciences sector.

Visit of Famous Philanthropist Marius Jakulis Jason

On 3 September 2020, famous businessman and philanthropist Marius Jakulis Jason visited the VU LSC.

The foundation of M. Jakulis Jason has been providing financial support to talented academics and students for a number of years with the aim to encourage talents living abroad to come or return to Lithuania, to create their own businesses here, to pursue the heights of scientific careers, having previously acquired knowledge in the best universities of the world.

The famous philanthropist supported many Lithuanian scientists including researchers working at the VU LSC. During the meeting, various possibilities of cooperation were discussed.

Invited Speakers

Speaker	*Institution*	*Title*
Aaron Ciechanover 2004 Nobel prize in Chemistry	Technion – Israel Institute of Technology, The Rappaport Family Technion Integrated Cancer Center (TICC), Israel	The Revolution of Personalized Medicine: Are We Going to Cure all Diseases and at What Price?
Jean-Marie Lehn 1987 Nobel Prize in Chemistry	University of Strasbourg Institute of Advanced Study (USIAS), Chair of Chemistry of Complex Systems, France	A Journey from Molecular towards Adaptive Chemistry
Peter Hegemann 2019 Warren Alpert Foundation Prize	Humboldt University – Berlin, Institute of Biology, Experimental Biophysics, Germany	Multicomponent Optogenetics ⟷ Sensing is not Understanding
Marius Bauža	University College London, UK	There and Back Again: How Do Animals Navigate?
Andrew Hammond	Imperial College London, UK	Gene Drives for Genetic Control of the Malaria Mosquito
Matthias W. Hentze	European Molecular Biology Laboratory (EMBL); Molecular Medicine Partnership Unit (MMPU) Heidelberg, Germany	A New Continent of the RNA World
Skirmantas Kriaučionis	University of Oxford, Ludwig Cancer Research, UK	The Roles of Chromatin in the Function of Transcription Factors and Gene Expression
Gražvydas Lukinavičius	Max Planck Institute for Biophysical Chemistry, Germany	Imaging Biomolecules with Super-resolution Microscopy
Luca Mazzitelli	10X Genomics; University of Dundee, Scottish Crop Research Institute, Italy	Biology at High Resolution with 10X Genomics: from Single Cell Applications to Spatial Transcriptomics
Valentin Nagerl	Interdisciplinary Institute for Neuroscience, University of Bordeaux / CNRS, France	Super-Resolution Microscopy for Neuroscience: New Development and Applications
Tomaš Paleniček	National Institute of Mental Health, Czech Republic	The Neuropsychological Effects of Psilocybin: Focus on Cognitive Processing and Brain Activity, Implications for Treatment
Augustas Pivoriūnas	State Research Institute Centre for Innovative Medicine (SRICIM), Lithuania	Extracellular Vesicles as a New Mode of Intercellular Communication and Potent Novel Therapeutic Tools against Neurodegenerative Diseases
Daan Swarts	Wageningen University & Research, The Netherlands	Exploring the Diversity of Prokaryotic Immune Systems
Joan Taylor	De Montfort University, Leicester School of Pharmacy, UK	Novel Insulin Technologies
Alina Urnikytė	Vilnius University, Faculty of Medicine, Lithuania	Inferring Microevolutionary Processes in Local Human Populations

Community Events

Celebrating the Day of Restoration of the State of Lithuania

Community of the Life Sciences Center continued the tradition of meaningfully commemorating February 16, the Day of Restoration of the State of Lithuania and watched Šarūnas Bartas' film "At Dusk", which tells the story of partisan movement resisting Soviet occupation, also known as the Lithuanian War, in Lithuania. This 10-year resistance period (1944–1953) is very important for the memory of the Lithuanian nation.

On the same day, our community had an exceptional opportunity to meet Juozas Jakavonis-Tigras (pseudonym - the Tiger), participant of Lithuanian freedom fights, actor Arvydas Dapšys, who played one of the main roles in the film "At Dusk" and historian Arvydas Anušauskas, who is author of numerous history books and articles about the resistance period.

From the left: G. Valinčius, J. Jakavonis-Tigras, A. Anušauskas, A. Dapšys

Diplomas Awarded to VU LSC Graduates

On 30 June 2020, the community of the Life Sciences Center held a diploma ceremony for graduates of VU LSC BSc and MSc degree programs. VU Vice-Rector for Research E. Sužiedėlienė, LSC Director G. Valinčius, LSC Deputy Director for Studies I. Prigodina Lukošienė, representatives of VU social partners Thermo Fisher Scientific Baltics congratulated the graduates.

Visit to the Branch of Svėdasai Nursing Home and Burbiškis Group Life Home Branch of Anykščiai Social Care Home

On February 24 2020, representatives of our community visited the residents of the Svėdasai Nursing Home and of Burbiškis Group Life Home Branch of Anykščiai Social Care Home.

"The initiative to visit the elderly at the Life Sciences Center has been around for several years. We try to brighten the daily life of these people with the gifts collected every year during the Christmas period", said J. Jachno, one of the promoters of this idea.

Public Engagement

Science and Business Professionals Presented the Development of Bioeconomy and Life Sciences in Lithuanian Regions

The LSC senior researcher Inga Matijošytė joined the tour of events organized by the Lithuanian Biotechnology Association *Bioeconomy and Life Sciences Development Opportunities in Lithuanian Regions*.

During these events, scientists and biotechnology industry representatives met with representatives of regional municipalities and local businesses with the aim to discuss the possibilities of regions to become more involved in the development of circular bioeconomy, to assess the potential for development of innovative technologies. By promoting cooperation between science and business in the regions in the development of bioeconomy and life sciences, regions could be more involved in international projects, in the development of high value-added production, in the increase of the number of employees and, of course, in the reduction of regional socio-economic exclusion.

In parallel during the tours, the LSC researchers Jokūbas Krutkevičius and Dovilė Daunoraitė organized seminars *Biotechnology in Daily Life* and mind game *BioSmart* for schools in the regions.

Bioeconomy and Life Sciences Development Opportunities in Lithuanian Regions event was partially funded by the Research Council of Lithuania.

Life Sciences Day for Schoolchildren

In October 2020, the Life Sciences Center of Vilnius University (VU LSC) and the Lithuanian Association of Biotechnologists organized a remote event for schoolchildren, the Life Sciences Day.

An event of this nature and scope was organized at LSC for the first time, as schoolchildren from more than 50 schools joined the event remotely. The speakers presented various scientific topics to schoolchildren.

Science Festival *Spaceship Earth*

Science festival *Spaceship Earth* is an annual event, organised since 2004 in the second week of September. The main aim of the festival is to foster scientific culture in society and to encourage schoolchildren to pursue careers in science. Over the years, it has become the largest science-promoting event in Lithuania, covering all fields of science. In 2020, it took place in 17 cities and districts of Lithuania. The program of the festival included over 300 free events (lectures, demonstrations, tours, exhibitions) and reflected the latest trends in science and technology in the world and in Lithuania. However, due to the threat of coronavirus, the majority of events were remote, or the number of participants was limited to 10–30 people.

Every year, the Life Sciences Center scientists actively participate in this festival, usually with interactive activities: tours, visits to research laboratories and practical activities. In 2020, scientists from the Department of Zoology, the Department of

Botany and Genetics and the Department of Biochemistry and Molecular Biology organized four tours, two lectures and two laboratory workshops. During this festival, the VU LSC microbiologists from the Department of Microbiology and Biotechnology organized three lectures dedicated to the international *Microorganism Day*, an annual international event that aims at raising awareness among the society on the essential role of microorganisms in our everyday life.

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 over a 100 schools in 70 cities of Lithuania.

Kaunas Fort VII – School for Natural and Exact Sciences

Collaboration with an informal, multifunctional children's education centre Kauno tvirtovės VII fortas (Kaunas Fort VII) includes consultations for teachers and secondary school pupils on protein purification and chromatography techniques.

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. The National Student Academy is a public educational organization for gifted high school pupils from forms 9–12. The Extramural School of Young Biochemists also focuses on 9–12 form schoolchildren, who show particular interest in life sciences. The Extramural School of Young Biochemists was founded in 1978 and until now, more than 1,600 graduates have finished this school.

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 students, who have ability to understand research design and conduct scientific research. In January 2020, both schools had classes in the LSC laboratories to develop practical skills.

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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)*
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 S*treptococcus 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)

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)*

PATENT APPLICATIONS

1. RNA-directed DNA cleavage by the Cas9-crRNA complex (US20150291961A1; US20180187195A1; JP2019030321A)
2. Systems and methods for barcoding nucleic acids (US20150298091A1; US20180304222A1; JP2017515469A)*
3. System and method for a biomimetic fluid processing (US20200316597A1; US16646593A1; CN105308452A1)*
4. System and method for producing target biological substances (EP3712612A1)*
5. Selective inhibitors of carbonic anhydrase (US20180222856A1; EP3328833A1)
6. Analysis of single-stranded RNA (US20180251814A1)
7. System and method for synthesis of DNA particles and use thereof (US20190002943A1)*
8. Production of cyclic adenylates and their use as allosteric regulators (EP3630966A1)
9. Methods for the identification and characterization of double-strand break sites and compositions and uses thereof (WO2019217816A1)*
10. Characterization of prostate cancer using DNA methylation assay (PCT/IB2019/056204)
11. N 4-modified cytidine nucleotides and their use (EP3681897A1; US20200270295A1)
12. Systems and methods for encapsulation and multi-step processing of biological samples (PCT/IB2020/057266)*;
13. Method for generating functional protein sequences with generative adversarial networks (PCT/IB2020/058401)*
14. Methods and compositions for noninvasive prenatal diagnosis through targeted covalent labeling of unmodified genomic sites (PCT/IB2020/053011)
15. Clear cell renal cell carcinoma biomarkers and uses thereof (US63128874)*

Key Performance Indicators	2020
New patent applications filed in 2020	7
Total number of EU and US patents	30
US patents granted in 2020	3
EU patents granted in 2020	4
Licenses	13

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

Cooperation

RESEARCH INSTITUTIONS

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AdventHealth for Children (USA)
Auckland University of Technology (New Zealand)
August Kirchenstein Institute of Microbiology and Virology (Latvia)
Australian Animal Health Laboratory - CSIRO (Australia)
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Finish Institute of Occupational Health (Finland)
Friedrich-Loeffler Institute (Germany)
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Poznan University (Poland)
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VTT Technical Research Centre (Finland)
Weizmann Institute of Science (Israel)
Wroclaw University of Science and Technology (Poland)
Zoological Institute of the Russian Academy of Sciences (Russia)

INTERNATIONAL INDUSTRY COLLABORATIONS

Abcam AG (UK), ArcDia (Finland), DuPont (US), Kalon Biological/Clin-Tech Ltd (UK), -Ramidus AB (Sweden), Santa Cruz Biotechnology Inc. (US), serYmun Yeast (Germany), Synthon chemicals (Germany), ThermoFisher Scientific (US), Experimentica (Finland).

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COMPANIES FOUNDED BY LSC RESEARCHERS

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

Names Index

CONTACT INFORMATION

Life Sciences Center
Vilnius University
Saulėtekio Ave 7, LT-10257 Vilnius, Lithuania
Phone: +370 5 223 4435 / +370 5 223 4426
Email: info@gmc.vu.lt
www.gmc.vu.lt/en/