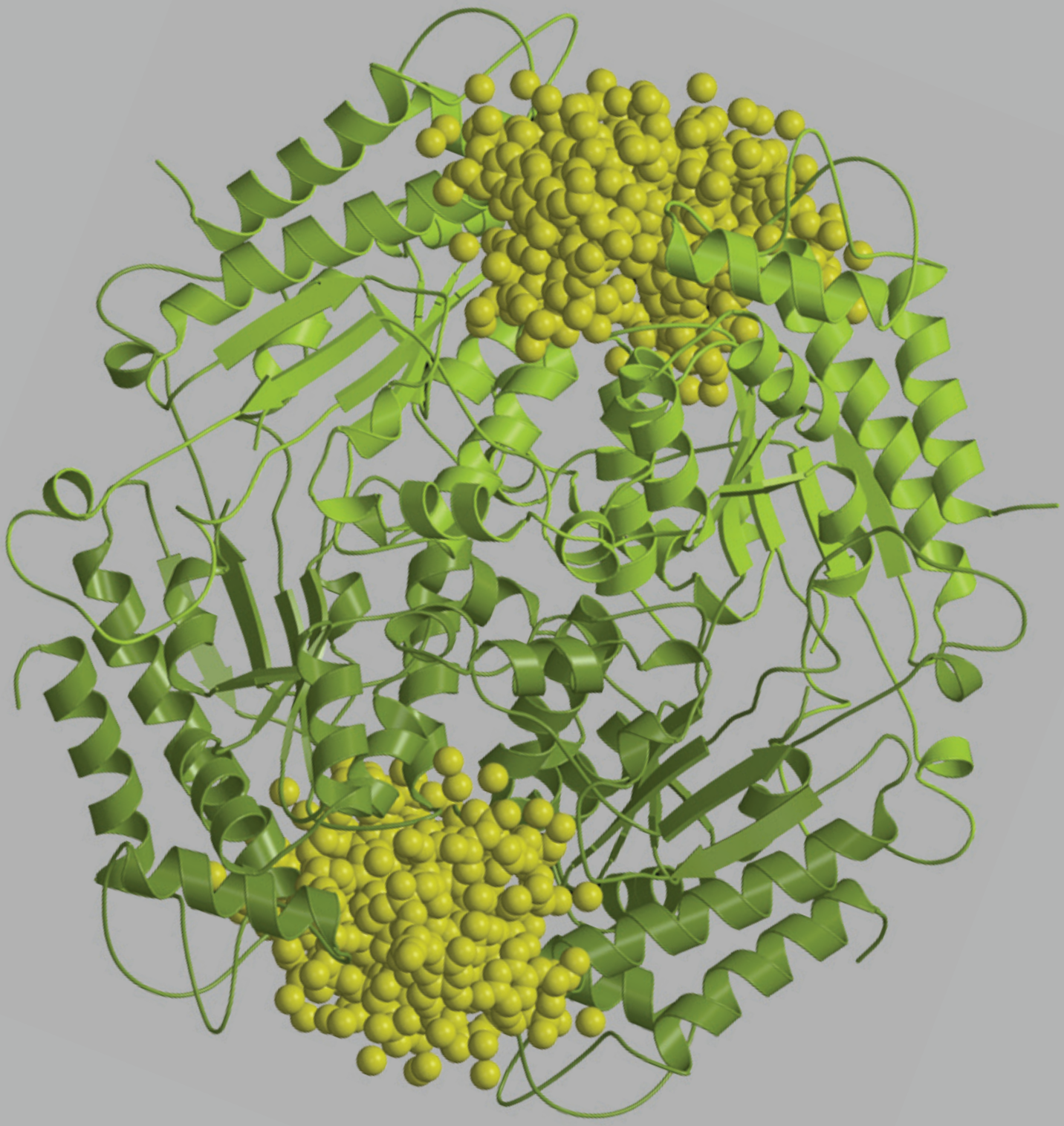
Life Sciences Center



Annual Report 2018

Front cover: the crystal structure of the NgoMIV restriction endonuclease in complex with DNA (PDB ID 1FIU, Deibert et al, 2000). NgoMIV restriction endonuclease belongs to the CCGG-family of restriction enzymes. This family was investigated by prof. Virginijus Siksnys' group (see page 6). Since 1996 Prof. V. Siksnys' group in cooperation with Prof. R. Huber's and Prof. M. Bochtler's laboratories has determined the crystal structures of 19 restriction endonucleases, which is ~30% of all known crystal structures of these DNA restriction enzymes.

CONTENTS

FOREWORD

2018 was an exceptional year for the Life Sciences Centre (LSC). Our Centre along with other Lithuanian research institutions was evaluated by a benchmarking exercise and was granted highest scores among peers in the physical, biomedical and technology sciences. The results of the benchmarking evaluation will determine government funding of Lithuanian research institutions for the next five years. Therefore, the LSC has a solid ground for the long-term development of research capacities and aspiring for excellence in science.

Last year was marked by exceptional recognitions of the scientific excellence of our researchers. One of our Centre's leading scientists, professor Virginijus Šikšnys, along with two other prominent researchers Jennifer Doudna and Emmanuelle Charpentier, was awarded the Kavli prize. On September 4th, in Oslo, the Kavli prize and medal were presented to Virginijus Šikšnys by the King Harald V of Norway. It was a historic event not only for our Centre and Vilnius University but for the whole country as well.

At the beginning of the year, our professor Saulius Klimašauskas was awarded the Lithuanian Science Prize for his contribution to the development of the "Molecular tools for epigenomics and RNAomics", which he and his team were developing in 2002–2016. The Lithuanian Science prize was also awarded to Virginijus Šiksnys and his team: Giedrius Gasiūnas, Toma Šinkūnas and Tautvydas Karvelis for the cycle of research works "Investigation of CRISPR-Cas systems: from immunity of bacteria to the gene-editing technology". The cycle includes research and development works performed from 2011 to 2016. I am sure, these awards will inspire generations of young scientists and provide momentum for the development of scientific excellence at the LSC.

The Graduate School for Life Sciences started operating in 2018. Daiva Baltriukienė was appointed the first director of the School. This is the first graduate school at Vilnius University, which aims at attracting young talents from both Lithuania and abroad. I believe, the brand new organisational structure of the graduate studies along with new student-centred, project-based Biotechnology study programs, approved by the Senate of Vilnius University, will provide ample opportunities for the LSC to become more internationally open to perspective students from other countries.

The LSC was highly visible nationally as well as internationally in 2018. Research papers in top-tier international journals, new discoveries and technology developments at the LSC, exhibitions which bring science and art together, vibrant seminars and talks at the LSC presented by prominent scientists, including the Nobel laureates Brian Kobylka, Harald zur Hausen and Eric Sackman, all these events saturated our academic life with the atmosphere of scientific excellence and meaningful deeds. Our students were a large part of our success. The Coins conference is becoming a prominent scientific event in Lithuania, while two VU iGEM teams in the Synthetic Biology contest in Boston won gold and bronze medals for their innovative projects carried out at the LSC. All these achievements demonstrate the strength and dynamism of the Life Sciences Centre's community at Vilnius University. I wish to thank all our community members for contributing so immensely to the academic growth of our University and to the intellectual and economic prosperity of our fellow citizens.

RESEARCH DESCRIPTIONS

Biomolecule Structure & Function

Cell & Molecular Biology

Ecosystems & Biodiversity

Molecular Biotechnologies

Molecular Mechanisms of Disease

Neurobiology

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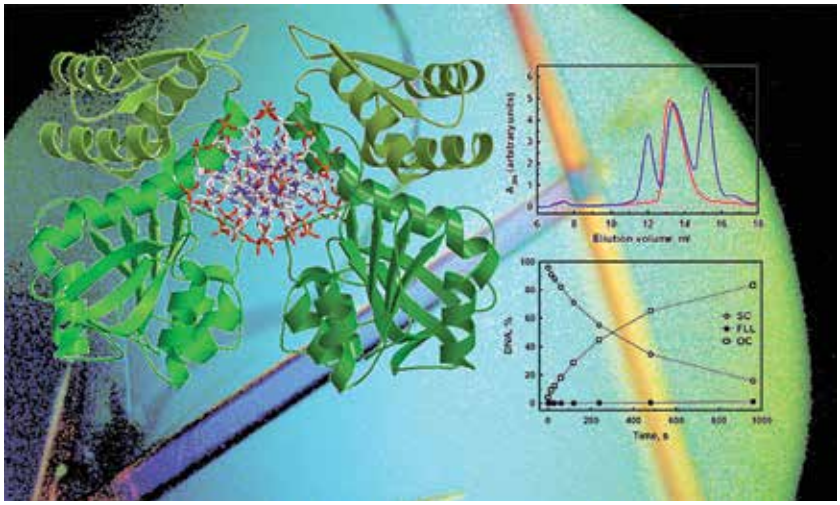

Antiviral Defense Systems in Bacteria

Phages are the most abundant organisms in the biosphere and the major parasites of bacteria. They infect bacteria in order to replicate and usually kill bacteria when the replication is completed. In response to the phage threat, bacteria developed multiple defence barriers for countering and fighting viral attacks. 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 restriction enzymes. We are using X-ray crystallography, mutagenesis and functional biochemical as well as biophysical assays to acquire more information on these systems.

Restriction-modification (R-M) systems often function as the first antiviral defence line and act as sentries that guard bacterial cells against invasions by bacteriophages. R-M systems consist of two complementary enzymatic activities, namely restriction endonuclease (REase) and methyltransferase (MTase). Typically, REase cuts foreign DNA but does not act on the host genome, because the target sites for REase are protected by accompanying MTase. In this respect, they function as an innate immune system of bacteria. REases have now gained widespread application as indispensable tools for the *in vitro* manipulation and cloning of DNA. We focus on the structural and molecular mechanisms of restriction enzymes.

CRISPR-Cas is a recently discovered 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 are subdivided into two classes depending on the composition of the effector complex. We aim to understand the molecular and structural mechanisms of immunity provided by different CRISPR-Cas systems.

In recent years, we have solved a number of 3D structures of REases by X-ray crystallography and characterized molecular mechanisms by a combination of biochemical and biophysical techniques including single molecule techniques in collaboration with Dr. R. Seidel (Universität Leipzig) and Dr. M. D. Szczelkun (Bristol University) [1, 2]. We have shown that the Csm effector complex of the type III CRISPR-Cas system provides immunity through a coordinated action of three different enzymatic activities and synthetizes a novel signalling molecule in response to target RNA binding [3, 4]. To explore type IIIA CRISPR-Cas systems as molecular tools initiated *in vivo*, we have initiated studies in *Danio rerio* in collaboration with Dr. M. Bochtler (International Institute of Molecular and Cell Biology, Warsaw) and in human cancer cells in collaboration with Dr. V. Starkuviene (University of Heidelberg).

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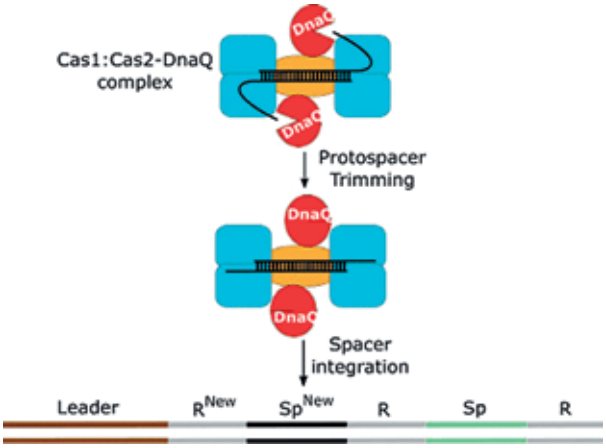
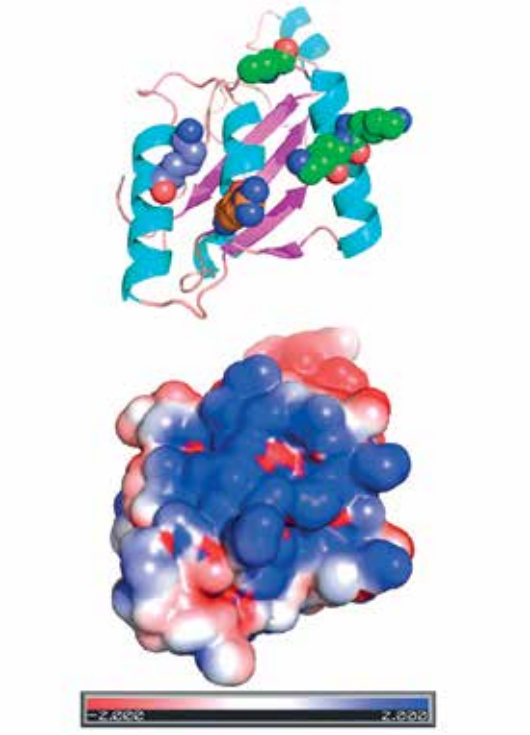
The Structural and Molecular Mechanisms of CRISPR-Cas Systems

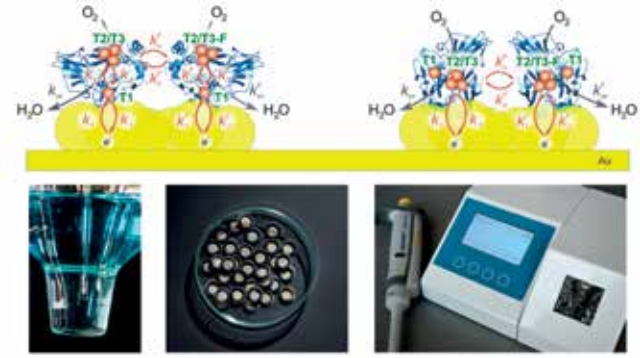
Upon invasion of foreign nucleic acids, some cells containing CRISPR-Cas systems integrate short fragments of foreign DNA as spacers into the CRISPR locus to memorize the invaders and acquire resistance in the subsequent round of infection. This immunization step called adaptation is the least understood part of the CRISPR-Cas immunity. We studied the adaptation stage of *Streptococcus thermophilus* DGCC7710 type I-E CRISPR4-Cas (St4) system. Cas1 and Cas2 proteins conserved in nearly all CRISPR-Cas systems are required for spacer acquisition. The St4 CRISPR-Cas system is unique because the Cas2 protein is fused to an additional DnaQ exonuclease domain. We demonstrated that St4 Cas1 and Cas2-DnaQ form a multimeric complex, which is capable of integrating DNA duplexes with 3′-overhangs (protospacers) *in vitro*. We showed that the DnaQ domain of Cas2 functions as a 3′-5′-exonuclease that processes 3′-overhangs of the protospacer to promote integration (Drabavicius et al. *EMBO Rep*. 2018, pii: e45543. doi: 10.15252/embr.201745543).



The Structure and Function of Restriction Endonucleases

CglI is a restriction endonuclease from *Corynebacterium glutamicum* that forms a complex between: two R-subunits that have site specific-recognition and nuclease domains; and two H-subunits with Superfamily 2 helicase-like DEAD domains, and uncharacterized Z1 and C-terminal domains. ATP hydrolysis by the H-subunits catalyses dsDNA translocation that is necessary for a long-range movement along DNA that activates nuclease activity. We provided biochemical and molecular modelling evidence that Z1 has a fold distantly related to RecA, and that the DEAD-Z1 domains together form an ATP binding interface and are the prototype of a previously undescribed monomeric helicase-like motor. The DEAD-Z1 motor has unusual Walker A and Motif VI sequences those nonetheless have their expected functions. Additionally, it contains DEAD-Z1-specific features: an H/H motif and a loop (aa 163-172) that both play a role in the coupling of ATP hydrolysis to DNA cleavage. We also solved the crystal structure of the C-terminal domain which has a unique fold, and demonstrate that the Z1-C domains are the principal DNA binding interface of the H-subunit. Finally, we used small angle X-ray scattering to provide a model for how the H-subunit domains are arranged in a dimeric complex (Toliusis et al. *Nucleic Acids Res*. 2018, 43: 2560–2572).





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Bioelectrochemical Systems in Biosensors and Bioreactors

Mediated and direct electron transfer (ET) coupling of enzymes to electrodes is important in realizing bioelectrocatalysis, which is often exploited as a basic 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, provided 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 to 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. Currently, the most efficient bioelectrocatalytic systems are based on direct ET, where enzyme exchanges electrons directly with electrode surface without redox mediators.

In this field, our team is proficient at constructing bioelectrochemical systems by wiring oxidoreductases to gold and carbon based electrode surfaces [1-3]. The enzyme-based amperometric biosensors for glucose, fructose, urea, glycerol and alcohols were designed, the core of which was based on either specially modified graphite or gold surfaces. For the most part the biosensors were produced by immobilisation of either *Canavalia ensiformis* urease, *Acinetobacter calcoaceticus* PQQ glucose dehydrogenase or *Gluconobacter industrius* D-fructose dehydrogenase on semi-permeable PET membranes mounted on working electrodes; nanostructured electrodes for biofuel cells were prepared by adsorbing laccases from various fungi. The most effective carbon-based bioelectrocatalytic systems were used in designing of prototypes of an analyser, which was applied for detection of urea in dialysate and other biologic liquids. For the next step to whole-cell biosensors field, a self-organization of *E.coli* in nutrient-rich microtiter wells is analysed and modelled [4]. On international level, we collaborate with scientists from institutions such as Malmö University (Sweden), Institute of Molecular Biology and Genetics of National Academy of Sciences of Ukraine, Moscow Kurchatov NBICS Centre (Russia).

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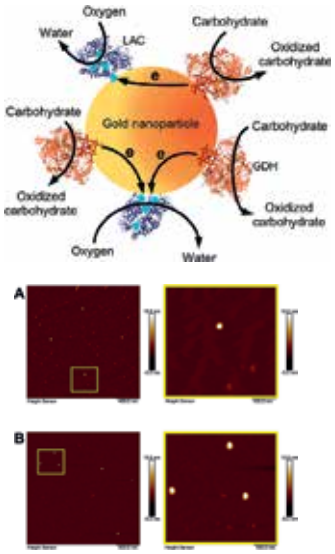
2. Gaidukevič, J.; Razumienė, J.; Šakinytė, I.; Rebelo, SLH.; Barkauskas, J. Study on the structure and electrocatalytic activity of graphene-based nanocomposite materials containing $(SCN)_n$. Carbon. 2017, 118: 156–167.
3. Ratautas, D.; Ramonas, E.; Marcinkevičienė, L.; Meškys, R.; Kulys, J. Wiring gold nanoparticles and redox enzymes: a self-sufficient nanocatalyst for oxidation of carbohydrates directly with the molecular oxygen. ChemCatChem. 2018, 10: 971–974.
4. Šimkus, R.; Meškienė, R.; Aučynaitė, A.; Ledas, Ž.; Baronas, R.; Meškys, R. Phoretic interactions and oscillations in active suspensions of growing *Escherichia coli*. Royal Soc Open Sci. 2018, 5: 1–16.

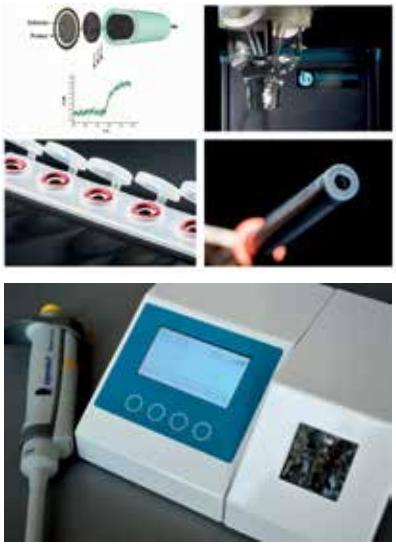
Hybrid Catalysis on Nanostructured Gold Surfaces

A prominent skill of our team is the ability to employ techniques for optimal enzyme adsorption on nanostructured gold surfaces obtaining an efficient bioelectrocatalysis. Recently, we demonstrated oxygen electroreduction catalysed by laccase oriented to gold nanoparticles via the trinuclear copper cluster [1]. Recently, we successfully created nanocatalysts composed of gold nanoparticles, glucose dehydrogenase, and laccase that were shown to act in concert and oxidized various carbohydrates directly with molecular oxygen [3]. From a fundamental viewpoint, this study demonstrated the possibility to wire oxidoreductases with gold nanoparticles to allow electron flow from one active centre to another. This is one of the techniques that is currently used in our project for the creation of bioreactor for oxidation of non-starch poly/oligosaccharides (RCL grant No. 01.2.2-LMT-K-718-01-0019).



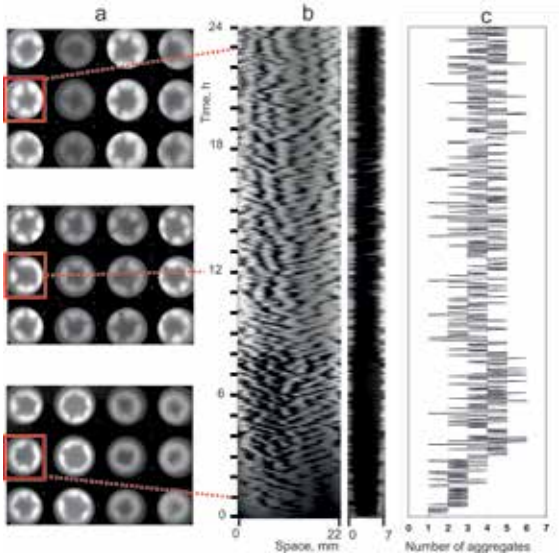
Biosensor with Graphene/Graphite-Based Materials

Currently, the electrocatalytic properties of carbonaceous materials doped with various functionalities are attracting attention in the field of biosensors or fuel cells. Recently, in collaboration with Faculty of Chemistry and Geosciences, graphene/graphite-based materials were synthesized, characterised by SEM, XRD, TGA analysis, Raman spectroscopy and BET measurements. The investigations of synthesized composites also revealed remarkable catalytic performance toward oxygen or hydrogen peroxide reduction reactions that opens new opportunities for creation of new technologies consuming oxygen or hydrogen peroxide [2]. Graphene-based materials were used in creation of urea analyser for fertilizer samples, developed together with our start-up company CC *Bioanalizės sistemos* (MITA grant No. TPP-01-054), as well as in utilization of biosensors for early diagnosis of acute pancreatitis (RCL grant No. 01.2.2-LMT-K-718-01-0025).



Self-Organization of Bacteria

Bioanalytical systems can be constructed by using whole-cell biosensors, where bacteria are grown on electrode surfaces. Bioluminescence imaging can be employed to provide new insights into the self-organization of such bacteria. We use bioluminescence imaging to record images of nutrient-rich liquid cultures of the *lux*-gene reporter *E. coli* and their mutants in microtiter plate wells. Analysis of the experimental data together with mathematical modelling of pattern formation suggest the following: 1) pattern-forming processes can be described by Keller-Segel-type models of chemotaxis with logistic cell kinetics; 2) active cells can be seen as biochemical oscillators that exhibit phoretic drift and alignment; 3) the spatiotemporal patterns in a suspension of growing *E. coli* form due to phoretic interactions between oscillating cells of high metabolic activity [4]. These studies were partly funded by RCL grant No. S-MIP-17-98.



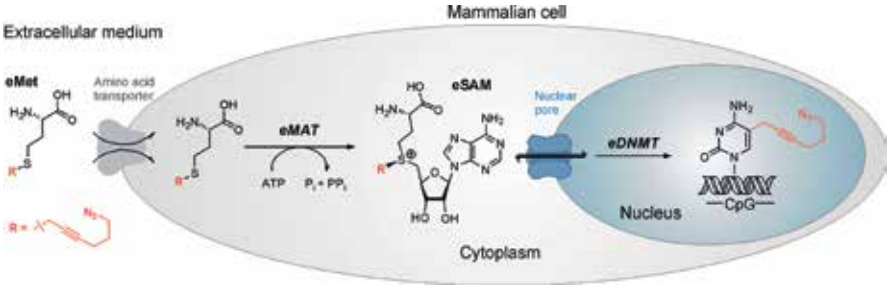


Fig. 1

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Biological Modification of DNA and RNA

Epigenetic Modifications of DNA and RNA in Mammals

In recent years, epigenetic phenomena have become a major focus in studies of embryonic development, genomic imprinting and complex human diseases. One of the best-understood epigenetic mechanisms is enzymatic DNA methylation. In the mammalian genome, cytosines in CpG dinucleotides are often methylated to 5-methylcytosine (m5C), which is brought about by combined action of three known AdoMet–dependent DNA methyltransferases (DNMTs). DNA methylation profiles are highly variable across different genetic loci, cell types and organisms, and are dependent on age, sex, diet and disease. Besides m5C, certain genomic DNAs contain detectable amounts of 5-hydroxymethylcytosine (hmC) and lower levels of 5-formylcytosine and 5-carboxylcytosine (caC), which are produced by the oxidation of m5C residues by TET oxygenases. However, many details of how these modifications are established at specific loci and how they control cellular events remain obscure [1,2].

The most abundant modification in RNA 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. Besides the HEN1 MTases [3], which modify 3'-terminal nucleotides in small RNAs, our studies focused on C/D box RNP MTases, which target sequence-defined internal nucleotide in tRNA and rRNA [4].

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 [5]. 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 (Fig. 1).

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5. Staševskij, Z.; Gibas, P.; Gordevičius, J.; Kriukienė, E.; Klimašauskas, S. Tethered Oligonucleotide-Primed sequencing, TOP-seq: a high resolution economical approach for DNA epigenome profiling. Mol. Cell. 2017, 65(3): 554–564.

Epigenomic Approaches for Deciphering Cell-Free DNA

Each cell type carries a unique DNA modification profile consisting mainly of patterns of 5-methylcytosine in CpG dinucleotides, which are critical for establishing and maintaining cellular identity. Blood plasma contains cell-free circulating DNA (cfDNA), which consists of the short cfDNA fragments that derive primarily from apoptosis of normal cells of the hematopoietic lineage, with some contributions from other tissues. The distribution of tissues comprising cfDNA varies in the context of specific physiological conditions or disease processes. Analysis of cfDNA has been instrumental for detecting foetal abnormalities in the maternal circulation, monitoring advanced cancers by quantifying mutations or aneuploidy and observing graft failure in transplant medicine. Epigenetic signatures of tumour-derived DNA, found in body fluids (liquid biopsy) of cancer patients as part of cfDNA, can serve as an analytical target for early cancer detection and monitoring. Recent studies demonstrate that detection of the tumour-derived changes in DNA modification combined with high-throughput analysis holds promise for developing highly specific noninvasive diagnostic tests (Daniūnaitė et al. *Curr. Opin. Biotechnol.* 2019, 55: 23–29).

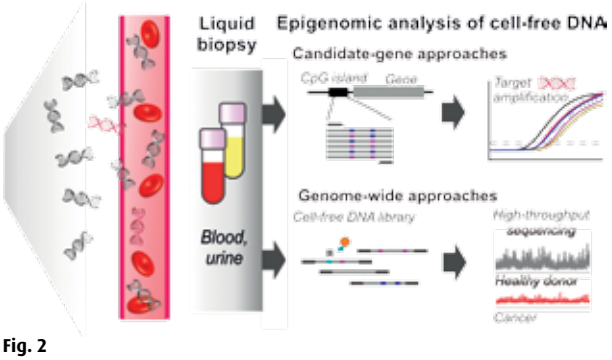


Fig. 2

Activity of Animal HEN1 2′-O-methyltransferases *in vitro* Permits Covalent 3′-labelling of Single-Stranded RNAs.

2′-O-methylation plays important roles in biogenesis of eukaryotic small non-coding RNAs, such as siRNAs, miRNAs and piRNAs. Here we demonstrate that, in contrast to plant and bacterial homologues, the Drosophila DmHen1 and human HsHEN1 piRNA methyltransferases require cobalt cations for their enzymatic activity *in vitro*. We also unveil the capacity of the animal Hen1 to catalyse the transfer of a variety of chemical groups from synthetic analogues of the AdoMet cofactor onto a wide range of ssRNAs permitting their 3′-terminal functionalization and labelling. Moreover, deletion of a C-terminal region from DmHen1 further increases its catalytic robustness as compared to the full-length protein. Finally, we demonstrate a successful FRET detection of the 3′- tagged ssRNA molecules in total RNA mixtures. These findings pave the way to developing novel chemo-enzymatic approaches for *in vitro* studies and monitoring of RNA pools (Mickutė et al. *Nucleic Acids Res*. 2018, 46: e104

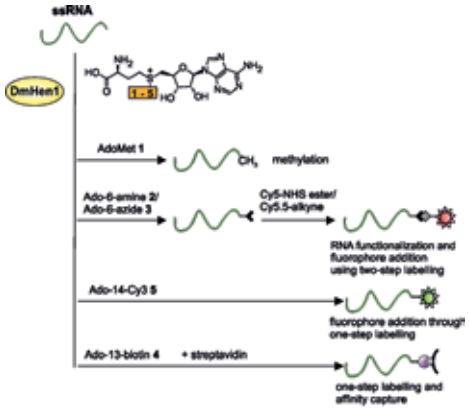


Fig. 3

Excision of a Doubly Methylated Base, *N*4,5-dimethylcytosine, from DNA by Nei and Fpg Proteins

Despite the significance of m^5C for epigenetic regulation in mammals, methylation damage to m^5C has received little attention. Chemical and enzymological evidence suggested that the doubly methylated base *N*4,5-dimethylcytosine ($m^{4,5}C$) can occur in DNA *in vivo*. In collaborative studies, we screened a series of glycosylases, from prokaryotic to human, and found DNA incision activity of the *E. coli* Nei and Fpg proteins at $m^{4,5}C$ residues *in vitro*. The activity of Nei was highest opposite cognate G followed by A, T and C. Fpg-complemented Nei by exhibiting the highest activity opposite C followed by lower activity opposite T. To our knowledge, this is the first description of a repair enzyme activity at a permethylated m^5C in DNA, and the first alkylated base allocated as a Nei or Fpg substrate. Based on our observed high sensitivity to nuclease S1 digestion (Fig. 4), we suggest that $m^{4,5}C$ occurs as a disturbing lesion in DNA and that Nei may serve as a major DNA glycosylase in *E. coli* to initiate its repair (Alexeeva et al. *Phil. Trans. R. Soc. B*. 2018, 373(1748): 20170337).

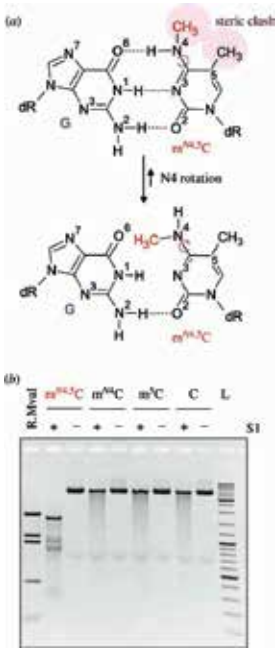
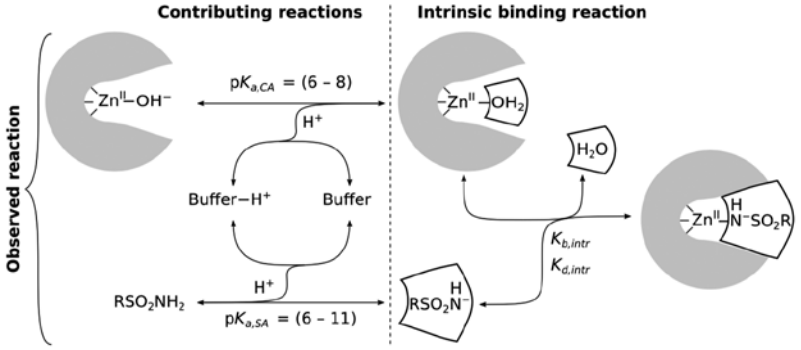


Fig. 4



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Drug Design via Structure and Thermodynamics

Rational drug design should be able to make chemical compounds that bind to disease-associated target proteins with high affinity and selectivity over all the remaining proteins to avoid toxicity. Unfortunately, such a design is not possible and currently, pharmaceutical companies instead perform various high-throughput screenings of available chemical libraries and develop compounds that perform best in such highly random screens. The reason for such a non-rational approach is that the recognition phenomenon between chemical compounds and proteins is poorly understood. It is not possible to design compounds *in silico* and predict their affinity to target proteins. There is a lack of suitable, well-determined datasets, where chemical compounds binding to proteins would be characterized, including (a) the crystal structures of protein-ligand complexes, (b) the thermodynamics of interaction of the same protein-ligand complexes (including the enthalpy, entropy, Gibbs energy, volume, heat capacity and other thermodynamic parameter changes upon binding) and (c) the kinetics of the same protein-ligand binding. In order to make drug design truly rational and make their success rate much higher in clinical trials, it is important to solve the structure-energetics relationships and be able to predict the binding efficiency of the designed compounds.

Our 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, primarily the family of human carbonic anhydrases and chaperones (Hsp90), biothermodynamicists determine the energetics of binding between the synthesized compounds and the target proteins by ITC or thermal shift and search for structure-energetics correlations, *in silico* modellers and crystallographers determine the X-ray crystallographic structures of protein-compound complexes, and the pharmaceutical scientists perform development studies of the effect of compounds in various biological systems including zebrafish and mice.

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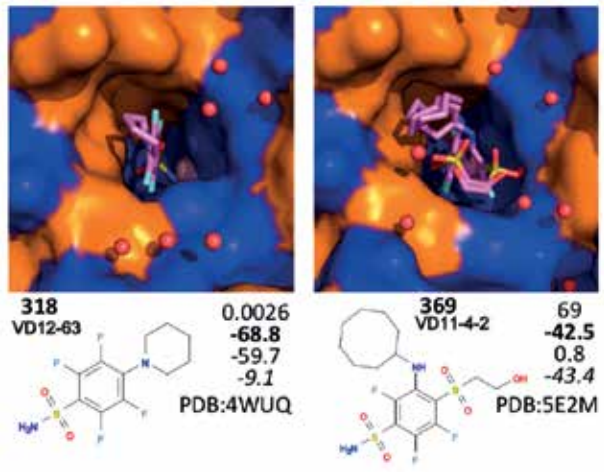
Structure Thermodynamics Correlations of CA Inhibitors

Last year we published a major review (Linkuviene et al, *Quart. Rev. Biophys*. 2018) that included a collection of intrinsic thermodynamic data of 402 inhibitors binding to 12 human carbonic anhydrase protein isoforms. This dataset included not only the affinities (standard Gibbs energy changes upon binding), but also standard enthalpies and entropies of binding enabling to determine a more precise mechanism of each inhibitor interaction and recognition of the protein active site.

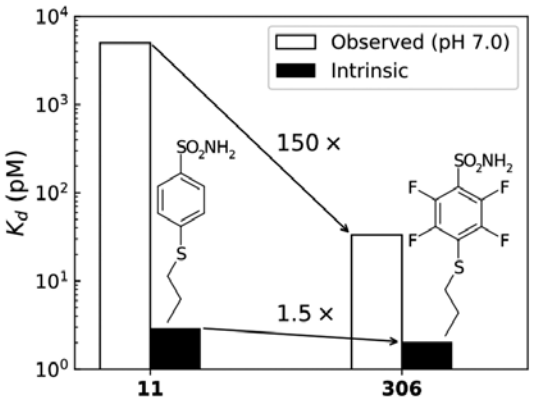
Together with a set of 85 crystal structures of compound complexes with 6 CA isoforms, the dataset allowed to evaluate and search for structure-thermodynamics correlations that are essential for rational drug design.



Intrinsic Thermodynamics of Inhibitor Binding to CAs

Most laboratories determine only the observed thermodynamic parameters of interaction between compounds and proteins. Such approach may lead to incomplete or even incorrect interpretation of the functional group contributions to the overall thermodynamics of compound binding thus leading to missed opportunities to understand the recognition phenomenon.

In this example, we see two compounds that both are very good inhibitors of CA I. Direct determination by any experimental technique, including enzymatic activity inhibition assay, isothermal titration calorimetry, or fluorescent thermal shift assay (that we consider advantageous in many instances), shows that the fluorinated compound on the right exhibits approximately 150-fold higher affinity for the protein than the non-fluorinated compound. However, dissection and accounting for the intrinsic parameters show that the fluorinated compound is only 1.5-fold stronger binder.

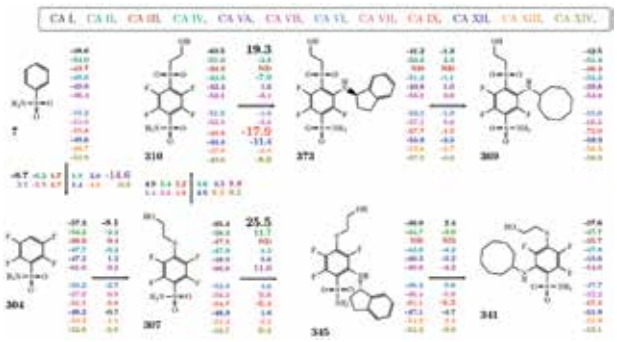


Fluorine atoms play a minor role in recognizing the protein surface, but instead they reduce the pKa of the sulfonamide group leading to stronger observed affinity.

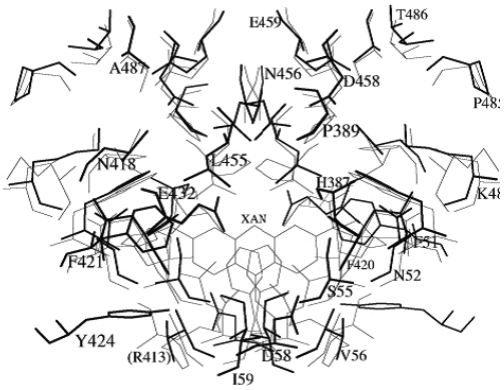
Chemical Structure-Thermodynamics Maps in Search of Selective Inhibitors

The goal of rational drug design is to understand the structure-thermodynamics correlations to predict the chemical structure of the drug that would exhibit an excellent affinity and selectivity to a target protein. In this figure, we explore the contribution of the added functionalities of inhibitors to the intrinsic binding affinity. Interestingly, the binding enthalpies of the compounds possessing similar chemical structures and affinities were highly different, spanning a range from -90 to +10 kJ/mol and compensated by a similar opposing entropy contribution.

The numbers next to compound structures show the intrinsic standard Gibbs energies of binding to all 12 human CAs (shown in different colours, labelled above). Introduction of particular



functional groups has increased the affinity thousand fold and at the same time often affected the selectivity toward the isoform that is targeted as a disease-associated protein.



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Mechanisms of Flavoenzyme Redox Reactions

Flavoenzymes contain flavinmononucleotide (FMN) and 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. For a long time, our studies were concentrated on the following aspects: 1) the mechanisms of electron/hydride transfer in catalysis of flavoenzymes electrontransferases and transhydrogenases; 2) single- and two-electron reduction of quinoidal and nitroaromatic compounds by mammalian or microbial flavoenzymes and their impact on their cytotoxicity; 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 2015–2018 were as follows: a) characterization of intraprotein electron transfer and mechanism of reduction of quinones and nitroaromatics by *S. aureus* flavohemoglobin, discovery of the activation of quinone reduction by azole antibacterial drugs that bind to the heme of flavohemoglobin (collaboration with dr. L. Baciou and F. Lederer, Universite Paris-Sud, France); b) characterization of intraprotein electron transfer and reduction of nonphysiological electron acceptors by flavocytochrome b2 (collaboration with dr. F. Lederer, Universite Paris-Sud, France); c) characterization of the mechanisms of two-electron reduction of quinones and nitroaromatic compounds by *E. coli* nitroreductase A and other nitroreductases (collaboration with dr. D.F. Ackerley, Wellington University, New Zealand); d) evaluation of nitroaromatic compounds as inhibitors and subversive substrates for Plasmodium falciparum glutathione reductase in the context of development of antiplasmodial agents (collaboration with dr. E. Davioud-Charvet, Universite de Strasbourg, France); e) studies of reduction of quinones, nitroaromatic compounds and aromatic N-oxides by P. falciparum ferredoxin:NADP+ oxidoreductase as a possible target for prooxidant antiplasmodial agents (in collaboration with dr. A. Aliverti, Universita di Milano, Italy) and f) 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 (European Social Fund, Global Grant Measure, Grant No. 09.3.3-lMT-K-712-01-0058, 2018–2021).

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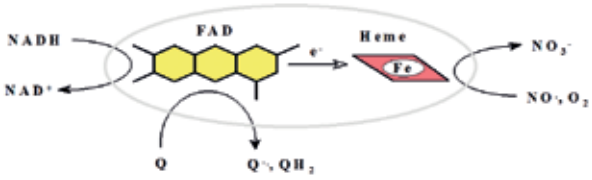
Quinones and Nitroaromatic Compounds as Subversive Substrates of Flavohemoglobin

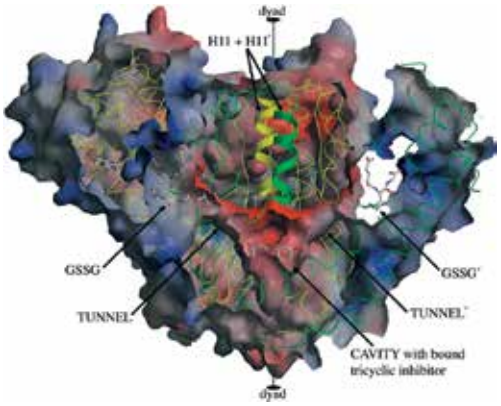
Bacterial flavohemoglobins (FHbs) contain FAD and heme redox moieties, and catalyse the NADH-dependent formation of NO_3^- from NO. FHb prevent the formation of toxic peroxynitrite from NO and superoxide, thus maintaining the viability and virulence of bacteria. We found that quinones and nitroaromatic compounds oxidize the reduced FAD but not heme of *Staphylococcus aureus* FHb, thus competing for the heme reduction. Besides, their redox cycling enhances the formation of superoxide and other reactive oxygen species, which in turn enhances the formation of peroxynitrite. Thus, these reactions may contribute to the bactericidal action of quinones and nitroaromatics. We also found that ketoconazole and other inhibitors of *S. aureus* FHb binding at the heme group activate quinone reduction. This may represent a novel combined approach in antibacterial chemotherapy (Moussaoui et al., *Free Radic. Biol. Med.* 2018, 123: 107–115). Our preliminary observations show that ketoconazole enhances the activity of juglone against *S. aureus*.



Electron flux in catalysis of flavohemoglobin

Interaction of Nitroaromatic Compounds with Biomedically Relevant Flavoenzymes

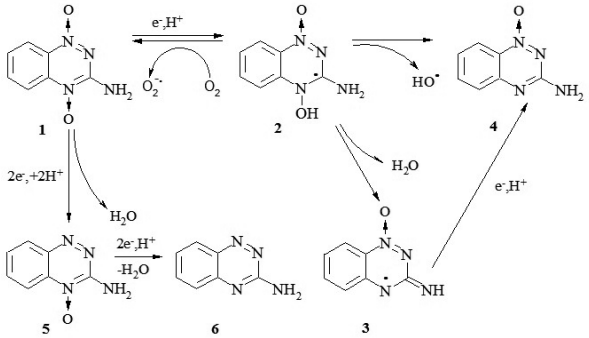
Tumour transfection by bacterial FMN-dependent nitroreductases (NRs) is an advanced tool in tumour chemotherapy and imaging. Clarifying the mechanism of *E. coli* NR-A, we found that in a net four-electron reduction of aromatic nitrocompounds to hydroxylamines the reduction of nitroso intermediates proceeds mainly by a direct reduction by NADPH, *i.e.,* nonenzymatically (Valiauga et al. *Molecules.* 2018, 23; 1672). We also 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.



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 Tirapazamine and Its Metabolites

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 cytotoxity of TPZ and, possibly, its 1-oxide metabolite are well above than expected from their E^1_7 values and reactivity towards single-electron transferring flavoenzymes. This was attributed to the formation of hydroxyl radical (OH·) which takes place during their reduction in partly hypoxic cell nucleus (Šarlauskas et al. *Chemija.* 2018, 29: 273–280). This is also characteristic for TPZ derivatives with electron-accepting and donating substituents.



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

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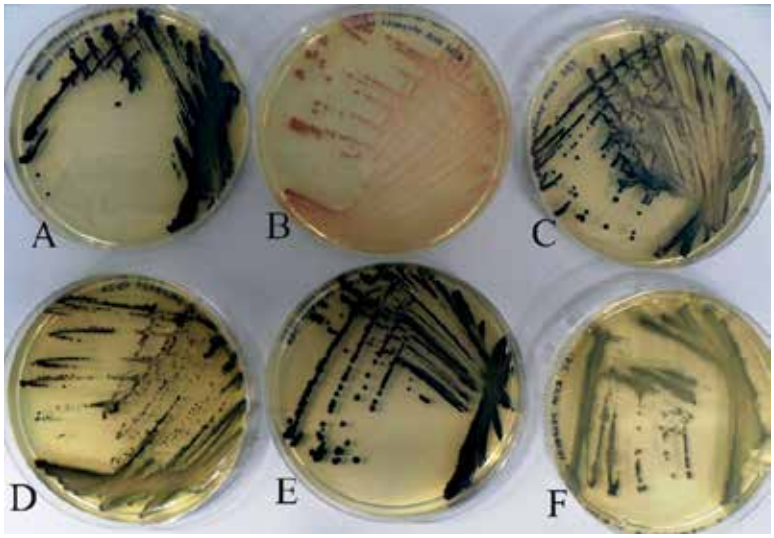

Microbial Diversity as a Source of New Biocatalysts

The current challenges for biotechnology include the discovery and implementation of green biocatalysis as an alternative to conventional chemical synthesis. Modern industrial biotechnology is based on the application of enzymes derived predominantly from microorganisms. Considering the number of species and the diversity of the environmental conditions in which they can grow, microbes dominate our planet. Hence, 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. Both the genetic and biochemical characterization of bioconversion processes are carried out. The modified nucleotides are among the many substrates the catabolism of which is being elucidated. A screening for novel enzymes is also carried out by applying metagenomic techniques – effective selection systems combined with tailored substrates. The screened enzymes are used for the development of biosensors, biofuel-cells and for the synthesis of industry-related chemical compounds.

The studies of heterocycles-utilizing or producing microorganisms, and the investigation of metabolic pathways of said compounds in individual bacteria are some of the aims of our group [1–3]. More than 50 bacterial strains belonging to *Rhodococcus*, *Arthrobacter*, *Burkholderia*, *Acinetobacter* and *Pusillimonas* genera capable of degrading indole, coumarines, pyridine, pyrazine as well as their various derivatives, including alkyl-, hydroxy-, carboxy- and aminopyridines and pyrazines, have been screened and characterized. The oxygenases active toward aromatic and related compounds are promising biocatalysts for organic chemistry. Unique multicomponent oxygenases active towards the pyridine ring as a primary substrate have been characterized [1, 3]. Modified nucleotides are present in many RNA species in all the Domains of Life. The biosynthetic pathways of such nucleotides are well studied. However, much less is known concerning the degradation or the salvage of modified nucleotides, their respective nucleosides or heterocyclic bases to the metabolism. Using an *E. coli* uracil auxotrophic strain and the metagenomic libraries, novel enzymes converting 2-thiouracil and isocytosine have been identified [4, 5].

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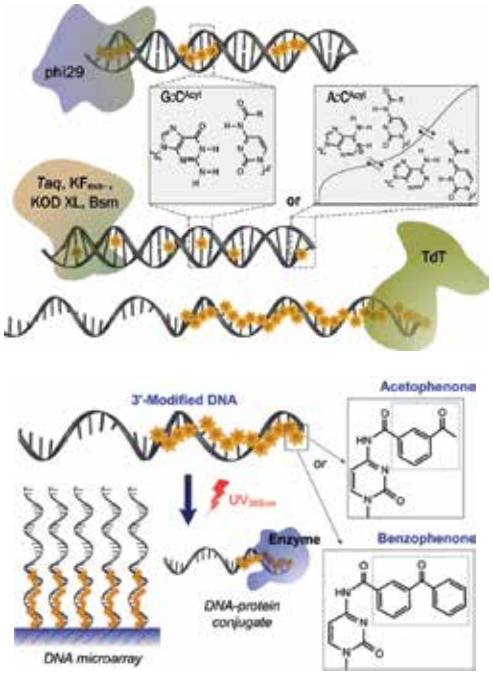
Investigation of *N*⁴-acyl-2′-deoxycytidine-5′-triphosphates

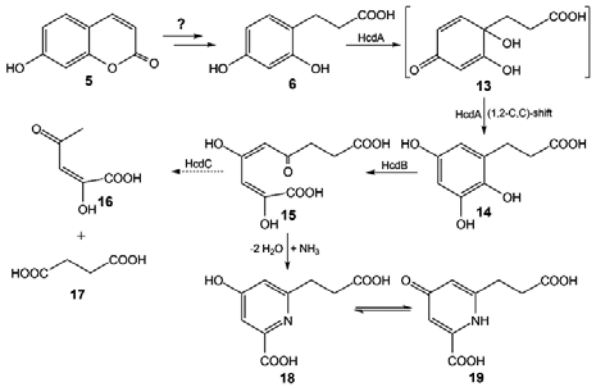
A huge diversity of modified nucleobases is used as a tool for studying DNA and RNA. Due to practical reasons, the most suitable positions for modifications are C5 of pyrimidines and C7 of purines. Unfortunately, by using these two positions only, one cannot expand the repertoire of modified nucleotides to a maximum. We demonstrate the synthesis and enzymatic incorporation of novel *N*⁴-acylated 2′-deoxycytidine nucleotides (dC^{Acyl}). A variety of family A and B DNA polymerases efficiently use dC^{Acyl}TPs as substrates. In addition to the formation of complementary C^{Acyl}•G pair, a strong base-pairing between *N*⁴-acyl-cytosine and adenine takes place when *Taq*, Klenow fragment (exo–), Bsm, and KOD XL DNA polymerases are used for the primer extension reactions. In contrast, a proofreading phi29 DNA polymerase successfully utilizes dC^{Acyl}TPs but is prone to form C^{Acyl}•A base pair under the same conditions. Moreover, terminal deoxynucleotidyl transferase is able to incorporate as many as several hundred *N*⁴-acylated-deoxycytidine nucleotides. These data reveal novel *N*⁴-acylated deoxycytidine nucleotides as beneficial substrates for the enzymatic synthesis of modified DNA, which can be further applied for specific labelling of DNA fragments, selection of aptamers or photoimmobilization. (Jakubovska et al. *Nucleic Acids Res*. 2018, 46: 5911; Jakubovska et al. *Sci. Rep*. 2018, 8: 16484).



Enzymatic incorporation of *N*4-acylated 2′-deoxycytidine nucleotides and photoimmobilization of the modified oligonucleotides.

Biodegradation of 7-hydroxycoumarin in *Pseudomonas mandelii* 7HK4 via ipso-hydroxylation of 3-(2,4-dihydroxyphenyl)-propionic acid

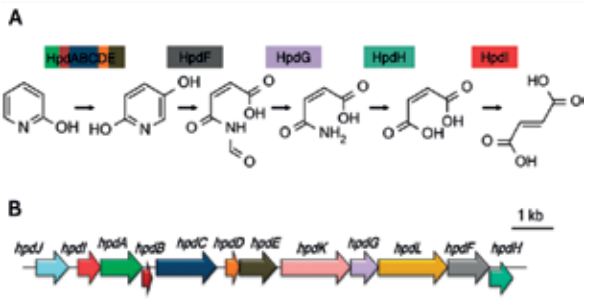
A gene cluster, denoted as *hcdABC*, required for the degradation of 3-(2,4-dihydroxyphenyl)-propionic acid has been cloned from 7-hydroxycoumarin-degrading *Pseudomonas mandelii* 7HK4. The analysis of the recombinant HcdA activity *in vitro* confirms that this enzyme belongs to the group of *ipso*-hydroxylases. HcdA catalyses the conversion of 3-(2,4-dihydroxyphenyl)-propionic acid to 3-(2,3,5-trihydroxyphenyl)-propionic acid through an ipso-hydroxylation followed by an internal (1,2-C,C)-shift of the alkyl moiety. Then, in the presence of HcdB, a subsequent oxidative meta-cleavage of the aromatic ring occurs, resulting in the corresponding linear product (2*E*,4*E*)-2,4-dihydroxy-6-oxonona-2,4-dienedioic acid. (Krikštaponis. *Molecules*. 2018, 23: 2613.).



The proposed metabolic pathway of 7-hydroxycoumarin in *Pseudomonas* sp. 7HK4 cells.

Catabolism of 2-hydroxypyridine by *Burkholderia* sp. MAK1

A gene cluster *hpd* from *Burkholderia* sp. MAK1 containing all the necessary genes for the degradation of 2-hydroxypyridine has been identified. The biodegradation pathway starts with hydroxylation of 2-hydroxypyridine to 2,5-dihydroxypyridine catalysed by the five-component monooxygenase HpdABCDE, an enzyme that shows similarity to soluble di-iron monooxygenases, which participate in the biodegradation of various aromatic and aliphatic pollutants but have never been implicated in the biodegradation of pyridines (Petkevičius et al. *Appl. Environ. Microbiol*. 2018, 84: e00387-18).



(A) Proposed 2-hydroxypyridine degradation pathway in *Burkholderia* sp. MAK1 and (B) organisation of *hdp* gene cluster.

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Protein Structural Bioinformatics

Proteins typically function as three-dimensional (3D) structures, often through interaction with each other and/or with other macromolecules. Protein 3D structure is also the most conserved property of evolutionary related proteins. Therefore, the knowledge of structures of individual proteins and their complexes is essential for understanding their evolution, function and molecular mechanisms. However, the experimental determination of protein structure is slow, expensive and not always successful. 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 intended for detecting protein homology (common evolutionary origin) from sequence data, comparative protein structure modelling, analysis and evaluation of 3D structure of proteins and protein complexes. In recent years, our team has 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. Although we have addressed a variety of biological problems, our major focus has been on studies of DNA replication and repair systems in viruses, bacteria and eukaryotes. In addition, we have recently entered a highly dynamic CRISPR-Cas research field and have already made important contributions in elucidating structural and mechanistic properties of CRISPR-Cas systems.

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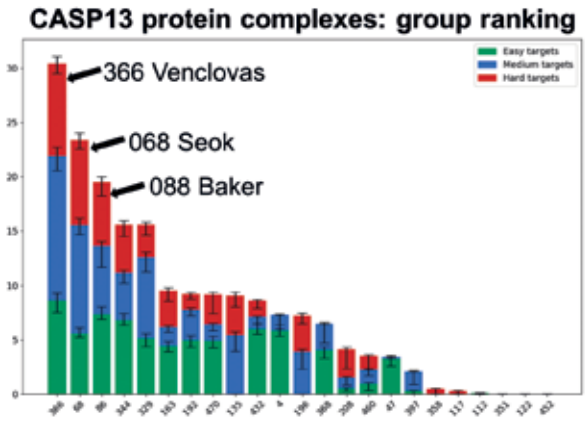
Participation in Community-wide CASP and CAPRI Contests

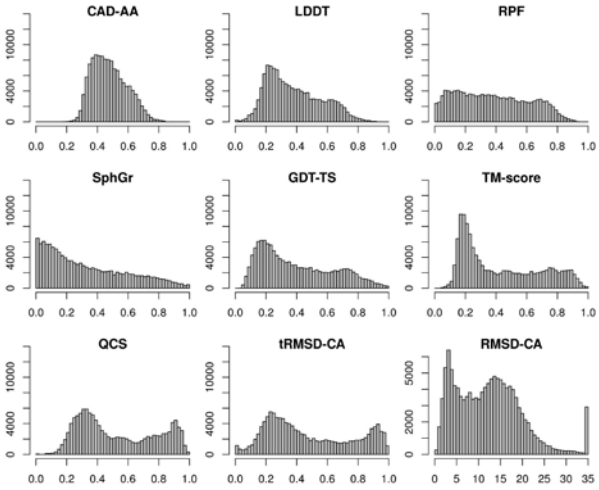
CASP and CAPRI, biennially held community-wide contests, correspondingly measure progress in prediction of 3D structures of proteins and protein-protein complexes. Both contests have become the gold standard for assessing computational modelling techniques. During CASP13/CAPRI experiment, which was conducted in the summer of 2018, we tested the latest versions of PPI3D and VoroMQA, methods developed in our group. PPI3D was developed for searching, analysing and modelling protein complexes, whereas VoroMQA is a method for estimating protein structure quality. We tested a combination of PPI3D, VoroMQA and free docking techniques in both CASP and CAPRI. Independent evaluation found our results (group 'Venclovas') in modelling protein complexes to be the best (http://predictioncenter.org/casp13/zscores_multimer.cgi). VoroMQA was also recognized as one of the best methods for estimation of model accuracy.



Group ranking in modelling protein assemblies, presented by Jose Duarte (the assessor) at the CASP13 meeting in December 1–4, 2018

Comparative Analysis of Methods for Evaluation of Protein Models against Native Structures

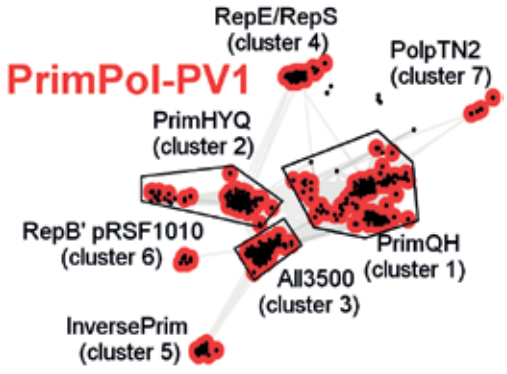
Measuring discrepancies between protein models and native structures is at the heart of development of protein structure prediction methods and comparison of their performance. To date, multiple reference-based model evaluation methods (scores) have been proposed; however, their comprehensive and unbiased comparison has never been performed. Therefore, we decided to perform a comparative analysis of several popular model assessment scores (RMSD, TM-score, GDT, QCS, CAD-score, LDDT, SphereGrinder and RPF) to reveal their relative strengths and weaknesses. Using a large and diverse set of protein models derived in the course of community-wide CASP experiments we analysed (1) how the scores differ from each other and (2) how each of the scores takes into account various structural properties of the models. We believe that our results provide a solid basis for an informed selection of the most appropriate score or combination of scores depending on the task at hand. The study was published in *Bioinformatics*.



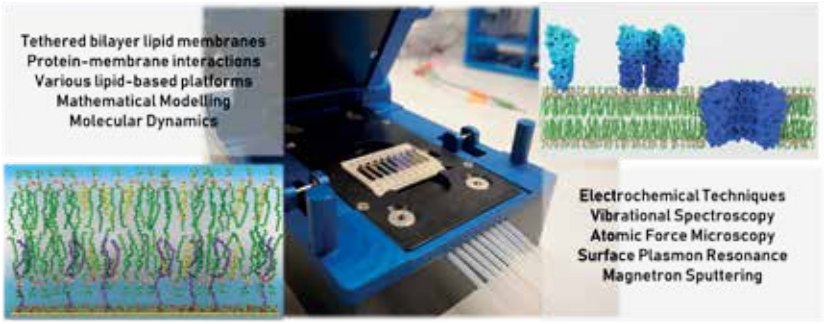
Empiric distributions of values for different model evaluation scores (Olechnovič et al. *Bioinformatics*. 2018, doi: 10.1093/bioinformatics/bty760).

Detection of Novel Archaeo-Eukaryotic Primase Families Associated with Mobile Genetic Elements of Prokaryotes

Cellular organisms in different domains of life employ structurally unrelated, non-homologous DNA primases for synthesis of a primer for DNA replication. Archaea and eukaryotes encode enzymes of the archaeo-eukaryotic primase (AEP) superfamily, whereas bacteria uniformly use primases of the DnaG family. However, AEP genes are widespread in bacterial genomes raising questions regarding their provenance and function. Using computational methods, we performed an extensive study of AEP in bacterial genomes and associated mobile genetic elements (MGE). We identified and described a new supergroup, PrimPol-PV1, consisting of several AEP families widespread in diverse bacterial and archaeal plasmids and viruses. The results of the study were published in the *Journal of Molecular Biology*.



PrimPol-PV1, a new supergroup of AEP families, identified in prokaryotic MGEs. (Kazlauskas et al. *J Mol Biol.* 2018, 430: 737-750).



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Protein Structure and Interactions in Phospholipid Membranes

The molecular organization of biological membranes and their interactions with extracellular and intracellular species are critical determinants of cell function. The structure and function of cell membranes is 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 those of MPs, and, unfortunately, such lack of knowledge seriously affects understanding of the membrane protein interactions and slows the development of new diagnostic tools and therapies. The major difficulties and challenges for structural and functional studies of MP's 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) systems. tBLMs are solid supported bilayers anchored to a surface via hydrophobic interactions between the molecular anchors and hydrophobic sheet of the bilayer. The molecular anchors are synthetic thiolipids or silanes covalently attached to a metal or metal-oxide surfaces. The anchors may contain oligoethylene 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 on multilamellar vesicle fusion. We showed that such tBLMs are capable of functionally 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 of both membranes and 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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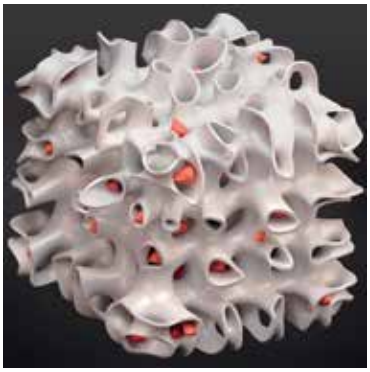
Vibrational Spectroscopy of Liquid Lipid Crystals

The lipid liquid crystalline phases (LLC) are widely used in a variety of fields such as drug delivery systems, biosensors, protein crystallization and immobilization. LLCs with an inverse bicontinuous cubic (Q) and sponge (L_3) phases attract most of the attention due to their ability to form large enough water pores to accommodate macromolecules such as proteins.

Vibrational spectroscopy and small-angle X-ray scattering study revealed that the sponge phase is structurally similar to the cubic and lamellar phases, however, the higher flexibility of this phase's carbohydrate chains was also observed. These findings demonstrate that the sponge like LLC has an increased capability to absorb various biologically relevant macromolecules. Raman spectroscopy revealed only a small change in structure for both LLC and protein indicating that protein remains intact after entering the sponge phase and is able to perform its biological function.

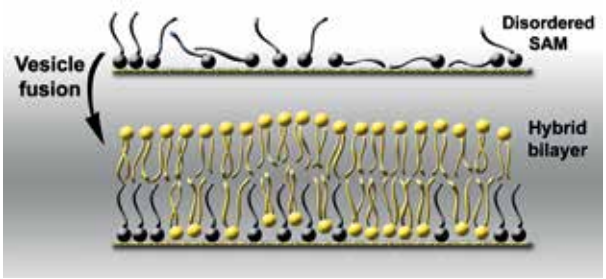


3D model of the protein (red) encapsulated in a sponge-like LLC phase.

(Talaikis, M. et. al. (in press). On the Molecular Interactions in Lipid Bilayer-Water Assemblies of Different Curvature. *Journal of Physical Chemistry,* 2019).

Phospholipid Bilayers on Metal-oxide Semiconductors

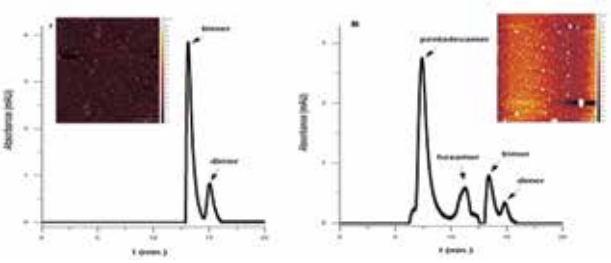
We developed a methodology for assembly of intact, highly electrically insulating phospholipid bilayers on metal-oxide semiconductors. Specifically, we demonstrate fast fusion of vesicles on octadecyltrimethoxysilane (OTS) decorated fluorine doped tin oxide and titania (Sabirovas et al. *J.Electrochem Soc*. 2018, 165: G109–G115; Gabriunaite et al. *Electrochimica Acta,* 2018, 283: 1351–1358) surfaces leading to a reproducible formation of phospholipid bilayers. The bilayers exhibit exceptional stability. They can be reversibly removed and re-assembled up to 10 times without losing their main physical properties. Such bilayers are stable with respect to the pore-forming proteins, but they are susceptible to phospholipase A2. The bilayer may provide milieu for transmembrane and peripheral proteins. The intimate contact between the bilayer and semiconducting surfaces allows utilization of these constructs in electrochemical biosensors, electrocatalysis and possibly electron transfer from photoactive substances, and charge separation.



Functionalization of metal-oxide surfaces by OTS leads to a formation of disordered layer of hydrophobic molecular anchors. Vesicle fusion leads to a self-assembly of phospholipid bilayer with no or very little water sub-phase between solid support and the membrane. They can be categorized as hybrid bilayer membranes with fluid like distal layer and partly mobile proximal layer of phospholipids and sterols.

Size-dependent Effects of Misfolded Protein Aggregates

There exists evidence that size exhibits a significant effect on the disruptive propensity of aggregates. We developed several protocols allowing to reproducibly obtain aggregates of different oligomerization degree (see Figure) and tested their ability to impair the dielectric barrier of bilayer membranes. Both biological tests and electrochemical impedance spectra (EIS) demonstrated a correlation between the size of amyloid oligomers and the propensity of amyloid aggregates to inhibit cancer cell growth. In addition, the damage to membrane integrity as measured by EIS was found to be dependent on membrane lipid composition. Dimers and trimers impair membrane integrity to a larger extent than large oligomers in membranes composed of phosphocholines and cholesterol. However, the effect reverses if sphingomyelin is added to the lipid composition. (Pavliukeviciene et al. *Plos One.* 2019, under review; Budvytyte R. et al., Electrochemical study of interaction of phospholipid membranes with β-amyloid oligomers. 2019, in preparation).



Size exclusion chromatograms of low (left) and high (right) oligomerization degree amyloid oligomers. Insets show atomic force microscopy images corresponding forms of oligomers adsorbed onto freshly cleaved mica surface.

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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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The Biosynthesis Genes of Bioactive Compounds: An Evaluation of the 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 under 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 an analysis of biosynthesis genes and not via the analysis 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 are under investigation in this project.

The Researcher teams' project "Discovery of Novel Bioactive Microbial Compounds in the Unique Environment: An Investigation of the Diversity, Prevalence and Expression" (grant No. S-MIP-17-21; 2017–2020).

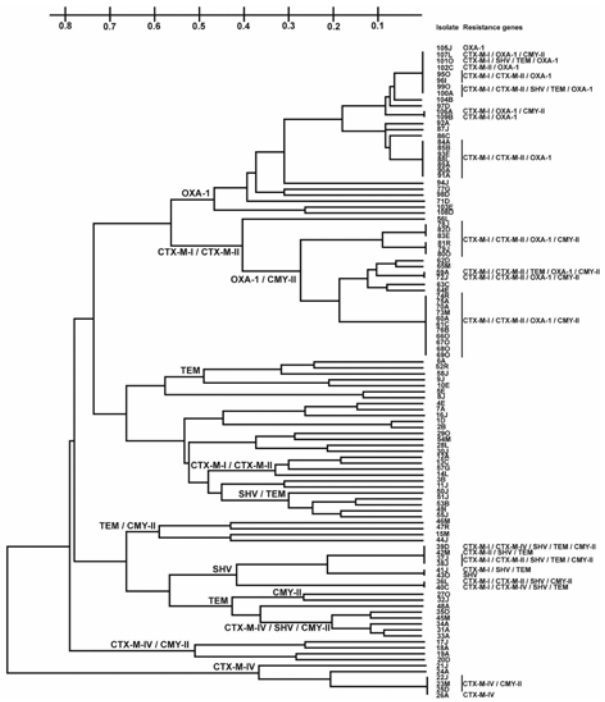
The Identification, Expression and Characterization of Bacterial Collagen-Like Proteins

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.

Postdoctoral fellowship project No. 09.3.3-LMT-K-712-02-0092 (2017-2019), funded under the Measure 09.3.3-LMT-K-712 "Development of Scientific Competences of Scientists, other Researchers and Students through Practical Research Activities" (Funding instrument – European Social Fund).

Molecular Epidemiology of Pathogenic Bacteria in Lithuanian Healthcare Institutions

The emergence and spread of drug-resistant pathogenic bacteria became one of the greatest threats in healthcare worldwide. Different strains of a single pathogenic species can be distributed differently in community and healthcare institutions, they can also differ in their level of virulence as well as susceptibility to antimicrobials. Therefore, it is very important to discriminate these strains in order to determine the ways of their transmission and to design highly efficient surveillance systems both at the hospital as well as at the national level. Molecular epidemiology is used in the current project to study conditions and trends of the dissemination of pathogenic bacteria in Lithuanian healthcare institutions (T. Kirtikliene et al. Evaluation of the inter- and intrahospital spread of multidrug resistant Gram-negative bacteria in Lithuanian hospitals. *Microb. Drug Resist.* 2019, in press).



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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 (bioremediation of polluted environments) is rather 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 of 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. Market of lipolytic enzymes predicted to uphold leadership and grow up to 2024 exceeding millions. Lipases and esterases 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, great stability in different reaction media and in reactions they are able to catalyse. Nevertheless, usually, for the industrial implementations, especially in the area of the organic synthesis, 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.

Another emerging topic nowadays is alternative antibacterial compounds such as bacterial ribosomally synthesized peptides with antibacterial activity (bacteriocins). These natural compounds have considerable diversity with respect to their size, structure, mechanism of action, inhibitory spectrum, immunity mechanisms and targeted receptors. In the era of antibiotic resistance, bacteriocins have been suggested not only as a potential alternative to antibiotics in clinics and veterinary settings, but also as food preservatives against spoilage and pathogenic microorganisms.

Moreover, 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.

Enzymes, antimicrobial and other biologically active compounds, which are identified, characterized and analysed by our research group, are attractive both, biotechnologically and in basic research. Ecologically- inspired method of immobilization of lipolytic enzymes on industrial waste products as carriers was developed. Some of the competences are achieved not only by introducing publications but also by participating in scientific projects co-financed by the EU funds and collaborating with the regional waste treatment company for the pilot study of biogas production from municipal waste. Moreover, LT patent was obtained concerning microbiological treatment methods of greasy plastic surfaces.

Our group members are also interested in characterization of new bacteriocins produced by thermophilic and lactic acid (LAB) bacteria, as well as in analysis of algal pigments. Some of our PhD students defended doctoral theses describing the identification of new post-translationally modified bacteriocins.

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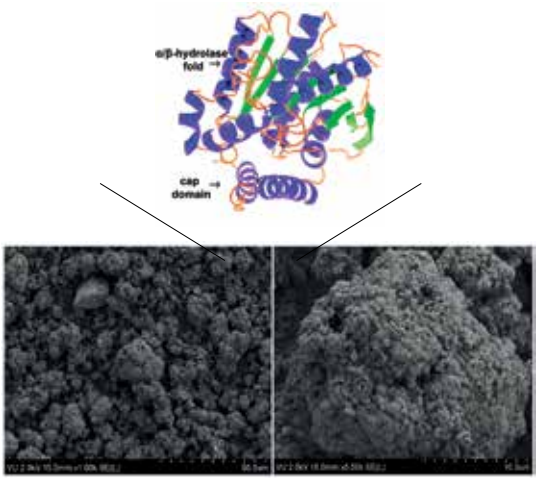
Industrially and Environmentally Relevant Microbial Enzymes: Immobilization Methods

Constant interest of the group is focused on novel enzyme searches using conventional as well as metagenomic and genome mining approaches. The latter represents a pool of unexplored genes coding enzymes that could be interesting both, biotechnologically and from the point of view of basic research. The main focus remains on uncharacterized and poorly described families of lipolytic enzymes. Moreover, by investigating different methods of immobilization, present research aimed to find fast, cheap and cost-efficient way of immobilization of lipolytic enzymes. The study revealed that physical immobilization of pyrolyzed sugar industry waste product (PSIWP) highly hyperactivated lipolytic enzymes - a hundred times higher activity in organic medium was achieved compared to the activity of corresponding free forms of the enzymes. Furthermore, such preparations showed to possess enhanced reusability offering great industrial potential.



Schematic illustration of lipolytic enzyme immobilized onto sugar industry waste pyrolysis product (figure below is the SEM photograph of the carrier using different magnifications)

Antimicrobial Compounds: Bacteriocins

Further, we have identified and characterized a biosynthetic machinery of a novel post-translationally glycosylated bacteriocin – pallidocin (Fig. 1). It exhibits extremely strong activity against specific thermophilic bacteria (Fig. 2). The biosynthetic machinery of pallidocin could be used not only for a biosynthesis of hypothetical glycocins, but also for the introduction of unique post-translational modifications, S-linked glycosylations, into other peptides with the aim to improve their bioactivities. Pallidocin is a good candidate to prevent bacterial contaminations in industrial fermentations operating under elevated temperatures.

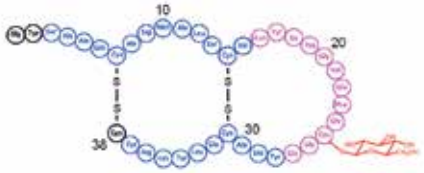


Fig. 1. Proposed structure of pallidocin. The α-helical structure shown in blue, coil structure in purple

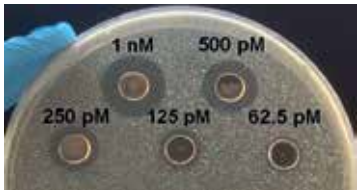


Fig. 2. Antibacterial activity of glycocin pallidocin against thermophilic bacteria P. genomospecies 1 NUB36187

Algal Pigments

Recent trends in drug research from natural sources have shown that algae are promising organisms to furnish novel biochemically active compounds. The main substances biosynthesized by algae have potential economic impact in food science, pharmaceutical industry and public health. The latter research is focused on algae pigments such as phycocyanin or astaxanthin. Based on a scientific service agreement, the original equipment for the production of algae biomass has been developed, which allows successful algal research to be carried out on a larger scale. (2017–2018 Reimbursable service contract with CC *Fortum Heat Lietuva*).

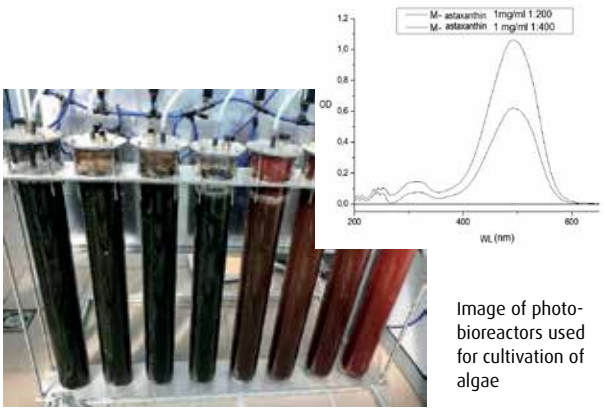


Image of photo-bioreactors used for cultivation of algae

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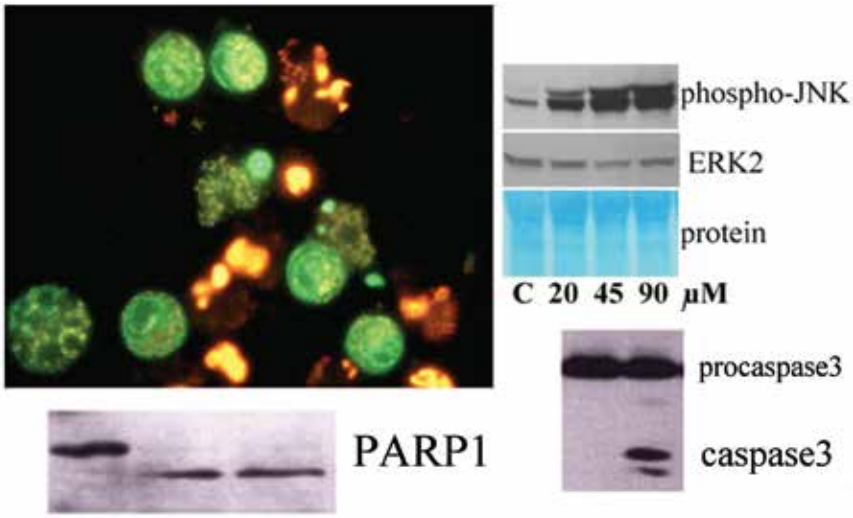

Cell Type-Specific Death/Survival Signalling in Cancer Treatment

Cell fate is determined by the balance of both survival and death signals, the roles of which may change depending on the cell type, the stage of development and the nature of stimuli.

Understanding cell death signalling networks upon anticancer drug responses is an essential approach for identifying new drug targets and biomarkers in cancer therapy. Manipulating chemotherapeutic drug-induced signalling provides a promising strategy for targeted cancer treatment.

Stem cells in an organism are also subjected to the toxic effect of antineoplastic drugs. The identification of signalling events leading adult stem cells to apoptosis should uncover new ways for regulating their survival *in vivo* and for improving the efficiency of cellular therapy.

The sequence of events and relations between 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). Chemotherapeutic drug-induced death signalling has been evaluated in lung cancer and muscle-derived stem cells as well as their differentiated progenies. It was shown that stem cells with distinct differentiation statuses were differently affected by apoptosis inducers; the protein kinase AKT activation was suggested to be responsible for that. The induction or down-regulation of different and opposite cell signalling pathways, which may counteract one another, were shown in cells during chemotherapeutic treatments (Kalvelyte et al., 2013; Stulpinas et al., 2012) [1, 2].

As a result of cooperation with Prof. Eltyeb Abdelwahid and other co-authors, we have published review articles [3, 4]. The involvement of various signal transduction pathways in cell death and survival processes as well as the various therapeutic options directed at controlling stem cell survival upon transplantation in patients with heart diseases were reviewed [3]. Approaches of manipulation of MAPK pathways for protecting cardiac cells in a diseased heart were discussed as a promising approach in cardiovascular medicine [4].

In our review published in "Advances in Molecular Toxicology" (Elsevier) [5], we described anticancer drugs (both conventional and targeted) and their molecular targets with advances and challenges in their use. The necessity to develop combination therapies with the application of multiple drugs with different modes of action, targeting different pathways both in cancerous and normal cells, as well as the microenvironment, has been proposed with the purpose to protect normal cells.

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Cancer Drug Resistance Dependency on Cellular Phenotype

Since 2011, we have been trying to get funding for the *ex vivo* patient tumour cell screening to identify potential therapeutic treatment combinations for individual therapy. The studies within the frame of the project, which has started in 2018, are focused on cell-phenotype-driven cancer resistance to therapeutic treatment.

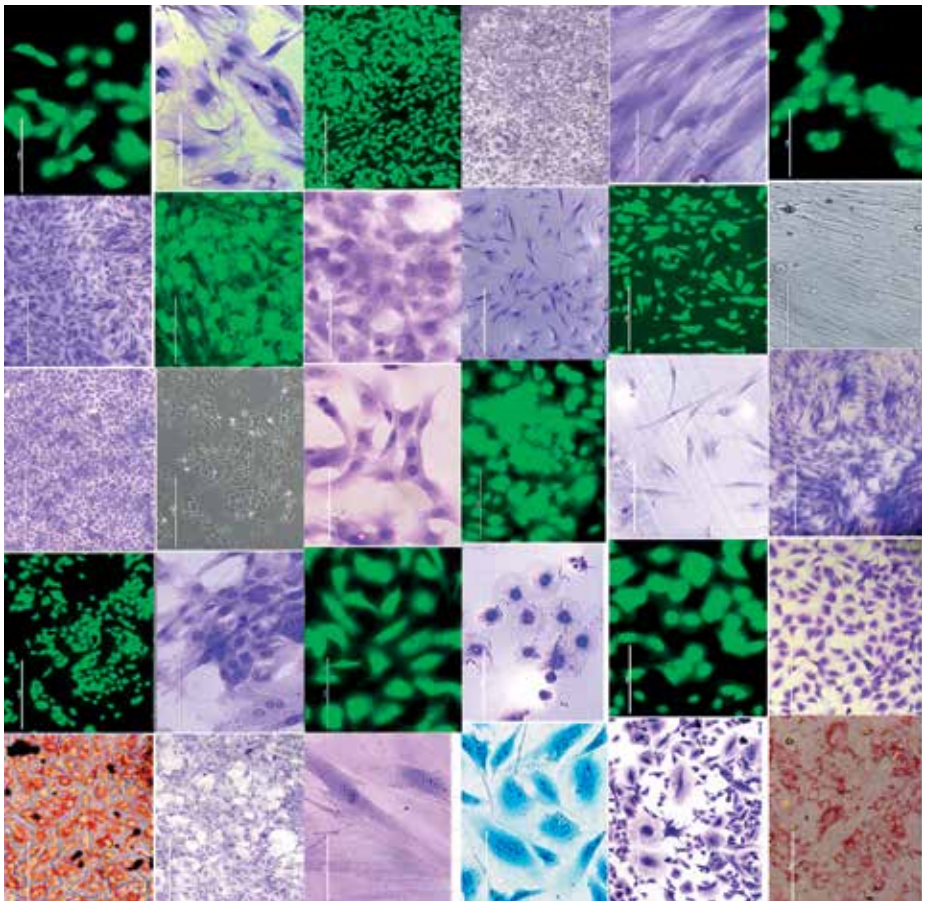
The effectiveness of conventional and targeted drugs, selected to inhibit the intracellular signal transducing protein kinases, are studied by using *ex vivo* patient-derived heterogeneous lung cancer cell sets as model system and functional testing of cells exposed to potential therapies. Phenotype switching, cell dynamic state, extracellular contacts, along with the identification of molecular mechanisms and molecular characterization of cells are assessed during the evaluation of drug effectiveness.

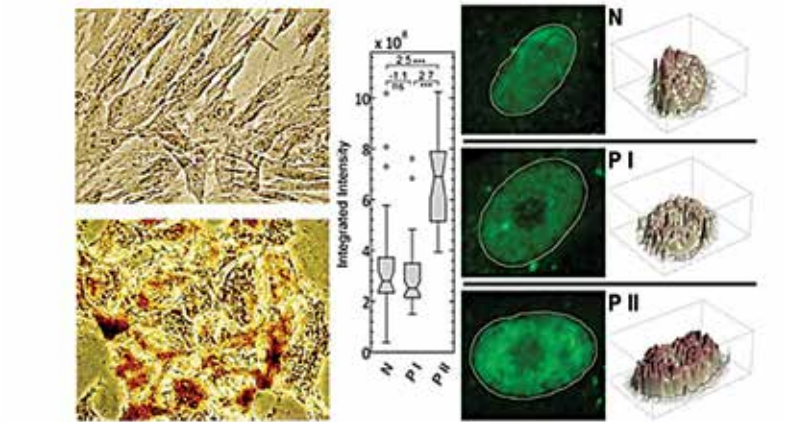
In our research, we are using a panel of targeted drugs, which are in various phases of clinical trials and FDA approval, including agents that target components of signalling components stand-alone or in combination with conventional drugs.

Functional cellular parameters, such as viability, proliferation, apoptosis and morphology, are assayed by various methods, e.g. evaluation of doubling time, measurement of ATP concentration, clonogenicity, growth in soft agar, migration, and susceptibility/resistance to the treatment etc. During signalling studies, cells are analysed for the expression, phosphorylation, localization of the targeted and other signalling molecules, as well as for the relationship of selected signal-transducing molecules in response to drug treatment.

MAP kinases ERK, JNK and AKT kinase-targeted drugs and their combinations with conventional chemotherapeutics are in the focus of our research. It has been known for decades that mitogen-activated protein kinases (MAPKs) regulate cell functions, such as proliferation, differentiation and programmed cell death. They are activated by various and different oncogenic mutations and are common to many cancers. These signalling pathways are considered to be the central transducers of oncogenic signals in tumour progression. MAPKs mediate cellular response to anticancer drugs and are candidates in improving the effectiveness of targeted and conventional chemotherapies. The results of our recent studies of the dynamic changes in phosphorylation of MAP kinases JNK, ERK and AKT during a well-known inducer of apoptosis cisplatin treatment were presented at the XVth International Conference of the Lithuanian Biochemical Society, Dubingiai, June 26–29, 2018.

Project "Designing of the patient-specific, heterogeneous lung cancer cell *ex vivo* model system for drug efficiency prediction in personalized oncotherapy" 2018–2022. European structural funds for Targeted Research in Smart Specialization Areas of Priority axis "Research projects implemented by world-class researcher groups"(Project No 01.2.2-LMT-K-718-01-0072, leader Dr. A. Kalvelytė).





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Functioning and Epigenetic Mechanisms of Human Stem Cells

Epigenetic regulation, when influenced by DNA and histone modifications as well as microRNA expressions, causes variances in gene expression and cell phenotype. They have a great influence on the development and functioning of stem cells as well as on epigenetic alterations. 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. Epigenetic and regulatory mechanisms governing stem and cancer cell identity and fate determination are still not well-understood.

We studied the differentiation potential of healthy amniotic fluid-mesenchymal stem cells (AF-MSCs) toward adipogenic, osteogenic, neurogenic, myogenic and even cardiomyogenic lineages and compared the stem cells of healthy and pathological pregnancies by determining histone modification patterns associated with the state of stem cells as well as senescence-associated molecular and epigenetic alterations during their propagation. We detected the expression of pluripotency genes and genes-markers and assessed the epigenetic changes with the main focus on chromatin remodeling proteins, such as DNMT1, PRC2, HDAC1 and 2, and on the activation or repression of histone modifications, i.e., H3K9ac, H4hyperAc, H3K4me3, H3K27me3 and others. Our findings provide useful insights for the future research and potential applications of amniotic fluid-derived stem cells.

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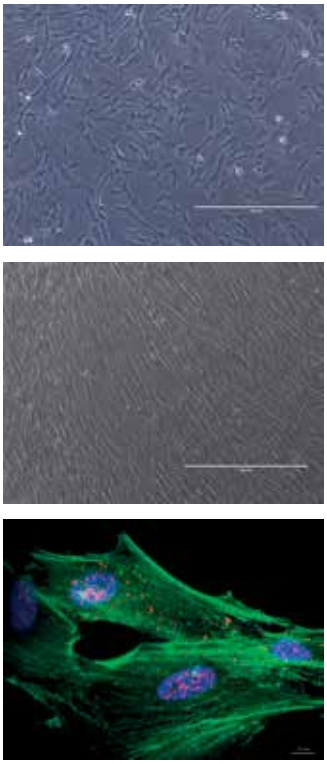
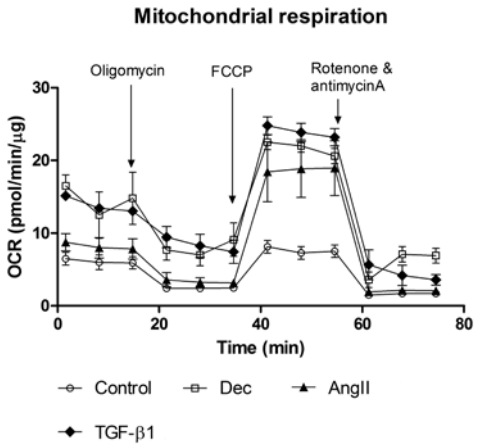
Cardiomyogenic Differentiation of Human Amniotic Fluid-Derived Stem Cells

Human amniotic fluid-derived mesenchymal stem cells (AF-MSCs) are obtained during amniocentesis procedure from pregnant women. These stem cells have a multipotent differentiation potential and may be proposed as a new and valuable source for cell therapy and regenerative medicine. One of our research topics was evaluation of AF-MSCs functionality during cardiomyogenic differentiation induction *in vitro*. For that purpose, two groups of inducers were tested: synthetic DNA methyltransferases (DNMT) inhibitors Decitabine, Zebularine, RG108 and p53 inhibitor Pifithrin-α as well as natural biologically active compounds, such as Angiotensin II and Transforming growth factor-β1 (TGF-β1). All of the applied agents caused changes in the cell phenotype, upregulated the relative expression of the main cardiac genes-markers and cardiac ion channels genes (sodium, calcium, and potassium). The increase in the expression of Connexin43, the main component of gap junctions, was also detected as a sign of successful differentiation into cardiomyocyte-like cells. In addition, we assessed cellular energetics and metabolic alterations during the induced cardiac differentiation. The obtained results revealed the switch towards more energetic phenotype and the initiation of AF-MSCs metabolic transformation into cardiomyocyte-like cells utilizing oxidative phosphorylation more than glycolysis for energy production. Furthermore, the evaluated levels of chromatin remodelling proteins, such as Polycomb repressive complex 1/2 components EZH2, SUZ12 and BMI1 together with DNMT1, HDAC1 and HDAC2, as well as the rate of activating histone modifications, exhibited rearrangements of chromatin after the induction of cardiomyogenic differentiation. Overall, both synthetic and natural cardiomyogenic differentiation inducers initiated alterations in human AF-MSCs at the gene and protein expression, metabolic and epigenetic landscape. However, different agents affected differentiation to the different extent with DNMT inhibitor Decitabine being the best. The generated cells can be considered as committed progenitors having the basis of becoming functional cardiomyocytes under relevant conditions *in vitro* or *in vivo* (Gasiūnienė et al. J *Cell Biochem*. 2018, 1–15).

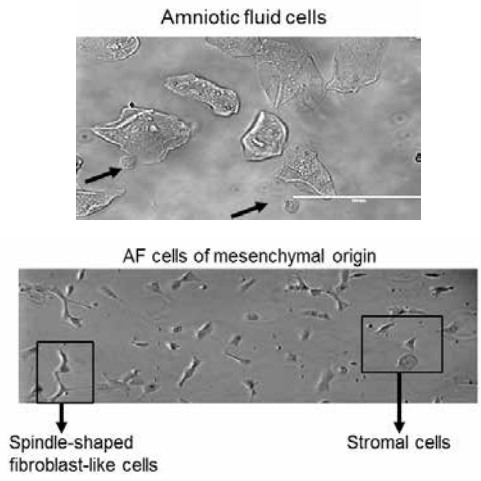


Metabolic landscape of AF-MSC induced to cardiomyogenic differentiation



Human Stem Cells of Perinatal and Endometrium Origin

Stem cells isolated from perinatal derivatives (such as amnion membrane, amniotic fluid, placenta etc.) and endometrium are a promising source for cell therapy and regenerative medicine because of the unique source (young and healthy donors) of these cells and not restricted accessibility of the initial substance. Therefore, we perform *in vitro* and *in vivo* experiments to properly characterize stem cells, standardize the protocols and evaluate the possibilities of the stem cells' application in the clinic.
In collaboration within COST Action CA 17116 International network for translating research on perinatal derivatives into therapeutic approaches (SPRINT).



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Genotoxicity of Anthropogenic and Natural Factors

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

Recent studies have confirmed the usefulness of biomonitoring chromosome damage in groups exposed to genotoxic agents by finding an increased risk of cancer in subjects with high levels of chromosome aberrations and thus proving the chromosome aberration assay as a reliable indicator of cancer risk. 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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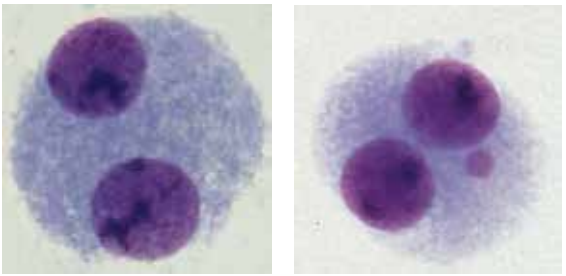
Genotoxicity and Antioxidant Activity of Plant Extracts

The numerous species of *Agrimonia* and *Filipendula* belonging to the *Rosaceae* family have been traditionally used in folk medicine as anti-inflammatory herbs. This study aimed at a more comprehensive evaluation of phytochemical composition, antioxidant activity and genotoxicity of five most common species, namely *A. eupatoria, A. procera, F. palmata, F. ulmaria* and *F. vulgaris.*

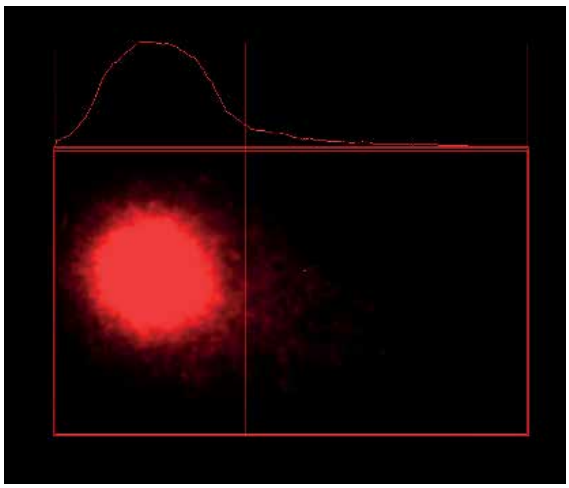
Thirthy five compounds belonging to the classes of phenolic acids, flavonoids, phenylpropanoids and ellagitanins were detected in methanolic extracts from the studied species. The extracts were particularly rich in different glycosilated flavonoids, the majority of which are described in literature as health-beneficial compounds. In addition, all extracts demonstrated strong antioxidant capacity *in vitro* which was in a good correlation with their polyphenolic content.

Genotoxic potential of *Agrimonia* and *Filipendula* plant extracts was tested using several genotoxicity assays covering different endpoints, namely micronuclei and DNA damage (detected by the Comet assay) in human lymphocytes *in vitro*, and gene mutations in the Ames *Salmonella*/microsome test. All tested extracts were clearly not mutagenic in the *Salmonella*/microsome test and micronucleus assay. However, they were mostly positive in the Comet assay. DNA damaging effect of the tested extracts could be attributed to the prooxidant activity of high concentrations of polyphenols.

Therefore, we propose that *Agrimonia* and *Filipendula* species could be further investigated as a potential nutraceutical agent with special interest for food and pharmaceutical industries, however additional toxicity and genototxicity evaluation *in vivo* is required (Pukalskienė, et al. *Food Chem. Toxicol*. 2018, 113: 303–313).



Binucleated human lymphocytes in the *in vitro* cytokinesis-block micronuclei assay: cells without and with micronuclei.

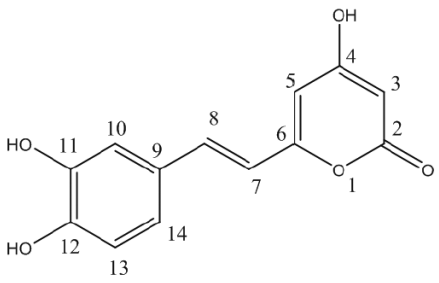


Damaged DNA in the Comet assay. DNA migration (% DNA in tail) was determined using Lucia-Comet image analysis system (Czech Republic).

Antioxidant and Genotoxic Properties of Hispidin

Hispidin was isolated from the acetone extract of *Phaeolus schweinitzii* mushroom more than 50 years ago. Later it was found in various related fungal species, such as *Inonotus xeranticus, Phellinus linteus* and *Gymnopilus spectabilis*. Previously reported bioactivities of hispidin include inhibition of protein kinase C (PKC), cytotoxicity against cancer cells, protective effects against peroxynitrite-mediated cytotoxicity, DNA damage and hydroxyl radical formation. However, the reports on the antioxidant activity and genotoxicity of hispidin are rather scarce. In the present study, we evaluated antioxidant and genotoxic properties of hispidin isolated from *P. schweinitzii* by various assays. Hispidin demonstrated strong free radical scavenging, oxygen radical absorbance capacity and ferric reducing antioxidant power: in all the applied assays hispidin exhibited similar or even higher antioxidant capacity than the reference antioxidant Trolox. Hispidin did not increase the frequency of chromosome aberrations, micronuclei or DNA damage (detected by the Comet assay) in human lymphocytes *in vitro* and gene mutations in the *Salmonella*/microsome test. However, in human lymphocytes we identified a statistically significant dose-dependent increase in sister chromatid exchange frequency and a decrease in replication index and nuclear division index values (Smolskaitė et al. Int *J Med Mushrooms.* 2017, 19(11): 967–980).

Velvet-top mushroom, *Phaeolus schweinitzii* (Agaricomycetes).



Chemical structure of hispidin, 6-(3,4-dihydroxystyryl)-4-hydroxy-2-pyrone.

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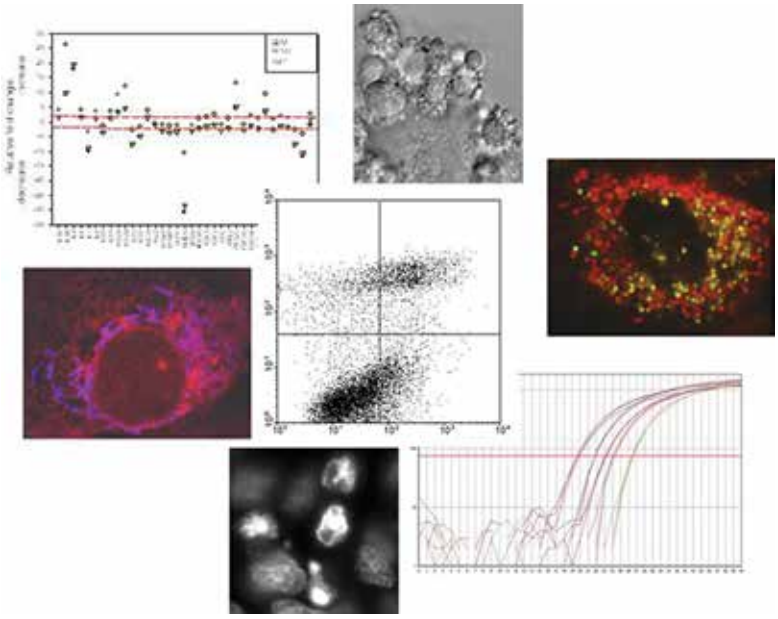

Molecular Mechanisms of Cell Death and Survival

Alterations in the molecular mechanisms of cell death and survival are important for the development of conditions caused by cellular overgrowth, cancer included, as they provide an increased proliferative capacity for malignant cells. Chemotherapy remains one of the main methods of cancer treatment; however, an acquired chemoresistance is one of the major reasons that limit the success of anti-cancer therapy. We focus our research on molecular mechanisms responsible for the cellular chemoresistance of colorectal cancer cells, in particular on the alterations in pathways of cell death and autophagy.

We have determined that Notch and Wnt signalling is upregulated in 5-fluorouracil (5-FU) or oxaliplatin (OxaPt) chemoresistant HCT116 cells. The roles of Notch and Wnt pathways for cell survival in 2D model after 5-FU and OxaPt treatment were different: in case of the 5-FU treatment, the Wnt pathway was cytoprotective (supported chemoresistance), while the inhibition of a Notch or Wnt pathway increased the cytotoxicity of OxaPt.

Another study was dedicated to cytokine effects on chemoresistant vs chemosensitive cells. We have demonstrated that cell stimulation with exogenous IL-1α increased chemosensitivity of both chemosensitive and chemoresistant colorectal cancer cell lines, treated with 5-FU. It was the result of increased cell death but not that of the cell cycle arrest. The combined exogenous IL-1α and 5-FU treatment changed the expression of cell adhesion molecules that can have the impact on adhesion-dependent chemoresistance and metastatic potential of the cells

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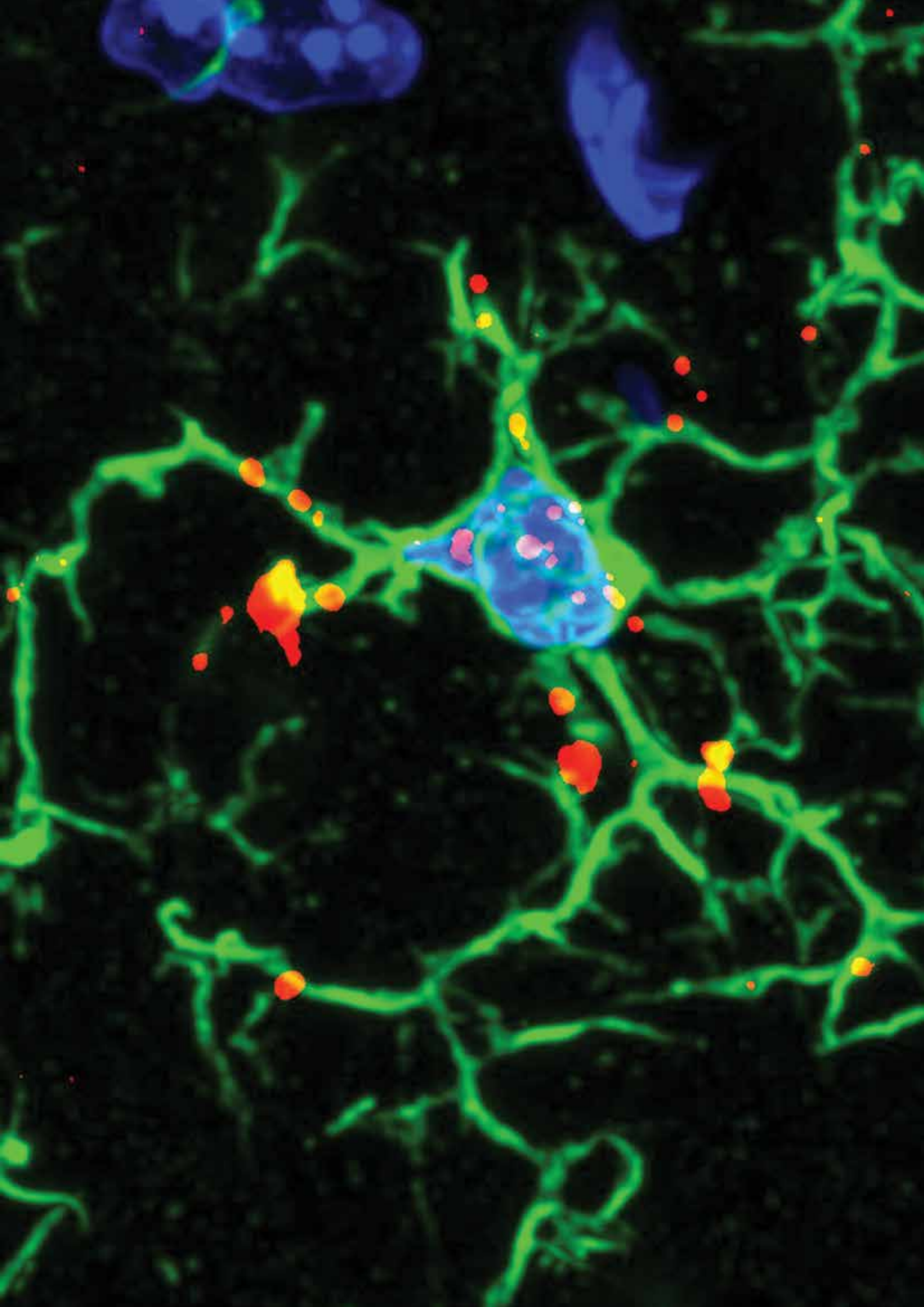

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Plant Polymorphism, Genome Stability and Its Changing Factors

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 (DNA sequencing, Differential display, ISSR, SSR), biochemical, statistical and bioinformatical methods. One of the traditional trends in our laboratory are studies of barley developmental mutants. Our attention was focused on the role of auxin in the induction of genetic instability in barley double mutants, which are characterised by wide spectrum of phenotype variations which cover the phenotypes of many known mutants [1]. 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 [2]. Another aspect of our investigation concerns plant evolution and ecology. Using haplotype analyses of three regions of chloroplast DNA (psbA-trnH, trnS-trn2GS and trnL-trnF) and a nuclear ribosomal DNA internal transcribed spacer region, we confirmed the role of the interspecific hybridization on the invasiveness of variegated alfalfa [2]. Some of our studies [3, 4] were carried out in collaboration with colleagues from the Nature Research Centre. 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 *Vicia faba* test-systems [5].

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Disturbed Local Auxin Homeostasis May Lead to Genetic Instability

Significant phenotype variations are common among mutants. However, the mechanisms underlying these variations remain to be characterized. Barley double mutants *tw*$_2$; *Hooded* are useful in the genetic instability studies for their inherited phenotypic instability and several new features. Since several of these features mimic the mutations in auxin pathway, the aim of this study was to link the genetic instability in the double mutants with the *tweaky (tw)* mutations, which are associated with an auxin imbalance in the developing spikes. The study revealed that the instability arose only if the *tw* allele was a constituent of the double mutants. 2,4-dichlorophenoxyacetic acid induced phenocopies of the *tw* mutation in *WT* plants and rescued the phenotypes of three allelic *tw* mutants, indicating the relationship of the instability of the double mutants and the *tw* phenotype to auxin imbalance. Several putative candidate genes in *tw* that may induce the instability in the double mutants by pleiotropic variations of their expression were also identified. Auxin imbalance as a cause of genetic instability was described for the first time (Šiukšta et al. *Planta*. 2018, 247(2): 483–498).

Fig. 1. Extreme phenotypes of flower/spike reversions, specific to barley double mutant tw;twmk lines

Genomic Changes and the Invasiveness of Alien Plants

Using ISSR fingerprinting, genetic structure of *Bunias orientalis* was studied. The eastern Baltic region and western Siberia represent the two opposite directions of *B. orientalis* spread in climatically different zones. AMOVA revealed considerable population differentiation in both the native and invasive ranges. Similar measures of genetic diversity and genetic structure were determined in the invasive populations in two geographically and ecologically distinct, non-native regions located in Europe and Asia. In both of these regions, higher genetic diversity was detected in the non-native populations than in the native ones. However, Bayesian clustering analysis revealed slightly different sources of invasive populations in the two non-native regions. Genetic diversity patterns revealed the lack of IBD between the populations and confirmed the influence of anthropogenic factors on the spread of *B. orientalis* (Patamsytė et al. *Plant Ecol*. 2018, 219: 101–114).

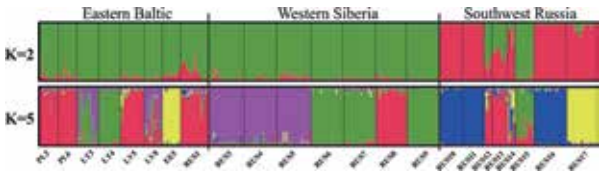

Fig. 2. Genetic structure of *Bunias orientalis* populations revealed by Bayesian assignment test

Human Impact on Stability of Vegetation in River Ecosystems

Difficulties of aquatic *Ranunculus* species delimitation according to morphological features are closely related to recognition of their communities. The diversity of species in the phytocoenosis characterized by the predominance of *Ranunculus* species and the main physical-chemical factors of water were studied at 15 sites of 10 different-sized rivers of Lithuania. The communities dominated by *R. fluitans* and *R. pseudofluitans* were recorded in different river systems. The association *Ranunculetum fluitantis* was distributed in the rivers on silicate substrates, in the waters with significantly higher flow velocity, but in about twice lower conductivity and alkalinity compared to the community with *Ranunculus pseudofluitans*. The latter was found in the river situated in the gypsum karst area covered by watertight clay, with waters of higher alkalinity, conductivity, concentration of calcium ions and rich in nitrogen compounds. Abundance of nutrients (concentration of total P and total N) negatively affected abundance of both species (Butkuvienė et al. *Pol J Ecol*. 2018, 66: 1–13).

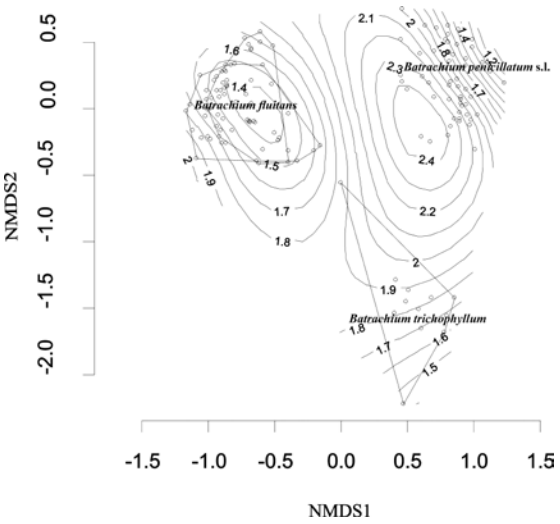

Fig. 3. NMDS analysis of amount of total nitrogen in river water for three *Batrachium* species

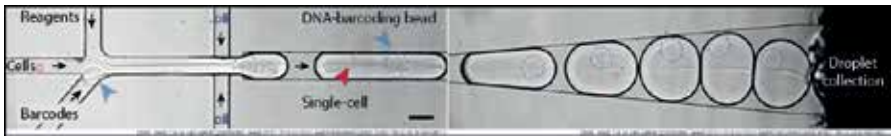

Fig. 1. The principle of *inDrops* technique. Digital micrographs of cell encapsulation together with hydrogel beads and reagents. Scale bars, 100 µm. Cell loading into droplets with hydrogel beads and assay reagents occurs at the flow-focusing junction. Hydrogel bead ferries ssDNA primers attached to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

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Single-Cell Transcriptomics and Genomics

Recent advances in high-throughput single technologies and computational methods have opened new horizons for biological and biomedical sciences. Just over the last four 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-liter 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 the genetic programs that drive tumour heterogeneity, progression and immune response.

In collaboration with Harvard University, our group has pioneered the droplet microfluidics technique *inDrops* (*in*dexing *Drops*) for barcoding the transcriptome of individual cells (Klein, *Cell*, 2015). Since then, the technique has triggered immense attention among many scientists across different disciplines. We are applying *inDrops* and other techniques to better understand the gene expression programs that drive the development of complex diseases (e.g., tumours) and how the immune system responds. In collaboration with the Harvard Medical School (Prof. 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 Centre and Columbia University (Prof. D. Peer), 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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Transcriptional Profiling of Tumour-Infiltrating Lymphocytes

A better understanding of the transcriptional landscape of tumour infiltrating lymphocytes has important implications for developing new immunotherapy treatment strategies. It is well established that the anti-tumour activity is mainly modulated via CD4 helper T-cells and CD8 cytotoxic T cells (CTLs), yet, despite their primary importance in immunotherapy, the cellular and molecular mechanisms that underlie the anti-cancer response remain poorly understood. In this project, we have built a large-scale, high-dimensional atlas of breast tissue immune cells at single-cell resolution. This atlas is built from the cells of hematopoietic origin in human breast tumours of various types – as well as paired normal breast tissue, peripheral blood, and a lymph node – using inDrops technology. We found a remarkably increased heterogeneity of intra-tumoural cells of both lymphoid and myeloid cell lineages, which occupy a markedly expanded continuous space in comparison to normal breast tissue (Fig. 2). The observed continuum likely reflects their progressive cellular activation and differentiation and argues strongly against the classical notion of a few discrete states of differentiation or the activation of individual cell types.

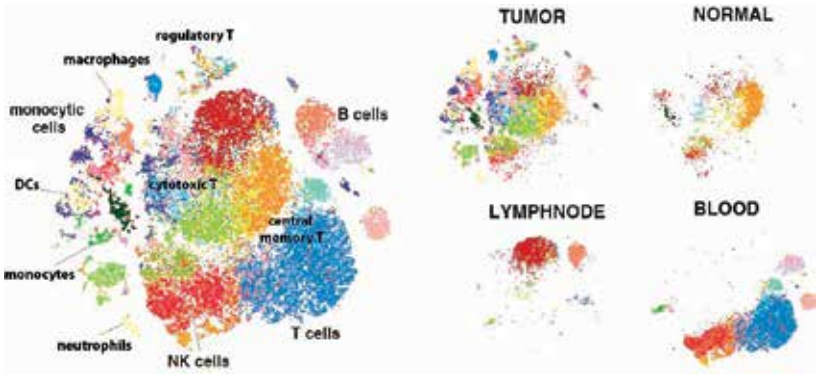


Fig. 2. The transcriptional atlas of breast tumour infiltrating immune cells

Computational Tools for Single-Cell RNA-Seq Data Analysis

Single-cell RNA-seq (scRNA-seq) is a powerful method that uncovers gene-gene relationships on a system-wide scale, based on naturally occurring variation. However, the data generated during scRNA-seq techniques capture only a small fraction of the whole transcriptome, typically 5–15%, which is known as a "dropout." This problem obscures gene-gene interactions, making interpretation of biological information retrieved from transcriptomic studies a challenge. In collaboration with Prof. Dana Peer (Columbia University) and Prof. Smita Krishnaswamy (Yale University), we developed an imputation method we call MAGIC (Markov Affinity-based Graph Imputation of Cells). We showed that MAGIC algorithm uncovers the dynamics of gene expression underlying the epithelial-to-mesenchymal transition (EMT), including known and novel regulatory interactions, demonstrating that our approach is able to successfully predict regulatory relations without perturbations. By using MAGIC, we explored the EMT and revealed a continuum of states, where the majority of cells reside in intermediate states that display stem-like signatures.

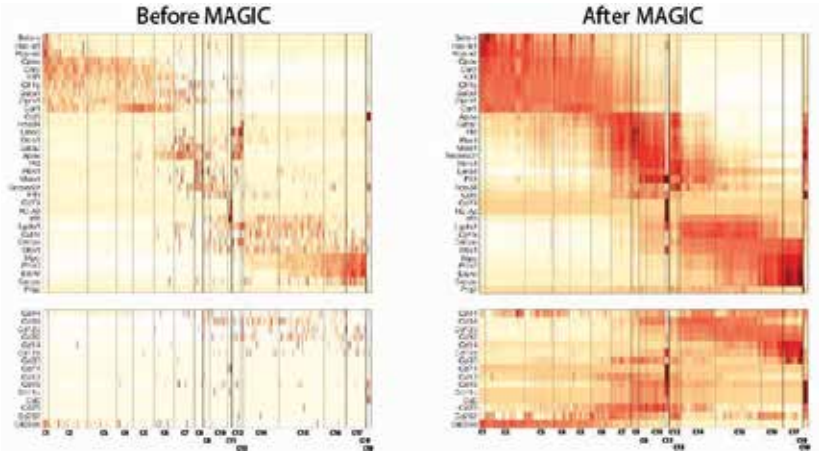
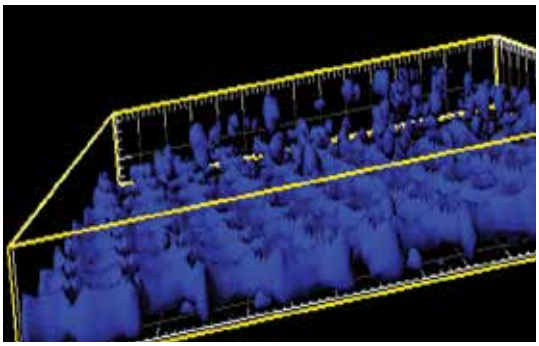


Fig. 3. The Markov Affinity-based Graph Imputation of Cells (MAGIC), a computational approach that shares information across similar cells to recover the information of missing transcripts



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Stem Cell Technologies for Tissue Engineering

The development of artificial tissues is one of the most exciting and rapidly developing areas in biomedical engineering with the goal of assembling scaffolds, cells and growth factors into functionally active constructs that can replace or restore damaged tissues. These constructs can also be used for non-therapeutic applications – as model systems to study cell behaviour, as biosensors to detect biological or chemical threat agents, as tissue chips that can be used to test the toxicity of an experimental medication etc. Tissue engineering principles focus on the use of the following: (a) stem cells, which have to be non-immunogenic, easily isolated and highly responsive to distinct environmental cues, (b) suitable carriers for the *in vitro* cell propagation and subsequent transplantation, and (c) a set of defined bioactive molecules driving the process of proliferation and maturation. Stem cells grown on purposely-designed artificial scaffolds seem to be an appropriate technology for the fabrication of autologous artificial tissues. Nowadays, multiform polymeric matrices for cell growth are being designed, which can serve as templates for artificial tissue fabrication. However, the demand for the development and improvement of technologies for fabrication of biocompatible 3D cell culture scaffolds persists. Moreover, different materials can exert diverse effects on cellular properties. Therefore, a deeper understanding of cell-scaffold interactions is critical for progressing tissue engineering toward clinical applications.

The tasks of our group are (1) to choose relevant materials for tissue fabrication, (2) develop microstructurization and/or chemical modification techniques for these materials, (3) evaluate the biocompatibility of the developed scaffolds, (4) elucidate the properties of cells grown on these scaffolds and (5) examine the functionality of artificial tissue constructs *in vivo*.

Over the last years, we have developed strategies for creating chemically modified materials with improved physical properties and studied their ability to modulate cellular properties. Our designed method of cell number assessment in 3D surroundings, which is substantially different from the flat tissue culture surface, is based on novel insight in the DNA content evaluation using DNA-binding dye DAPI. This way might give a convenient snapshot of cell number at a given time point. To represent the structure of real tissue and the cellular interactions within, we have optimized the parameters of a co-culture of two types of cell populations. These complex systems mimicking the interactions of the native tissues combined with 3D scaffolds are a favourable strategy for tissue engineering.

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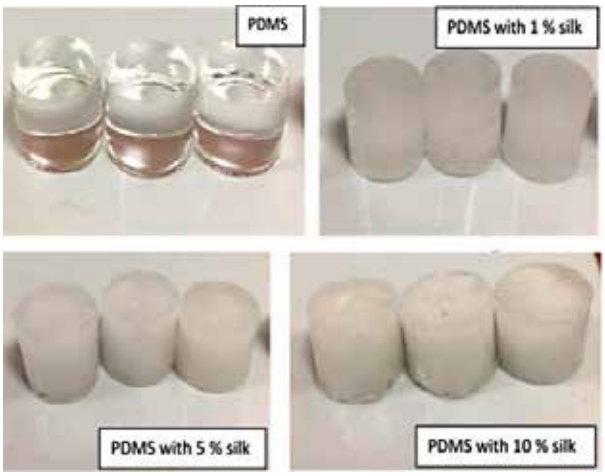
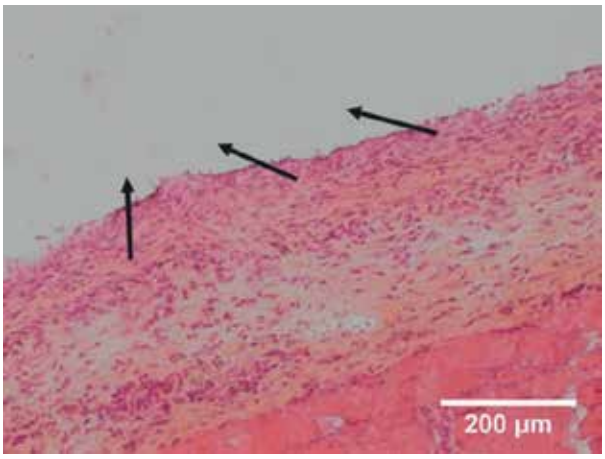
Soft Tissue Engineering: From Cell to Artificial Tissue

Polydimethylsiloxane (PDMS) is widely used in biomedical research and technology, but its mechanical properties should be tuned according to the desired product specifications. Mixing ratio of base polymer to curing agent or additives enables its mechanical properties to be manipulated and fit to the mechanical properties of biological tissues. Therefore, we analysed the effect of mechanical load on silk-reinforced PDMS depending on silk concentration. The results obtained suggested that silk-reinforced PDMS scaffolds might be used for soft tissue engineering applications, where it is essential to finely tune the mechanical properties of the artificial cell niches to both integrate with the surrounding tissues and to guide cellular differentiation towards the desired lineages (Kilikevičius et al., 2018).

We have also developed a new strategy of one-pot three-step synthesis of novel biocompatible hydrophilic copolymers containing siloxane units. In the first step, free radical copolymerization of acrylic acid, butyl methacrylate and 2-hydroxyethyl methacrylate in dioxane solution in the presence of 2,2′-azodiisobutyronitrile was proceeded. In the second step, obtained copolymers were modified with diepoxypropoxypropyl terminated polydimethylsiloxane, and in the third step copolymers were additionally modified with glycidyl methacrylate. Modified copolymers were characterized by FTIR and NMR spectroscopy as well as elemental analysis. UV-curing technique was used for obtaining films from modified copolymers. SEM studies showed microphase-separated morphologies with distribution of PDMS domains. The mechanical properties of films were dependent on the amount of silicone modifier incorporated. The films were more hydrophilic to compare with PDMS films. All novel copolymers demonstrated high biocompatibility *in vitro* and *in vivo* (Budriene et al., submitted).



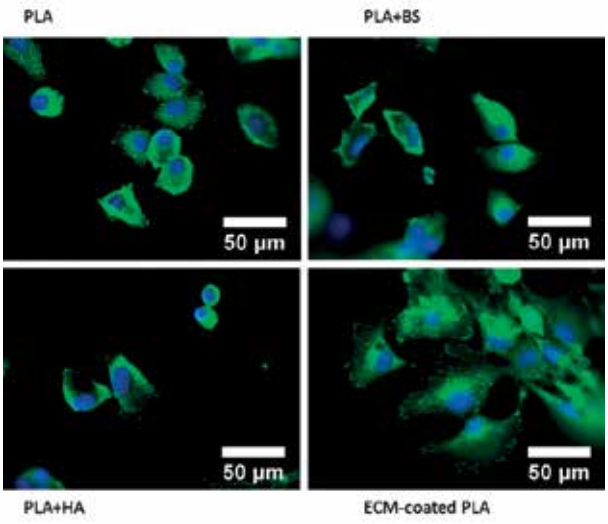


This work was supported by the "Resoft" grant from the RCL, No SEN-13/2015.

Impact of Scaffold's Modifications on the Fate of Stem Cells

In tissue engineering, the structure and material of the scaffold can help control cell functions and behaviour. The aim of this study was to evaluate polylactic acid (PLA) scaffold structure, PLA additives and the impact of surface modifications on rat's dental pulp stem cells (DPSC) fate *in vitro*.

Two differently microstructured scaffolds were created by 3D printing – wavy (consisted of 188 µm joined threads) and porous (with ~300 µm diameter pores). Both surfaces resulted in alterations in morphology and cytoskeleton arrangement of DPSC that potentially initiated osteogenic differentiation. According to these results, in subsequent experiments porous scaffold's structure was improved by using two-layer threads to form a groove. PLA composites with hydroxyapatite (HA) or bioglass (BS) and cell-derived extracellular matrix (ECM)-coated PLA scaffolds were used to analyse their effect on DPSC functions. Results indicated that ECM proteins improved cell adhesion, proliferation and osteogenic differentiation compared to composite PLA material.



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Strategies in Antimicrobial Therapy and Protein Engineering

Skin infections caused by microorganisms represent a clinical challenge, due to them being widespread and their ability to cause morbidity and mortality of the patients. The increasing resistance of the microorganisms to antibiotics and antifungal agents is promoting the search for new compounds and methods in the treatment of skin diseases as well as understanding the physiology and metabolic plasticity of the infectious microorganisms. 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. The discovery of the natural antifungal and antibacterial substances, synthesized by microorganisms, is promising a substitution for the antibiotics and antifungal drugs. Our laboratory has successfully isolated the *Geobacillus* strains capable to synthesize the silver nanoparticles (AgNPs) that are showing the promising results in antimicrobial therapy. Another group of the microorganisms displaying the selective antifungal activity is *Streptomyces* genera bacteria. The selective way of action is very important in keeping the intact microflora of the human skin, avoiding the elimination or the bacteria that are suppressing the growth of *Candida* yeasts. 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 skin pathogens [2].

Another powerful tool for developing new antibiotics or biocatalysts for industrial and pharmaceutical applications is protein engineering allowing us to overcome the limitations of natural enzymes and holding the potential for transforming the metabolic drug landscape. In our research, we applied various protein engineering methods (random and site-specific mutagenesis; DNA shuffling; the design of new fused biocatalysts) to investigate lipolytic enzymes produced by Geobacillus lipases [3, 4]. Lipases as biocatalysts can be used in organic synthesis reactions to produce precursors of drug and bioactive compounds against microorganisms related to skin infections.

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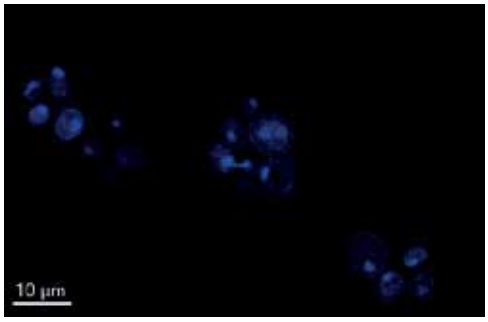
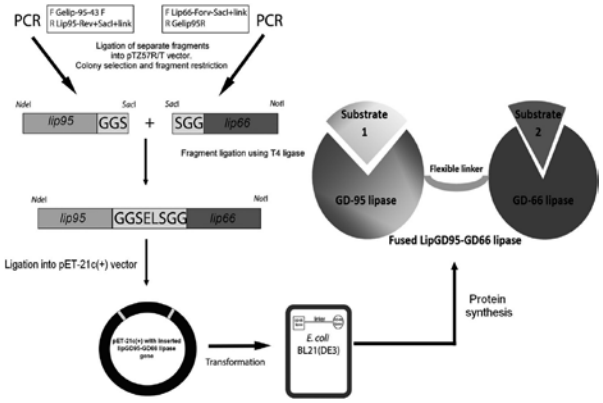
The *Candida* genera yeast-caused infections are frequent and difficult to treat, as the physiology and metabolisms of yeast are similar to the host. Induction of the programmed cell death is of the great medical relevance, since during apoptosis peptides, nucleotides, amino acids et al are released 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. For the apoptotic phenotype detection, we perform active caspase staining, a TUNEL reaction and a phosphatidylserine externalization analysis in the *Candida* yeast.



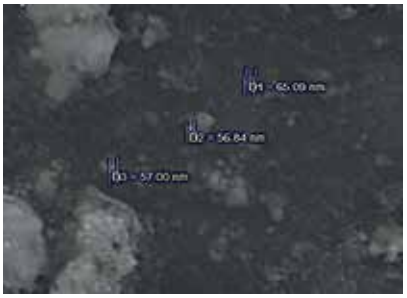
Confocal laser scanning microscopy of yeast cells

The rapid evolution of bioconversion and ecotechnology strongly increases the focus on the enzymes that possess novel properties. These new enzymes can change the chemical-less, eco-friendly synthesis of various industrial products. One of the most interesting enzymes are the lipases from *Geobacillus* bacteria as they can be active in extreme conditions. In our current study, new fused lipolytic enzyme LipGD95-GD66 was constructed using two *Geobacillus* lipases with different properties – GD-95 and GD-66 [6]. This enzyme provides a novel example of lipolytic chimeric enzymes, which might expand the knowledge of how such multifunctional enzymes operate. This study also shows for the first time that GD-66, GD-95 and LipGD95-GD66 lipases retained high lipolytic activity after incubation in organic solvents for periods longer than several weeks. GD-95 itself possesses attractive physicochemical and kinetic features for application in biofuel or fragrant esters production via transesterification of vegetable oils [4].



Construction scheme of chimeric LipGD95-GD66 lipase

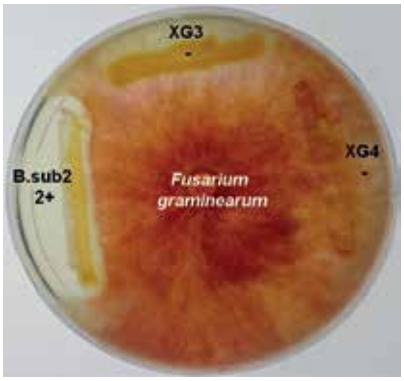
Silver nanoparticles (AgNPs) are of interest due to their unique physical and chemical properties, which can be incorporated into a wide range of applications. Recently, AgNPs have received significant attention due to their potent antimicrobial properties. We focus on the biosynthesis of silver nanoparticles by using a reduction of Ag^{+} with the cell-free extracts of four *Geobacillus* bacteria strains. Only a few studies that involved *Geobacillus* bacteria in metal nanoparticle synthesis, including AgNPs, have been performed recently.



SEM analysis of obtained AgNPs produced by *Geobacillus* sp. 18

Fusarium head blight (FHB) is a global concern. This study sought to identify bacterial isolates, which could be applicable for the control of *Fusarium graminearum* in wheat. In this study, eight newly cultured isolates (GB31, GB41X, XJ11, XG11, B. sub2, XJ2, XJ8 and XI4), which can effectively reduce the growth of more than two *F. graminearum* strains *in vitro*, were identified. In this investigation, four laboratory-selected strains, GB31, B. sub2, MBK-a3 and MBK-r4, were evaluated for their potential for biocontrol of *F. graminearum in vivo* and tested under the field conditions. Results suggest the possibility of using MBK-a3 isolate to control FHB by natural antagonism [5].

Antifungal activity of selected bacteria cultures against different strains of *F. graminearum*. Size of clear zones around the tested isolates indicated inhibition of the growth of tested fungus: high inhibition (3+), small (2+), very small (+), not clear (±), not detectable (−). The number indicates different strains of F. *graminearum*



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Animal Biodiversity, Structure and Ecology of Populations

Animals are the largest component of biodiversity, both in terms of known species (almost 5 million species, over 80% of all known species) and the biomass and significance in ecosystems. Therefore, it is important to reveal the basic principles of systematic and ecological animal evolution based on studies of certain model animal groups. This involves the mapping and catalogisation of the Lithuanian fauna with a particular concern on the ecology of rare and endangered, also alien and invasive species of animals, changes in their abundance and distribution. The principal aims include: 1) the developing of the principles of taxonomic and ecological research of the animal world based on the studies of particular animal groups; 2) the studying of the ecology of rare animal species, their abundance and distribution patterns in Lithuania; 3) the carrying out of research concerning the diversity and abundance of fauna in the protected areas of Lithuania.

The ongoing research of our team concerns insects (Diptera: Tipulomorpha; Hemiptera, Sternorrhycha: Aphididoidea and Adelgoidea; Hymenoptera: Bombidae and Braconidae), spiders, slugs (Mollusca: Gastropoda and Bivalvia), freshwater fishes, birds of prey and owls, black storks. Research topics include taxonomy and systematics, distribution, ecology and economic importance as well as the monitoring of local faunas in the protected areas of Lithuania and elsewhere.

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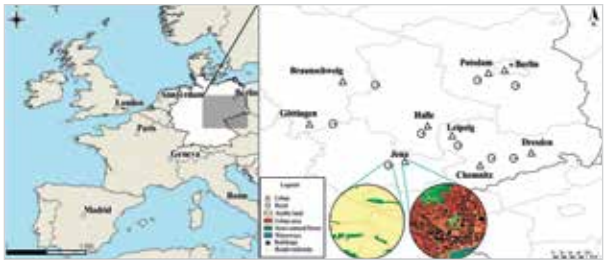
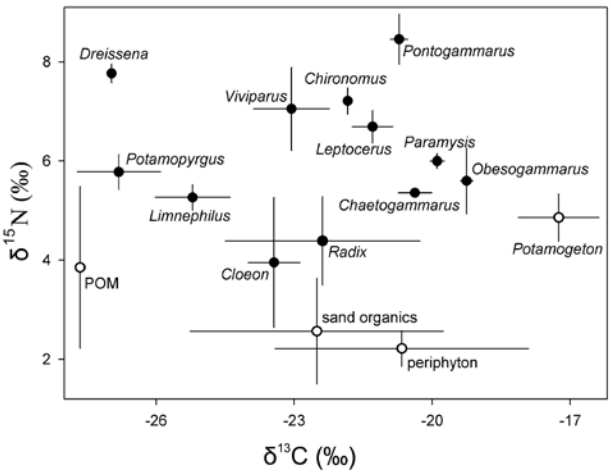
Genome-Wide Single Nucleotide Polymorphism Scan Suggests Adaptation to Urbanization in an Important Pollinator, the Red-Tailed Bumblebee (*Bombus lapidarius* L.)



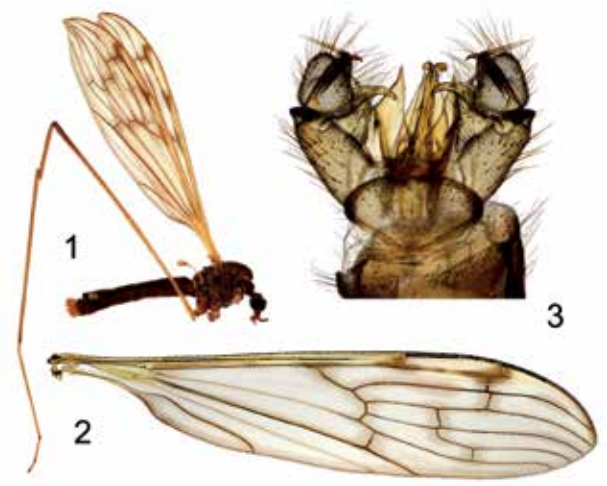
Genome-wide effects of urbanization on putative neutral and adaptive genomic diversity in a major insect pollinator, *Bombus lapidarius*, collected from nine German cities and nine paired rural sites was investigated using 110 314 single nucleotide polymorphisms generated by restriction-site-associated DNA sequencing. Genetic differentiation among sites was low, and there was no obvious genome-wide genetic structuring, suggesting the absence of strong effects of urbanization on gene flow. Overall, the results provide evidence of local adaptation to urbanization in the face of gene flow in a highly mobile insect pollinator (Theodorou et al. 2018, doi: 10.1098/rspb.2017.2806).

Effect of the Invasive New Zealand Mud Snail (*Potamopyrgus antipodarum*) on the Littoral Macroinvertebrate Community in a Temperate Mesotrophic Lake



The chief aim of the present study was to reveal changes in a littoral macroinvertebrate community of the lake Dusia (Lithuania) induced by the invasion of the New Zealand mud snail (*Potamopyrgus antipodarum*). For that purpose, relevant aspects of the lake littoral macroinvertebrate community in pre- and post-invasion periods were compared, and the trophic position of *P. antipodarum* in the lake food web was determined by performing the stable isotope-ratio analysis. It appeared that invasion of the *P. antipodarum* caused a significant increase in the local macroinvertebrate family richness and diversity, shifting the community composition from crustacean- to gastropod-dominated. However, the biomass of local macroinvertebrates remained unchanged (Rakauskas et al. 2018, doi: 10.1071/MF17059).

Libnotes Crane Flies (Diptera: *Limoniidae*) from Jeju Island (South Korea)



The Korean species of *Libnotes (Laosa)* Edwards, 1926 and *L. (Libnotes)* Westwood, 1876 from Jeju Island were taxonomically revised. *L. (Laosa) charmosyne* (Alexander, 1958) and *L. (Libnotes) divaricata* (Alexander, 1924) are new records for South Korea, and *L. (Libnotes) byersiana* n. sp. is described. An identification key for all Korean Libnotes, redescriptions and illustrations of the three currently known Jeju species are presented (Podenas & Byun. 2018, doi: 10.11646/zootaxa.4483.2.9).

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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, morphologies, 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.

Our research group focuses on the diversity and ecology of plants, algae, fungi and lichens. The following are several examples of conducted research. A field investigation based on phytosociological methods revealed the functioning patterns and structure of *Lycopodium* and *Diphasiastrum* populations with an emphasis on gametophytes and juvenile sporophytes [1]. The life, scientific activities and discoveries of the botanist Abromas Kisinas (1899–1945) were investigated by the analysis of the historical herbarium collections and a biographical approach [2]. Investigation on *Equisetum telmateia*, a vulnerable (IUCN) plant species, showed that the species occurs in seven localities in Lithuania, it usually grows in river valleys or close to rivers and occupies alluvial forest habitats [3]. Experimental study showed that green algae *Chlorella vulgaris* is capable of a very efficient nutrient removal from municipal wastewater [4]. To assess the post-fire development of mycobiota following fires in pine forests, we, in collaboration with mycologists from the Nature Research Centre in Vilnius, have used field mycosociological methods, light microscopy in taxa identification and a chemical analysis of the environmental samples [5].

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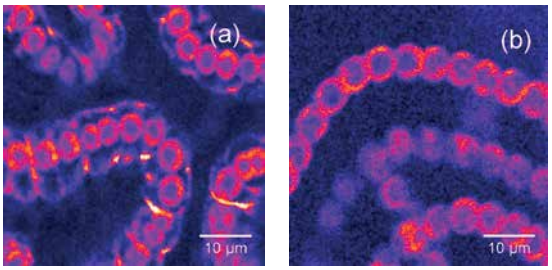
CARS Microscopy of Scytonemin in Cyanobacteria *Nostoc commune*

We studied the scytonemin in cyanobacteria applying coherent anti-stokes Raman spectroscopy (CARS) microscopy. The chemically specific CARS response from particular chemical compounds in the algae cell is usually weak because of their low concentration and are often overwhelmed by optical emissions from other cellular constituents. The study demonstrated that for scytonemin localized in cyanobacteria *Nostoc commune*, the vibrational response is sufficient for its reliable identification. Scytonemin is known as sunscreen sheath enclosing the cells of microalgae against ultraviolet radiation. We showed effectiveness of CARS microscopy as a mapping technique to visualize the scytonemin sheath in live culture such as cyanobacteria *N. commune* (Venckus et al. *J. Raman Spectrosc.* 2018, 49(8): 1333–1338).



Images of laboratory-cultivated *Nostoc commune* microalgae cells after exposure to UV-A light (a) and from control sample (b)

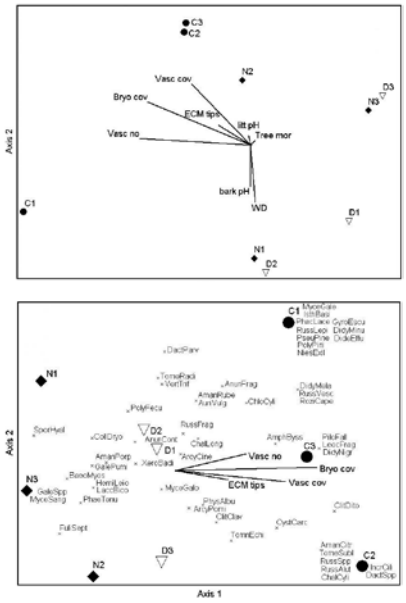
S. B. Gorski's Bryological Collection in the Herbarium of Vilnius University

Stanislaw Batys Gorski (1802–1864) was a pharmacist, botanist and entomologist. At the beginning of the 19th century, he headed the Botanical Garden of Vilnius University and lectured on botany. Gorski's bryological collection comprises 729 specimens. The species' names of bryophytes were written in Latin in the central part of the label. Other label information was written either in Latin or Polish. Only 20% of the labels contain information about localities. As a collecting locality, Lithuania was indicated in 21% of such labels. The most common cities or settlements recorded on the labels were Vilnae, Vilna, Kalwarya, Werki, Boltupie. Other specimens were collected from Italy, Belarus, and Germany. The date of specimen collection indicated on some labels, e.g., 1823, 1834, and 1835, refer to the time when Gorski collected his vascular plant herbarium in the *Białowieża* Forest and in the vicinity of Vilnius (Jukonienė et al. *Acta Soc Bot Pol*. 2018, 87(3): 3588).

Bryological collections of the 19th century in the Herbarium of Vilnius University

Effect of Water Stress and Nitrogen Addition on the Mycobiota

Mycobiota (fungi, lichens and myxomycetes) was examined in the permanent plots following experiments of artificial drought and nitrogen addition, and compared with untreated forest C), in a Scots pine stand. Species diversity and relationships between fungal community structure and environmental variables (plant species numbers and cover, bryophyte cover, soil and bark pH, tree mortality) were explored. Both draught and nitrogen addition treatments lead to decrease of fungal species in general, however, responses of individual trophic and ecological groups varied. The strongest effect of the treatments was observed for soil fungi, especially mycorrhizal: numbers of fruiting species and ectomycorrhizal root tips decreased, and species composition has changed. Saprotrophic fungi reacted by changes in species composition but not in numbers (Motiejūnaitė et al. *Russ J Ecol*. 2018, 49(3): 236–241).



Relationship between fungal communities and environmental variables

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Environmental Assessment & Ecosystem Development

Our main research goal is the impact of various anthropogenic and natural stress factors on ecosystem state dynamics and environment assessments. During the last decades, the ecosystem development 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. A 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. An 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. An introduction of alien species provokes new infochemical interactions and changes in the behaviour of organisms, which leads to a reorganization of the functional groups within an impacted ecosystem.

Our interdisciplinary team has contributed with different methods and different levels of ecosystem organization. The pollution of the bottom sediments of water bodies and sapropel quality in the lakes of Lithuania as well as the contents of heavy metals in the bottom sediments were assessed by geological core techniques and a consequent chemical analysis. Wildlife-vehicle collisions are analysed using GIS, spatial and temporal modelling [1]. Response of model insects to food quality changes on the genetic level under conditions of simulated parasite attacks suggests new implications for the pest control [2]. The antidepresant treatment tests have revealed different responses in metbolism and behaviour of Gryllus integer [3]. A chemoreception of model indicator species *Plodia interpunctella* on substrates infected and not infected by Micromycetes allowed us to discover 3-methyl-1-butanol as the main biomarker. The ecotoxicity of surface water sewage and the filtrates of landfills were tested with a standard bioassay using the marine bacterium *Aliivibrio fischeri*. Biotest species and different toxicity result scoring systems, which are app lied in effluent/wastewater and landfill leachate toxicity assessments, were reviewed.

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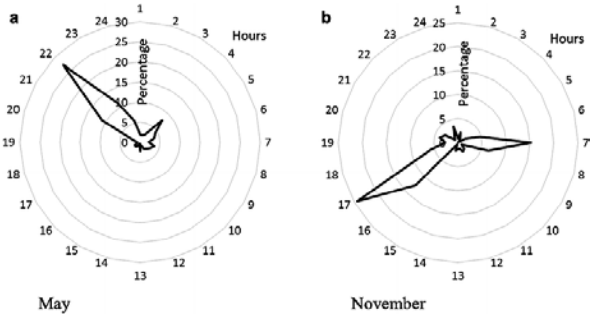
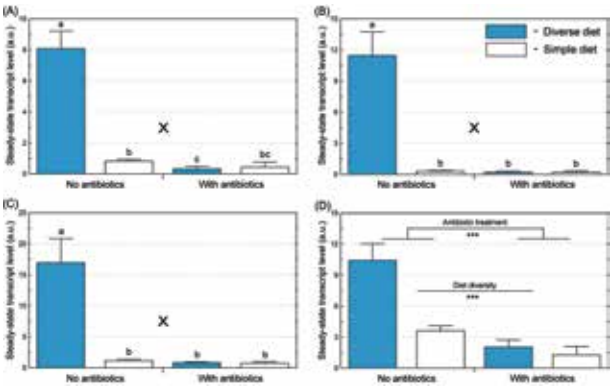
The Influence of Time Factors on the Dynamics of Roe Deer Collisions with Vehicles

Roe deer are the most common large animals involved in wildlife-vehicle collisions (WVC) in Europe. The collisions were analysed in terms of monthly and daily data for each month separately, and the results are compared with the time of sunrise and sunset in Lithuania. By analysing trends of natural factors that influence the number of collisions, we show that the frequency of WVC strongly correlates with seasonal and yearly changes in sunrise and sunset. Results show that these natural factors are extremely important for the dynamics of WVC. Future analysis of these factors and application of appropriate preventative measures should significantly reduce the risk of WVC (Ignatavičius et al. *Landsc. Ecol. Eng*. 2018, 14 (2): 221–229).



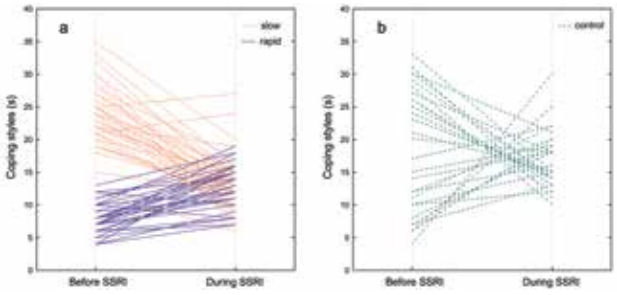
Food Diversity Affects the Expression of Antimicrobial Peptide Genes upon Simulated Parasite Attack in the Larvae of Greater Wax Moth

Immunity is a costly function for the organism, as it often competes with other life-history traits for limited nutrients. We tested whether the expression of antimicrobial peptides (AMP) of the larvae of *Galleria mellonella* (L.) (Lepidoptera: Pyralidae) changes as a consequence of insertion of a nylon monofilament, which acts like a synthetic parasite. The treatment was done for larvae grown on a high-quality vs. a low-quality diet. Food quality seems to affect AMP gene expression in *G. mellonella* larvae, therefore it should always be controlled in studies on bacterial and fungal infections in *G.mellonella* (Krams et al. *Entomol. Exp. Appl*. 2017, 165(2–3): 129–137.).



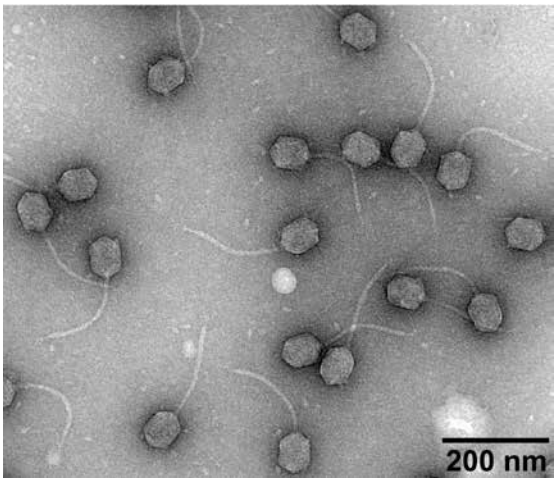
Linking Organismal Growth, Coping Styles, Stress Reactivity and Metabolism via Responses against a Selective Serotonin Reuptake Inhibitor in an Insect

Evidence suggests that brain serotonin (5-HT) is one of the central mediators of different types of animal personality. We tested this assumption in field crickets *Gryllus* integer using a selective serotonin reuptake inhibitor (SSRI). Crickets were selected for slow and rapid development and tested for their coping styles under non-stressful conditions (time spent exploring a novel object). Before the SSRI treatment, a strong negative correlation was observed between coping style and stress reactivity, which suggests the existence of a behavioural syndrome. After the SSRI treatment, the syndrome was no longer evident. The results of this study show that 5-HT may be involved in regulating behaviour not only along a stress reactivity gradient but also along a coping styles axis (Krams et al. *Sci. Rep.* 2018, 8: 8599).



The Biomarkers of Exposure to Stressors in Aquatic Organisms

The toxicity of chemical and physical stressors to water organisms at different development stages will be evaluated. The experiments will be conducted with a few aquatic species (fish and mussels). The effects of stressors will be assessed using an integrated assessment – from the subcellular to organism level. The biological response will be analysed using cytogenetic, biochemical, cytological and physiological biomarkers. The integrated multibiomarker response will be estimated, which is useful in ecological risk assessment and can be applied in environment protection management (Project ACTIS. Dr. L. Butrimavičienė (Nature Research Centre, Vilnius). Dr. V. Kalcienė. 2017-2020).



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Genetic and Structural Diversity of Bacteriophages

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

The phage group of the Department of Molecular Microbiology and Biotechnology has recently focused on the isolation and molecular characterisation of novel phages having unique structure, host range or physiology. Over the last five years, researchers of the Department carried out five different projects funded by the Research Council of Lithuania. The projects were conducted by a group of scientists of the Department (MIP-002/2014) or in collaboration with the research groups of the Institute of Biotechnology (S-MIP-17-47), the Nature Research Centre and the Institute of Biosciences, and Kyoto University (SIT-7/2015, P-MIP-17-6, 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 the impact of global warming on the diversity and co-evolutionary dynamics between microorganisms and virus populations in Lithuanian agroecosystems and aquatic environments. A number of unique *E. coli*, *Arthrobacter,* and *Pantoea* phages have been isolated, characterised and published [1–5]. In total, eleven peer-reviewed scientific publications have been published by the research group during the five-year period.

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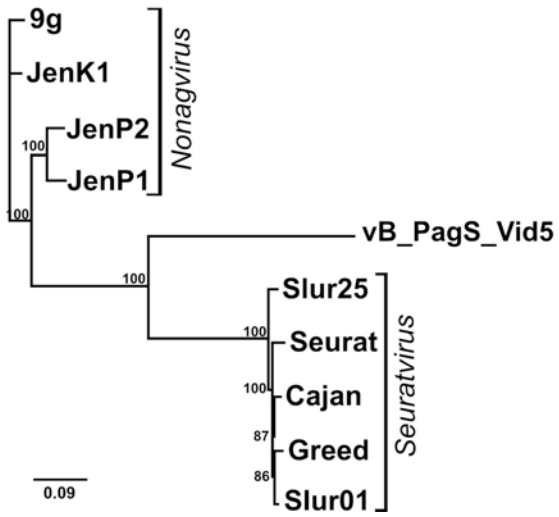
Pantoea Bacteriophage vB_PagS_Vid5: A Low-Temperature Siphovirus that Harbours a Cluster of Genes Involved in the Biosynthesis of Archaeosine

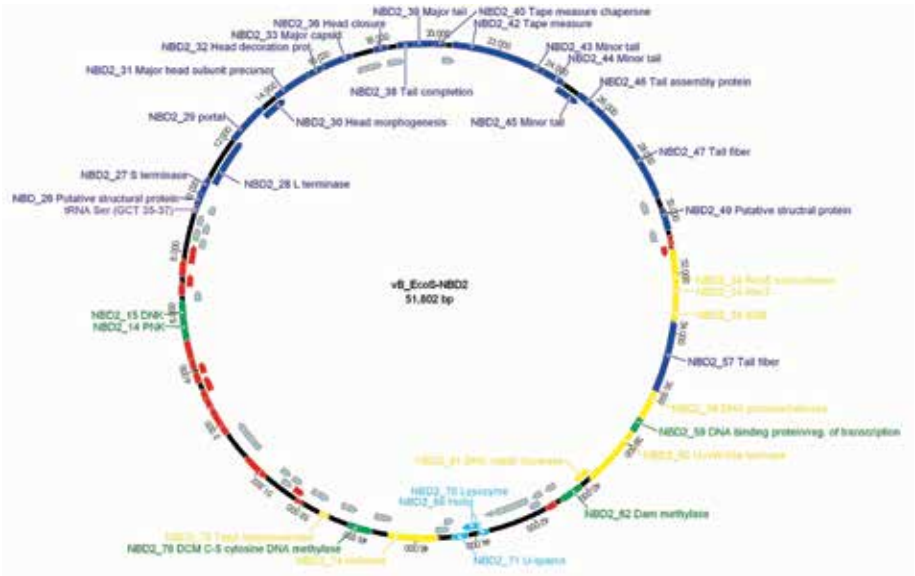
A novel low-temperature siphovirus, vB_PagS_Vid5 (Vid5), was isolated in Lithuania using *Pantoea agglomerans* isolate for the phage propagation. The 61,437 bp genome of Vid5 has a G–C content of 48.8% and contains 99 probable protein-encoding genes and one gene for tRNASer. A comparative sequence analysis revealed that 46 out of 99 Vid5 open reading frames (ORFs) code for unique proteins that have no reliable identity to database entries. In total, 33 Vid5 ORFs were given a putative functional annotation, including those coding for the proteins responsible for virion morphogenesis, phage-host interactions and DNA metabolism. In addition, a cluster of genes possibly involved in the biosynthesis of 7-deazaguanine derivatives was identified. Notably, one of these genes encodes a putative preQ0/preQ1 transporter, which has never been detected in bacteriophages to date. A proteomic analysis led to the experimental identification of 11 virion proteins, including nine that were predicted by bioinformatics approaches. Based on the phylogenetic analysis, Vid5 cannot be assigned to any genus currently recognized by ICTV, and may represent a new one within the family of *Siphoviridae* (Šimoliūnas et al. *Viruses* 2018. 25: 10(11).



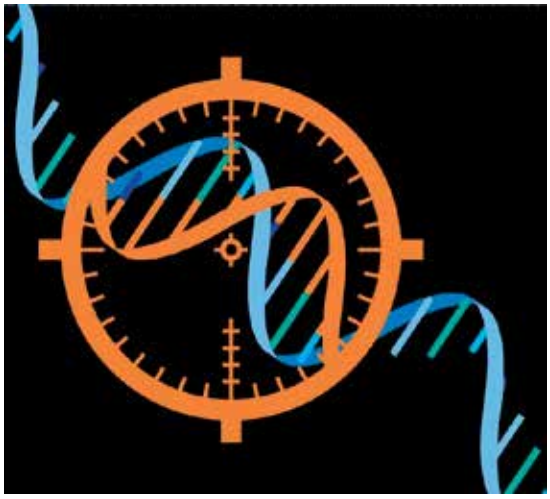
Neighbour-joining tree based on the alignment of vB_PagS_Vid5 and *Nonagvirus* and *Seuratvirus* phage genome sequences

Molecular Analysis of the Low-Temperature *Escherichia coli* Phage vB_EcoS_NBD2

A novel low-temperature *Escherichia coli* phage vB_EcoS_NBD2 was isolated in Lithuania from agricultural soil. With an optimum temperature for plating around 20 °C, vB_EcoS_NBD2 efficiently produced plaques on *Escherichia coli* NovaBlue (DE3) at a temperature range of 10–30 °C, yet failed to plate at temperatures above 35 °C. Phage vB_EcoS_NBD2 virions have a siphoviral morphology with an isometric head (65 nm in diameter), and a non-contractile flexible tail (170 nm). The 51,802-bp genome of vB_EcoS_NBD2 has a G+C content of 49.8%, and contains 87 probable protein encoding genes as well as 1 gene for tRNASer. Comparative sequence analysis revealed that 22 vB_EcoS_NBD2 ORFs encode unique proteins that have no reliable identity to database entries. Based on homology to biologically defined proteins and/or proteomics analysis, 36 vB_EcoS_NBD2 ORFs were given a putative functional annotation, including 20 genes coding for morphogenesis related proteins and 13 associated with DNA metabolism. Phylogenetic analysis revealed that vB_EcoS_NBD2 belongs to the subfamily *Tunavirinae*, but cannot be assigned to any genus currently recognized by ICTV (Kaliniene et al. *Arch Virol*. 2018, 163(1): 105–114).



Functional genome map of bacteriophage NBD2



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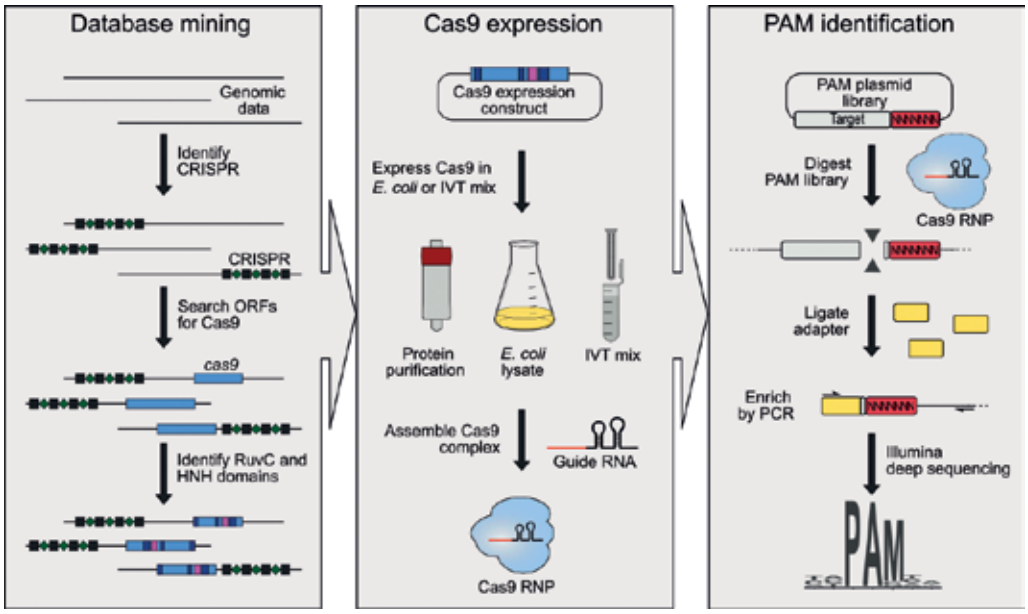
CRISPR-Cas Tools and Technologies

In recent years, Cas9 has revolutionized the genome-editing field and enabled a broad range of applications from basic biology to biotechnology and medicine. Cas9 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. There are thousands of type II CRISPR-Cas9 sequences available in sequence databases. 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 [5]. 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.



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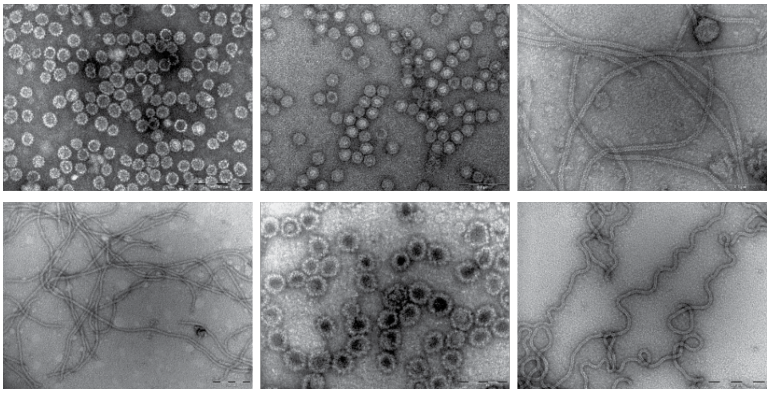

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Biosynthesis of Chimeric and Native Proteins

Protein engineering, i.e. the generation of proteins with desired features, is a fascinating opportunity for producing very sensitive diagnostic tools for the detection of viral infections and the development of highly efficient and safe vaccines against different pathogens.

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 a 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.

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Development and Application of Biocatalytic Systems

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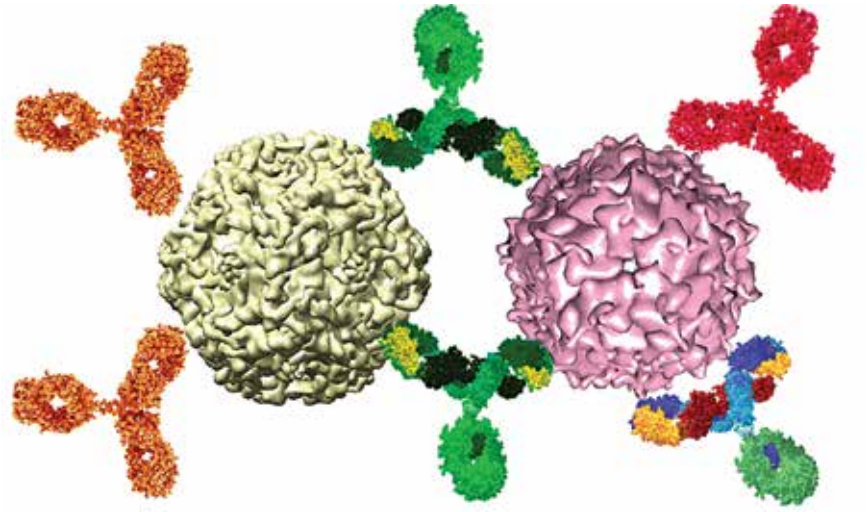

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Generation and Analysis of New Antibodies

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

Our team has strong expertise in the development and characterization of monoclonal and recombinant antibodies. We have generated more than 500 monoclonal antibodies against different targets: viral antigens, bacterial virulence factors, cellular proteins, cytokines, hormones. 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 [1] and others. These antibodies are valuable tools for investigating the antigenic structure of viruses [2], the development of diagnostic assays and the prevalence studies of viral infections. Virus research is carried out in collaboration with Prof. Dr. R.Ulrich (Friedrich-Loeffler-Institute, Greifswald, Insel-Riems, Germany), Prof. Dr. D. Glebe (Giessen University, Germany), J. O. Koskinen (ArcDia International Oy Ltd., Turku, Finland) and other partners. We have also generated a collection of antibodies against bacterial cytolysins and exploited them both for structural studies and a quantitation of cytolysins [3]. In collaboration 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. 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 as demonstrated for vaginolysin-specific antibodies and neutralizing antibodies against the hepatitis B virus [4].

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Microfluidic Tools 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.

Many useful microfluidic techniques have been developed to analyse single-cells or bio-molecules; however, there is an unmet demand for methods with improved analytical sensitivity and high-throughput capabilities. Our multidisciplinary team is working at fulfilling this demand by developing microfluidic tools for a diverse set of quantitative experiments in cell biology and biomedicine. In 2018, our group members have collaborated with Harvard University, Harvard Medical School, Oxford University and MSKCC to advance single-cell biology research in cancer and immunology. Among the most important achievements are two *Cell* and two *Nature Group* articles.

System and Method for Synthesis of DNA Particles and Use Thereof Patent application: US patent application No 16/069,404 and EP patent application No EP17704298,3.

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Single-Cell Barcoding and Sequencing Using Droplet Microfluidics

We have previously reported a droplet microfluidic technology, known as inDrops (for indexing droplets), for barcoding thousands of transcriptomes of single-cells (Zilionis et al. *Nature Protocols*, 2017). The basic principle is relatively simple: a mixture of cells is encapsulated into microfluidic droplets together with barcoding oligonucleotide primers (attached to hydrogel beads) and a mix of RT and lysis reagents. The mRNA released from the lysed cells remains trapped inside the same droplet and is tagged (barcoded) with oligonucleotide primers during the RT reaction. After barcoding, the material from all cells is pooled by breaking the droplets, and the cDNA library is processed for the next-generation sequencing. We are applying this technology to solve various biological questions, such as heterogeneity's role in cancer, anti-tumour immune responses and the development of complex diseases.

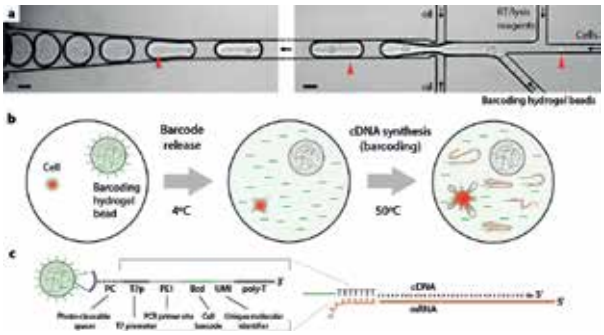

Fig. 1. Single-cell transcriptome barcoding in microfluidic droplets

In vitro Directed Evolution

Protein expression *in vitro* has broad applications in directed evolution, synthetic biology, proteomics and drug screening. However, most of the *in vitro* expression systems rely on relatively high DNA template concentrations to obtain sufficient amounts of proteins, making it challenging to express proteins from gene libraries. We developed a microfluidic technique for the generation of condensed DNA particles from the single DNA molecules. We used droplet microfluidics to encapsulate single-DNA molecules in 3-picoliter (pL) volume droplets and converted them into 1 µm-size DNA particles by the multiple displacement amplification reaction. In the presence of magnesium ions and inorganic pyrophosphate, the amplified DNA condensed into the crystalline-like particles, making it possible to purify them from the reaction mix. Using purified DNA particles, we performed an *in vitro* transcription-translation reaction and successfully expressed the complex enzyme β-galactosidase in droplets and in the 384-well format. The yield of protein obtained from the DNA particles was significantly higher than that from the corresponding amount of free DNA templates, thus opening new possibilities for high throughput screening applications.

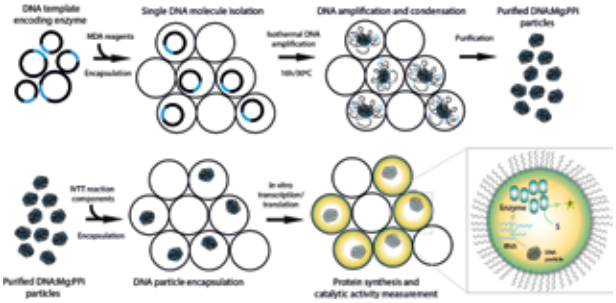

Fig. 2. Single-molecule derived DNA microparticle generation

Microfluidic Tools for Screening Antibody Secreting Cells

Droplet microfluidics provides much higher throughput and screening capabilities as compared to conventional microtiter plate assays. Because compartmentalized cells stay alive for extended periods of time, it becomes possible to assay millions of individual cells and sort positive clones at high-throughput rates. These features are particularly relevant when screening individual cells producing proteins of therapeutic value (e.g. antibodies). Furthermore, in contrast to conventional FACS-based assays, where the detection is based on membrane-bound proteins, using droplet microfluidics makes it possible to screen extracellular (e.g. secreted) biomolecules. Due to a small volume of the droplets, antibodies secreted by single cells quickly achieve detectable concentrations enabling rapid functional screening. Hence, single-cell screening options are highly flexible and are not limited to the surface-bound antibodies. Finally, encapsulated cells can be lysed, and their genetic material can be further analysed, thus extending researchers' capabilities of linking the cellular genotype and phenotype.

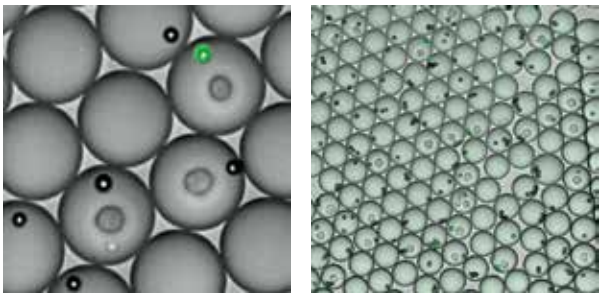

Fig. 3. Two images of encapsulated cells recorded under fluorescence microscope. On the left, the cells that produce and secrete antigen-specific antibodies are detected, when co-encapsulated antigen-coated bead becomes fluorescent (green). The cells that do not produce antibodies, or those antibodies are not binding the antigen, will not generate fluorescent-bead signal. On the right, the encapsulated cells under lower magnification

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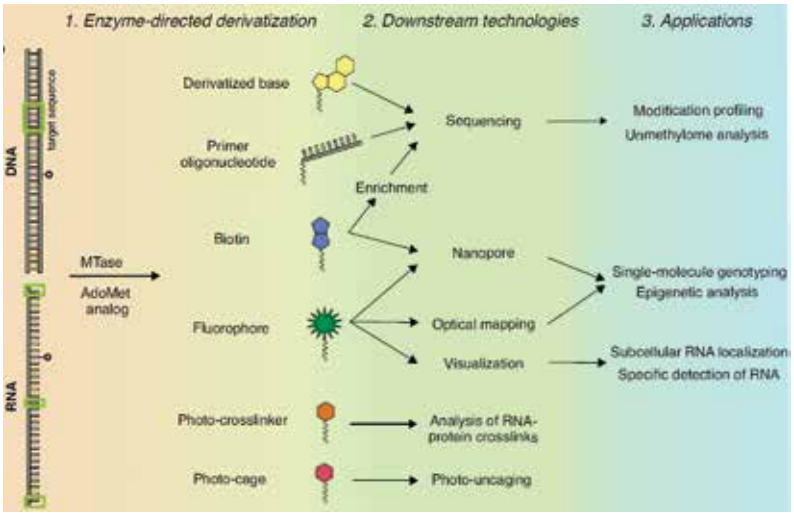

Molecular Tools for Covalent Labelling and Profiling of Epigenome

Molecular Tools for Targeted Covalent Derivatization of DNA and RNA

Nucleic acids are linear polymers comprised of four major types of building blocks encoding the genetic blueprint of life. Selective chemical or physical identification of different loci of such largely uniform biomolecules is rather problematic, unless they can be furnished with suitable 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. Although DNA and RNA methylation plays important roles in biological signalling, the naturally transferred methyl group is a poor reporter and is hardly amenable to chemical derivatization. To unlock the technological potential of these enzymes we seek to repurpose them for the transfer of prederivatized (extended) versions of the methyl group. A series of synthetic analogues of the AdoMet cofactor were developed that allowed MTases to tag DNA and RNA with extended moieties [1]. This novel technology named mTAG (methyltransferase-directed Transfer of Activated Groups) enabled targeted covalent derivatization and labelling of DNA and RNA which is opening new avenues in genomic research, diagnostics, and bionanotechnology.

Advanced Methods for Epigenome Analysis

Epigenetic regulation in vertebrates involves enzymatic methylation of cytosine in certain CpG dinucleotides to 5-methylcytosine, followed by enzymatic oxidation to 5-hydroxymethylcytosine, 5-formylcytosine and 5-carboxylcytosine. Although many aspects of how these modifications are established and perform their functions remain obscure, research into the epigenetic regulation is hampered by limitations of available analytical techniques. Using the mTAG and related technologies, we are developing new experimental approaches for profiling of DNA modifications and miRNA pools for epigenome studies and improved diagnostics [2-4]. 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].

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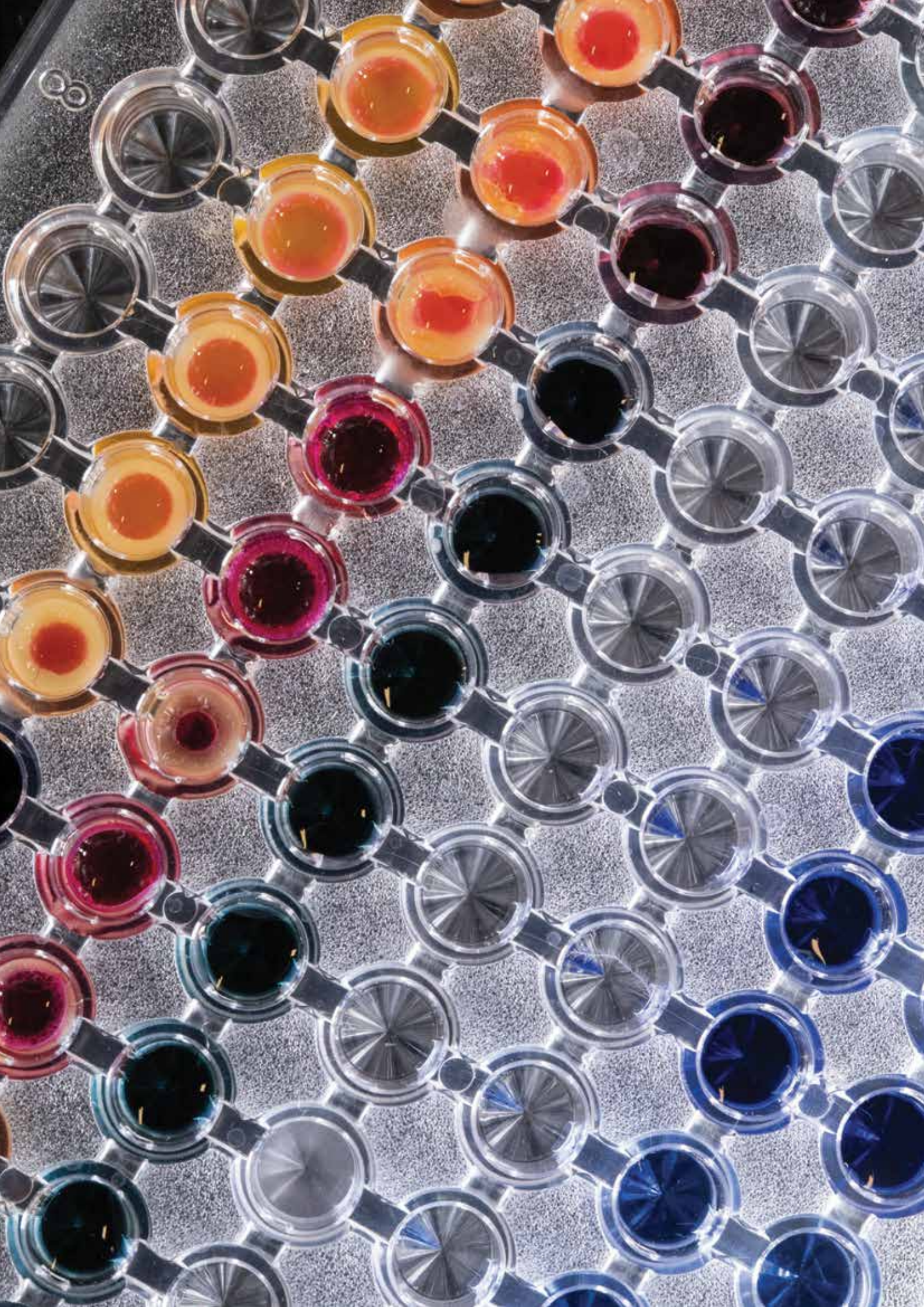

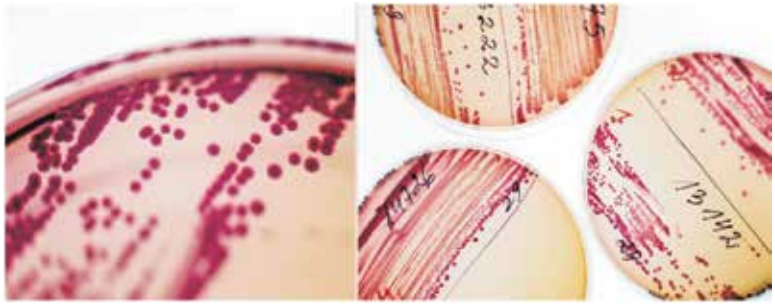

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Antibiotic Resistance and Pathogenesis

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 ones of the most important hospital infection agents. The opportunistic pathogen *Acinetobacter baumannii* causes a variety of 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, transposons and integrons, as well as the ability to adhere and colonise the host cells. The bacterial toxin-antitoxin (TA) systems are widely spread chromosome and plasmid-borne gene loci, proposed to be involved in a variety of functions such as plasmid stabilisation, regulation of cell growth and death under the stress, mediation of bacterial persistence through generation of cells tolerant to antibiotics. All listed features are crucial in the life of pathogens and understanding the role of TAs might bring novel insights into pathogenicity and development of novel antibacterial strategies.

We focus our research upon 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 features contributing to pathogenesis. Towards this goal, we have recently characterised a set of *A. baumannii* TA systems including a pair of TAs carried by ubiquitous resistance plasmid carried by multidrug resistant clinical isolates and show their involvement in *A. baumannii* stress response and plasmid stabilization functions. Our recent comprehensive analysis of *A. baumannii* isolates belonging to most worldwide spread clonal lineages showed that they markedly differ in the series of cell surface-related features suggesting that *A. baumannii* strains evolved features, which could provide an advantage in the specific conditions outside or within the host. We also search for the novel antibiotic resistance mechanisms that have evolved in other gram-negative bacteria present in clinical environment as well as in non-clinical habitats such as soil and water.

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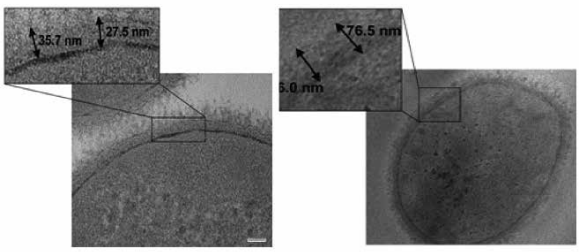
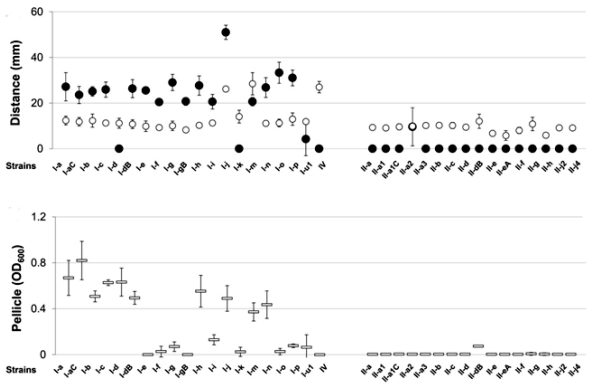
Surface-related Features and Virulence among *Acinetobacter baumannii* Clinical Isolates

The worldwide spread of *A. baumannii* in clinical settings is characterized by the expansion of several predominant clones. The strains belonging to the international clonal lineages I (IC I) and II (IC II) are associated with the hospital outbreaks and a high virulence. In particular, the IC II lineage strains are characterized through their high carbapenem-resistance and nosocomial spread in many countries during the recent years. In this paper, we show that IC II strains are non-motile, do not form specific biofilm called pellicle and display distinct capsular polysaccharide profile compared with the IC I strains. Moreover, in contrast to the overall highly hydrophobic IC I strains, IC II strains showed a greater variation in cell surface hydrophobicity. Within the IC II lineage, hydrophilic strains demonstrated reduced ability to form biofilm and adhere to the abiotic surfaces, also possessed two-fold thicker cell wall and exhibited higher resistance to desiccation. Importantly, these strains showed increased adherence to the lung epithelial cells and were more virulent in nematode and mouse infection model compared with the hydrophobic IC II strains. Hence, this study shows that the most widespread *A. baumannii* clonal lineages I and II markedly differ in the series of cell surface-related phenotypes including the considerable phenotypic diversification of IC II strains at the intra-lineage level what might contribute to a recent spread of strains belonging to this lineage in clinical settings worldwide including Lithuania (Skerniškytė et. al. *Frontiers Microbiol*. 2019, 9: 3116).



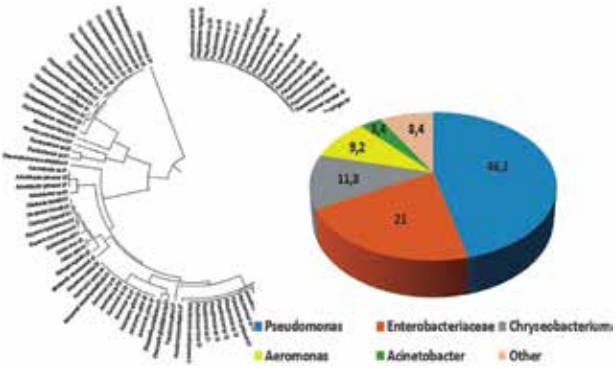
The features of cell wall of clinical *A. baumannii* isolates belonging to predominant clones accessed by TEM



The ability to form biofilm (pellicle) and motility of most spread *A. baumannii* clonal lineages differ considerably

Composition and Antimicrobial Resistance of Gram-negative Microbiota in Aquacultured Fish

Aquatic environments are one of the most favourable settings for acquisition and dissemination of antimicrobial resistance. This work aimed to isolate, identify and characterise resistant Gram-negative bacteria that are prevalent in fish in open aquaculture ponds. The most predominant genera were *Pseudomonas, Chryseobacterium*, Enterobacteriaceae and *Aeromonas*. The highest numbers of multiresistant isolates were found within *Pseudomonas* spp. Resistance to beta-lactams was the most common among all of the bacterial genera. Some strains, particularly the family *Enterobacteriaceae*, contain clinically important resistance determinants that potentially can be transmitted to human microbiota in cases of inappropriate processing of fish intended for food (Ružauskas et al. *Journal of Food Safety*. 2018, 38: e12447).



Antibiotic resistant gram-negative bacteria isolated form fish living in open aquaculture ponds harbour clinically important resistance determinants

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Epigenomic Studies of Human Disease

The uncovering of the origins of human disease, such as schizophrenia, diabetes, and cancer, is the most important goal of biomedical research. All such diseases exhibit inherited predisposition, which implies that DNA sequence variation is involved. Some important features of common diseases, however, are difficult to explain by DNA sequence-based mechanisms. Such include discordance of identical twins, delayed age of onset, significant fluctuations of the disease course and partial or even full recovery. The cases when identical genomes result in different phenotypes point at the involvement of epigenetic factors. It is well known that the same gene can "behave" very differently depending on its epigenetic profile, which is modified by developmental, environmental, and stochastic factors. Epigenetic issues in the cell may be just as dangerous as mutant genes, and disruption of the normal epigenetic regulation of a gene can be harmful to a cell, which may result in a diseased state.

Our recently formed research group at the Life Sciences Centre, Vilnius, has teamed up with the Epigenetics Laboratory, Centre for Addiction and Mental Health, Toronto. The new laboratory represents an extension of the long-term collaboration between bioinformatics experts in Lithuania and their counterparts in Canada. The international group of Canadian and Lithuanian scientists has significantly contributed to the development of the epigenetic theory of human disease, established the required infrastructure, developed the expertise necessary for large-scale epigenomic studies and performed a series of pioneering epigenomic studies.

Since the Laboratory of Epigenomic Studies was established in January 2018, we have been working productively on three different but related epigenomic projects. First and most important, we finished a series of large-scale experiments documenting the cyclic epigenomic oscillations, a new and very interesting epigenetic phenomenon which may play a direct role in epigenomic aging and account for epigenetic disease risk. Millions and millions of DNA bases in the nucleus of each cell are subjected to epigenomic "tides" every day and night, and even small deviations accruing over time may result in major epigenetic misregulation. Second, we investigated the diagnostic potential of cell-free circulating DNA, and its modification profiles, in predicting the outcomes of prostate cancer treatment. Translational potential of such pharmacoepigenetic study is evident: only patients who are responders to a specific drug should undergo a full course of treatment, otherwise the expensive treatment is wasted. Third, we investigated the behavioural effects of a small organic molecule which inhibits an important enzyme which methylates lysine 9 of histone H3 (H3K9), a suppressive mark of chromatin.

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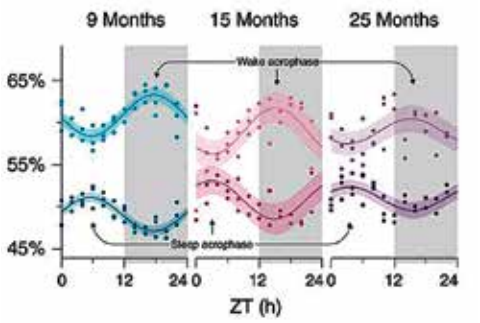
Cytosine Modifications Exhibit Circadian Oscillations that are Involved in Epigenetic Diversity and Aging

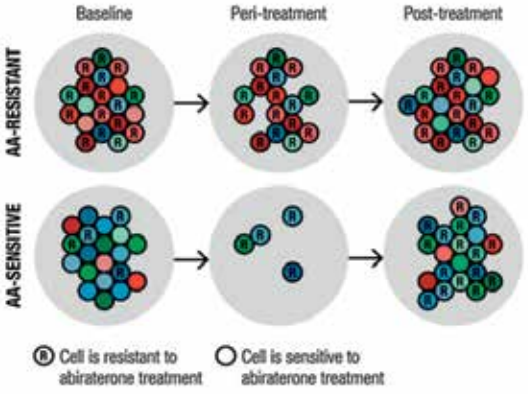
We discovered evidence of circadian cytosine modification patterns in mice. This discovery is at odds with the traditional perception that in somatic differentiated cells cytosine modification is static, albeit with some gradual and unpredictable life-long "epigenetic drift". Presence of oscillating cytosine modification may help elucidate several poorly understood epigenetic phenomena, such as the occurrence of ongoing active demethylation and production of hmC in differentiated somatic cells and large stochastic variation in cytosine modifications levels. Importantly, the cytosines with circadian epigenetic oscillations were associated with the aging epigenome, and the amplitude of the oscillation correlated with the magnitude of the aging effect, implying common molecular mechanisms. Our findings suggest that evolutionary advantageous processes such as circadian rhythmicity can also contribute to an organism's deterioration later in life (Oh et al. *Nature Communications*. 2018, Feb 13; 9(1): 644).



Averaged profile of oscillating cytosine modifications in 3 age groups of mice

Cell-Free DNA Modification Dynamics in Abiraterone Acetate-Treated Prostate Cancer Patients

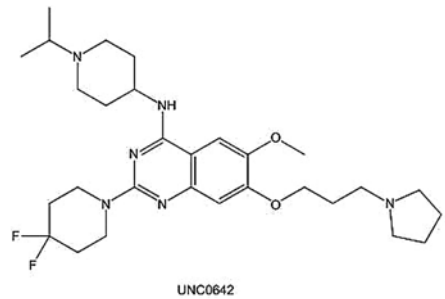
Prostate cancer is the second most common type of cancer in men, and abiraterone acetate is one of the most effective drugs used for castration-resistant prostate cancer treatment. It inhibits androgen synthesis by blocking CYP17A1 mediated reactions and substantially improves survival. However, 20% to 40% of patients show primary resistance to treatment and all eventually develop secondary resistance. We performed a longitudinal cell-free DNA modification study to identify epigenetic biomarkers related to treatment response and understand its mechanisms. We identified 30 potential biomarkers and demonstrated epigenetic variability as an additional layer when investigating cancer dynamics. This was also the first cell-free DNA modification study performed using Infinium Human Methylation 450K BeadChip microarrays (Gordevičius et al. *Clinical Cancer Research*. 2018, 24(14): 3317—3324).

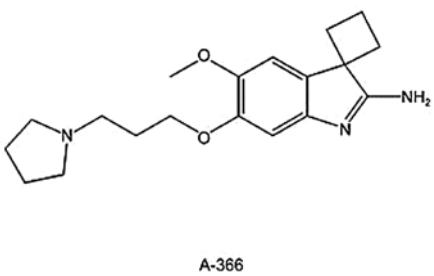


Proposed hypothetical clonal complexity dynamics in patients that react differently to abiraterone treatment

Inhibition of the Histone Methyltransferase Complex Modulates Anxiety-Related Behaviour in Mice.

Current anxiolytic drugs have significant shortcomings, and development of new medications is warranted. Two proteins, G9a and GLP, which methylate lysine 9 of histone H3 (H3K9), could be promising anxiolytic targets. Postnatal genetic knock-out of G9a reduces anxiety-related behaviour, consistent with the reduction of G9a levels by some medications used to treat anxiety. Conversely, there is increased anxiety-like behaviour in mice with GLP haplodeficiency. We sought to determine whether two pharmacological inhibitors of G9a/GLP, UNC0642 and A-366, would have similar effects to genetic G9a/GLP insufficiency. We found that G9a/GLP inhibition with either compound reduced anxiety-like behaviours, when administered to adult mice, in conjunction with decreased H3K9 methylation in the brain. These and other findings reinforce genetic evidence that G9a/GLP has different effects on anxiety-like behaviour at different stages of brain development and suggest that targeting this histone methyltransferase pathway could be useful for developing new anxiolytic drugs (Wang et al. *Acta Pharmacologic Sinica*. 2018 Feb 8, doi: 10.1038/aps.2017.190).





Chemical structures of histone methyltransferase inhibitors, which modulate anxiety-related behaviour in mice

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Genetic and Epigenetic Mechanisms of Cancer Development and Progression

The incidence of cancer is continuing to rise, and early diagnostic and/ or primary prevention strategies, as well as tools for predicting disease progression and resistance to therapy, are needed. Recently, genome-scale analyses have provided novel insights into the genomic and epigenomic landscape of various cancers, including mutational profiles, DNA methylation, protein-coding and microRNA (miRNA) expression patterns. Despite the validation of previously known alterations, various newly discovered genetic and epigenetic features have been proposed as measures of cancer aggressiveness as well as tools for diagnosis. During the last decade, the increased understanding of genetic alterations in tumours has encouraged the development of molecular tests 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. However, many of such tests assess similar molecular features and, thus, occupy only some overlapping clinical niches, whereas the full phenotypic spectrum of various malignancies is not properly covered.

Using a variety of genome-wide and target-oriented methodologies [1], our group aims at the (epi)genetic characterization of various human tumours (prostate, kidney, breast, lung and others) and the development of molecular biomarker systems for cancer detection, prognosis and early identification of resistance development [2–5]. Primarily focusing on altered DNA methylation and miRNA expression patterns, we have recently proposed biomarker panels for prostate cancer detection using liquid biopsy samples [3, 5]. Genome and epigenome-wide profiling allows us to identify novel biomarkers for cancer as well as novel mechanisms of therapy resistance [2–4]. In collaboration with the National Cancer Institute of Lithuania, we investigate the molecular profile of various tumours and apply modern 3D multicellular spheroid culture, single-cell, genome-wide approaches to explore tumour complexity and mechanisms of treatment resistance, as well as to develop novel solutions for cancer therapy [2].

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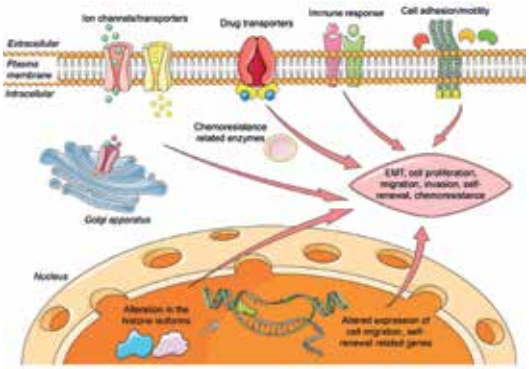
Chemoresistance Development in Cancer Cells

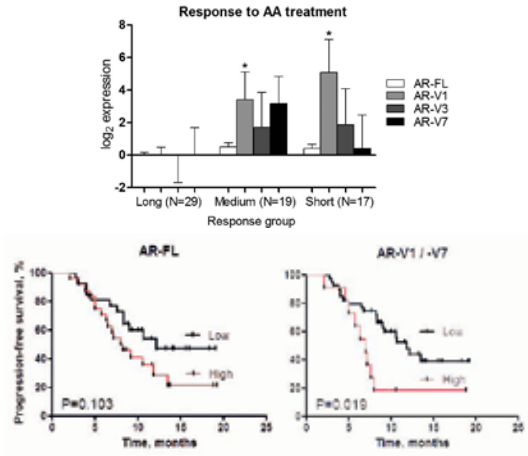
Despite significant advances in cancer diagnosis and treatment, resistance to chemotherapy remains an important barrier to successful cancer therapy. Multidrug resistance, known as the simultaneous resistance to different classes of drugs, is the leading cause of cancer recurrence and lethal outcomes. The most common cancer chemoresistance mechanisms are the hyperactivation of ABC transporters, especially ABCB1 (P-glycoprotein), and induction of epithelial-mesenchymal transition (EMT), however the interaction between these pathways remains poorly perceived. In the present study, the mechanisms of acquired cellular chemoresistance were studied in breast cancer cell line exposed to genotoxic DNA intercalating chemotherapeutic compound and non-genotixic ABC transporters stimulating agent. At least two possible scenarios of chemotherapeutic drug resistance were described in the derived unique chemoresistant



cell sublines: (1) the gradual activation of the canonical EMT pathway with later activation of ABCB1; and (2) the hyperactivation of ABC transporters followed by non-canonical EMT changes.

Blood-circulating Androgen Receptor Variants as Biomarkers of Treatment Response in Castration-resistant Prostate Cancer

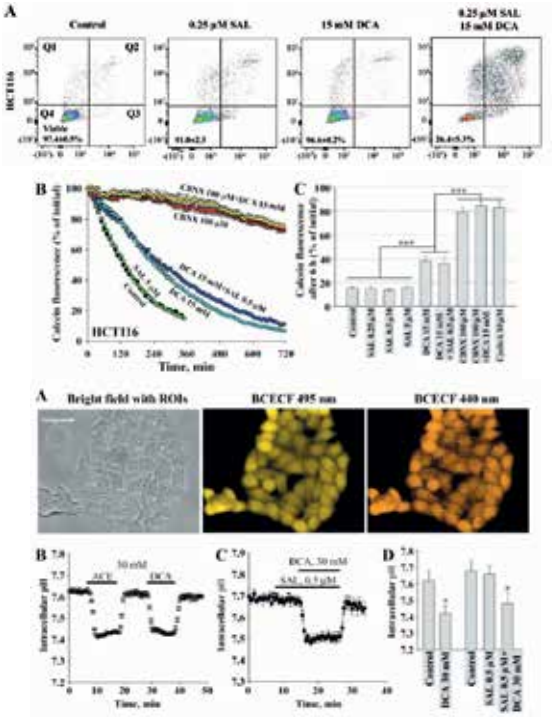
In the last decade, understanding of molecular mechanisms played a major role in castration-resistant prostate cancer (CRPC) treatment, and new androgen receptor (AR)- targeted drugs such as abiraterone acetate (AA) were introduced. Despite the progress, around 25% of CRPC patients show primary resistance to therapy, and AR signalling axis remains the core of CRPC development. One of the mechanisms might be the expression of alternative AR variants (AR-Vs) that lack ligand-binding domain and, therefore, are constitutively active. In our study, AR-V1, -V3, and -V7 were analysed in blood of AA-untreated CRPC patients. In 82% of blood samples, at least one AR-V type was detected. The lowest abundance of full-length AR (AR-FL) and AR-Vs was detected in blood samples of patients with long response to treatment, while marked difference in the amount of AR-V1 was revealed in the blood of cases with

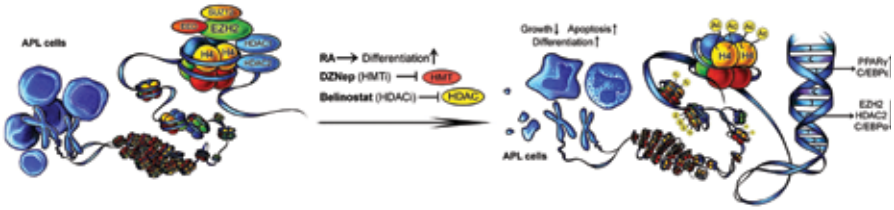


medium or short response to AA. Kaplan-Meier analysis confirmed that progression-free survival was significantly shorter of those patients who had high levels of either AR-V1/AR-V7 or AR-FL/AR-V1.

Dichloroacetate and Salinomycin Exert a Synergistic Cytotoxic Effect in Colorectal Cancer Cell Lines

In the present study, we examined a hypothesis that dichloroacetate, a metabolic inhibitor, might efficiently potentiate the cytotoxic effect of salinomycin, an antibiotic ionophore, on two human colorectal cancer derived cell lines DLD-1 and HCT116. First, we performed a series of dose response experiments in the 2D cell culture by applying mono- and combination therapy and by using the Chou-Talalay method found that salinomycin in combination with dichloroacetate acted synergistically in both cell lines. The compounds were also tested in the 3D multicellular spheroid culture. The effect of combination of dichloracetate and salinomycin on multicellular spheroid size was stronger than the sum of both monotherapies, particularly in HCT116 cells. Further, we demonstrate that the synergistic effect of compounds may be related to the inhibitory effect of dichloroacetate on multidrug resistance proteins, and, in contrast, it is not related to dichloroacetate-induced reduction of intracellular pH. Our findings indicate that the combination therapy of salinomycin and dichloroacetate could be an effective option for colorectal cancer treatment and provide the first mechanistic explanation of the synergistic action of these compounds.





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Molecular Mechanisms and the Treatment 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, 3) 1.3 years; 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 or pursue aggressive therapy. For many types of cancer, finding 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.

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

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Acute and Chronic Myeloid Leukaemia: Epigallocatechin-3-gallate and BIX-01294 Have Different Impact on Epigenetics and Senescence Modulation

We used the NB4 cell line, which possesses the characteristic APL chromosomal translocation t(15;17) and CML cell line K562, which has Philadelphia chromosome. We examined the potential of EGCG and BIX-01294 to cause epigenetic changes and cellular senescence in both APL and CML cells. EGCG and BIX-01294 impair myeloid leukaemia cell proliferation and survival: APL cells show higher sensitivity than CML cells. Moreover, acute promyelocytic leukaemia NB4 cells undergo apoptosis, whereas chronic myeloid leukaemia K562 cells remain apoptosis-resistant. We suggest that epigenetic modifier EGCG as cellular senescence inducing agent could be important for myeloid leukaemia therapy with an advantageous epigenetic modulation on acute promyelocytic leukaemia cells. BIX-01294, although not inducing cellular senescence, could cause anti-cancerous epigenetic changes in both acute and chronic myeloid leukeamias. Indeed, both EGCG and BIX-01294 might be beneficial for the development of new myeloid leukaemia treatment strategies (Vitkeviciene et al. *Eur J Pharmacol*. 2018, 838: 32–-40).

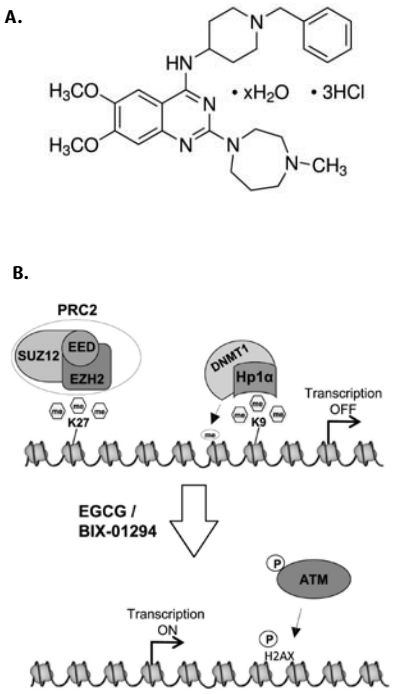
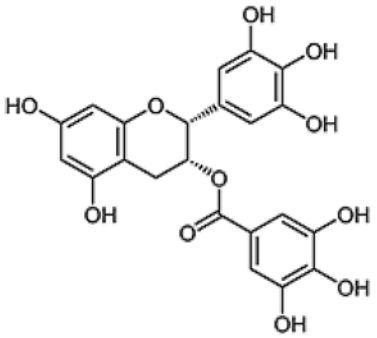


Fig. 1. (A) – structure of 2-(Hexahydro-4-methyl-1H-1,4-diazepin-1-yl)-6,7-dimethoxy-N-[1-(phenylmethyl)-4-piperidinyl]-4-quinazolinamine trihydrochloride hydrate, BIX 01294; (B) – EGCG and BIX-01294 modulate acute promyelocytic leukaemia cell chromatin: reduce levels of PRC2 complex proteins, of histone modification H3K9me3 and HP1α, DNMT1 proteins thus causing heterochromatin decondensation and tumour suppressor gene transcription activation. Also, both agents cause DNA damage response: induce ATM phosphorylation leading to histone H2AX phosphorylation

Acute Promyelocytic Leukaemia Could Be Treated with Natural Compound from Green Tea

This study was designed to determine EGCG effects on leukemic cell growth and viability as well as on gene expression. In our studies we used NB4 cell line with the characteristic reciprocal translocation t(15;17) and fusion protein PML-RARα. Our results showed the changes in the expression of genes and proteins associated with molecular mechanisms of inhibition of proliferation and chromatin remodelling. We observed C/EBPα and C/EBPε up-regulation in cells treated with EGCG. 40 μM EGCG treatment for 48 h up-regulated C/EBPε gene expression level even up to 8 times. In addition, ChIP experiments revealed that EGCG treatment enhanced the binding of H4 hyperacetylated histone and acetylated H3K14 histone to the promoter regions of C/EBPα and C/EBPε genes.

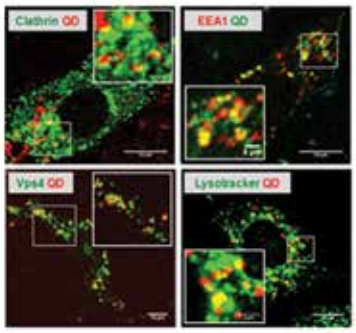
We determined down-regulated gene expression of these three PRC2 core components after the treatment with EGCG in NB4 cells. Protein expression analysis confirmed significantly decreased expression of EZH2 and SUZ12. Furthermore, ChIP experiments showed that EGCG treatment reduced hyperacetylated H4 and acetylated H3K14 binding effect on the promoter of PCR2 complex genes (EZH2, SUZ12, EED). It has been previously shown that EGCG

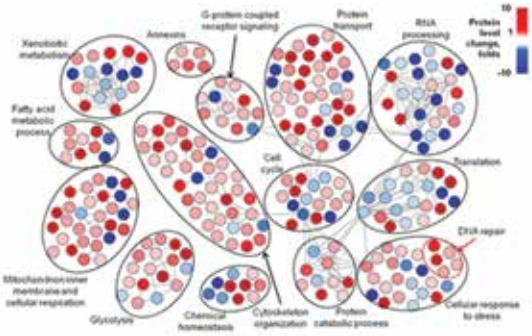


[(2R,3R)-5,7-dihydroxy-2-(3,4,5-trihydroxyphenyl) chroman-3-yl] 3,4,5-trihydroxybenzoate, Epigallocatechin-3-gallate, EGCG

treatment reduces Bmi-1 (main component of PRC1) and Ezh2 levels of skin tumour SCC-13 cells.

These findings are important for epigenetic therapy in the treatment of leukaemia (Borutinskaitė et al. *Leukemia and Lymphoma*. 2018, 59(2): 469–478).





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Molecular Mechanisms of Cancer Cell Chemoresistance

Neoplastic diseases are one of the major causes of death worldwide. An early 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 successful tumour therapy. However, chemotherapy often fails due to the ability of the tumour cells to adjust to the therapy and to become even more malignant. There are several strategies for overcoming these problems. First, one must understand the fundamental mechanisms of cancer genesis, target the crippled processes with specific agents and/or deliver drugs specifically to cancer cells to avoid unwanted side effects. Second, to deal with the constantly rising drug resistance, it is necessary to choose and individually apply 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 search for early diagnostic protein markers as well as markers for successful treatment; III) to create drug delivery systems based on nanomaterials and direct targeting via cell surface proteins.

Recently, we have found that the surface properties of quantum dots play a crucial role in defining their cellular routes and biological activity. The data has shown for the first time that the covalent modification of these nanoparticles with a growth factor enables the visualization of cancer cells via their cell surface receptors. This suggests the potential application of quantum dots for cancer diagnostics and drug delivery based on their surface modifications as well as specific targets on the cell plasma membrane [1]. In collaboration with the V. P. Lehto Lab (Kuopio University Hospital, Kuopio, Finland), we have developed a drug carrier system based on mesoporous inorganic nanoparticles. Dual PEGylation has dramatically improved the stability of these particles due to their avoidance of clearance by accumulation in the spleen. A proteomic analysis has shown that PEGylated nanoparticles have different corona protein formations, which allows them to escape macrophage phagocytosis. Since these particles can also be magnetically modified, they can be applied for targeting cancer cells and their visualization by magnetic resonance imaging [2].

We have investigated the mechanism of cytotoxicity of a novel anticancer drug RH1 and acquired resistance to RH1 in liver and breast carcinomas. High throughput proteomic analysis with subsequent extensive bioinformatics predicted the potential molecular mechanism of RH1 [3]. Computer modelling revealed that this drug targets protein kinases [4].These data provide basis for the search of RH1-dependent resistance biomarkers and also predict targets for second-line therapy for the drug-resistant cells.

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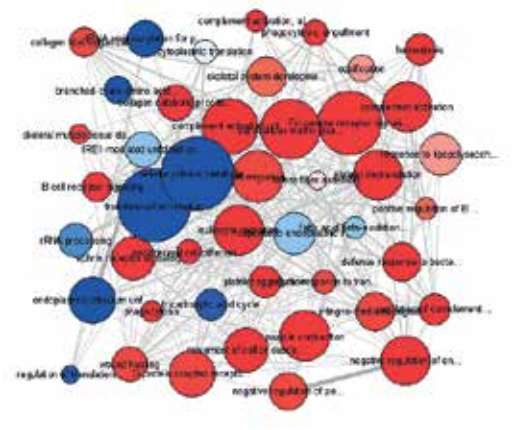
Novel Prognostic Markers for Pancreatic Cancer

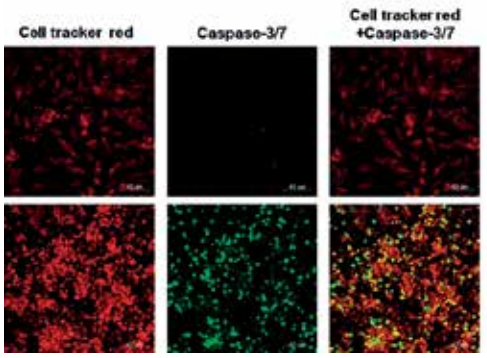
Pancreatic ductal adenocarcinoma (PDAC) is one of the deadliest types of cancer, particularly due to its aggressive course and challenging diagnostics in the early stage of the disease. Moreover, some of candidate biomarkers lack specificity in distinguishing PDAC from the chronic inflammation of the pancreas (pancreatitis). The aim of this study was to discover new potential prognostic and diagnostic pancreatic cancer biomarkers. We compared 37 samples from pancreatic cancer, inflammatory or healthy pancreatic tissue through in-depth differential proteomic analysis. PDAC-specific differentially expressed proteins enabled us to identify biological processes specific to pancreatic cancer but not pancreatitis. We also identified a set of candidate proteins for pancreatic cancer-specific diagnostic or prognostic biomarkers. Survival data of patients after two-year follow-up show the potential of FLT3 and PCBP3 proteins as biomarkers for favourable PDAC prognosis. The level of PCBP3 correlated with the tumour stage, and FLT3 level was evaluated as



an independent prognostic marker. Thus, FLT3 and PCBP3 represent potential tool for improved individualized pancreatic cancer prognosis (Ger et al. *Anticancer Research*. 2018, 38: 5759–5765, doi:10.21873/anticanres.12914).

Novel Approach to Predict Anticancer Drugs for Individualized Therapy

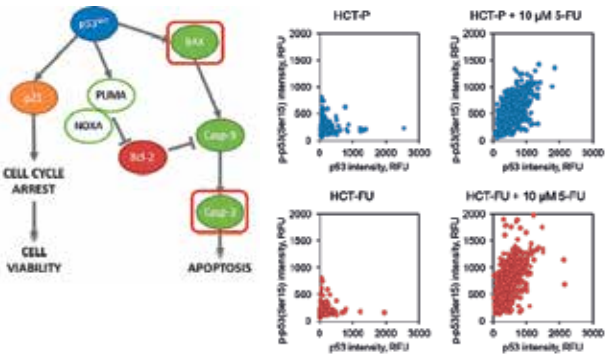
Low efficiency of current chemotherapeutic approaches and lack of molecular signatures for individualized treatment make pancreatic ductal adenocarcinoma (PDAC) one of the deadliest forms of cancer. We combined in-depth proteomic analysis of PDAC surgical specimens with drug testing in patient-derived primary cell culture to predict and validate potential chemotherapeutic agents for the PDAC treatment. Differentially expressed PDAC-specific proteins enabled us to identify a set of proteins specific to pancreatic cancer but not to chronic pancreatitis or healthy patients. Using various databases of gene expression combined with bioinformatic analysis, we generated a shortlist of chemotherapeutic compounds for the evaluation as potential PDAC drugs. The efficiency of the drugs was assayed using primary patient-derived PDAC cell cultures. Five of the six predicted compounds significantly slowed down the growth



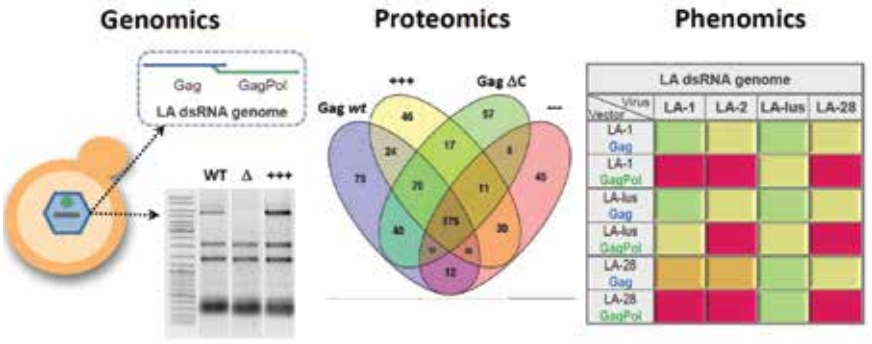
of the primary cells in PDAC cell cultures. Most of the drugs also inhibited to a various extent cell dissemination from spheroids and cell cycle. The results show promising potential of this integrative pipeline for anticancer drug discovery and evaluation (National science program's project No SEN-01/2016; Group leader: M. Valius).

Mechanisms of Acquired Chemotherapy Resistance in Colorectal Cancer

Colorectal cancer (CRC) is the third most common cancer in men and the second in women worldwide, affecting mainly people over 50 years old. Adjuvant combinational chemotherapy by 5-fluorouracil and oxaliplatin (FOLFOX) is the established second line treatment of the CRC. The potential efficiency of FOLFOX has to be evaluated before applying the treatment. However, although FOLFOX has been used for CRC treatment for more than a decade, no successful predictive biomarkers have been introduced into clinic. The combination of the latest proteomic, bioinformatic and cell analysis techniques enables the elucidation of FOLFOX-acquired resistance mechanism and establishment of the set of predictive FOLFOX resistance biomarkers. Introduced into clinic, such set of



biomarkers will provide successful personalized treatment of CRC. (National science program's project No SEN-17/2015; Group leader: M. Valius).



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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, involving advanced-level manipulations on the genomic material and proteome. 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 an 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.

Nucleoside and nucleotide-based antivirals constitute an essence of modern high-efficacy antiretroviral HIV treatment. While being 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, 3, 4], 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.

To create novel and 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 [1]. 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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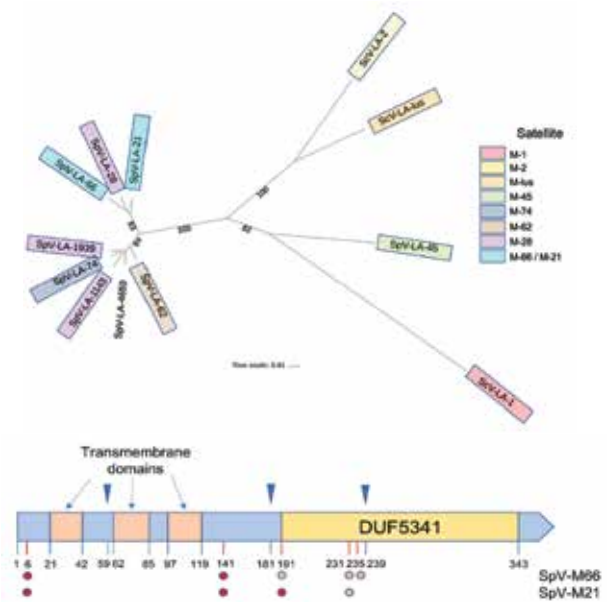
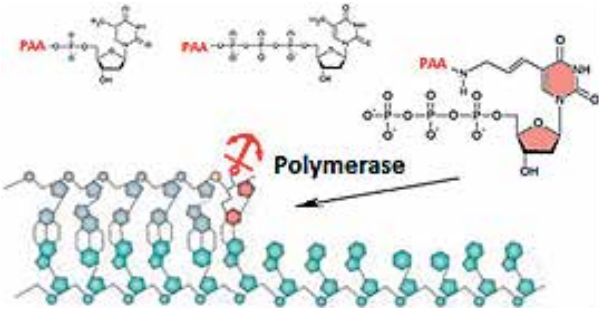
Novel Yeast Killer Systems

For the first time, we demonstrated the coherent habitation of different types of helper and satellite viruses in a wild-type *Saccharomyces paradoxus* strain. We isolated the *S. paradoxus* AML-15-66 killer strain from spontaneous fermentation of serviceberries and identified helper and satellite viruses of the family *Totiviridae*, which are responsible for the killing phenotype. The corresponding full dsRNA genomes of viruses have been cloned and sequenced. Sequence analysis of SpV-LA-66 identified it to be most similar to *S. paradoxus* LA-28 type viruses, while SpV-M66 was mostly similar to the SpV-M21 virus. A genetic screen performed on *S. cerevisiae* YKO library strains revealed 125 gene products important for the functioning of the *S. paradoxus* K66 toxin, with 85% of the discovered modulators shared with *S. cerevisiae* K2 or K1 toxins. Investigation of the K66 protein binding to cells and different polysaccharides implies the -1,6 glucans to be the primary receptors of *S. paradoxus* K66 toxin (Vepštaitė-Monstavičė et al. *Viruses*. 2018, Oct 16: 10(10).



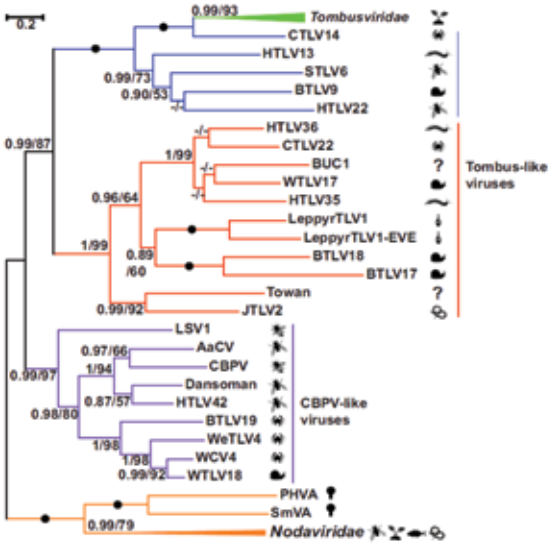
Mechanism-Based Antivirals

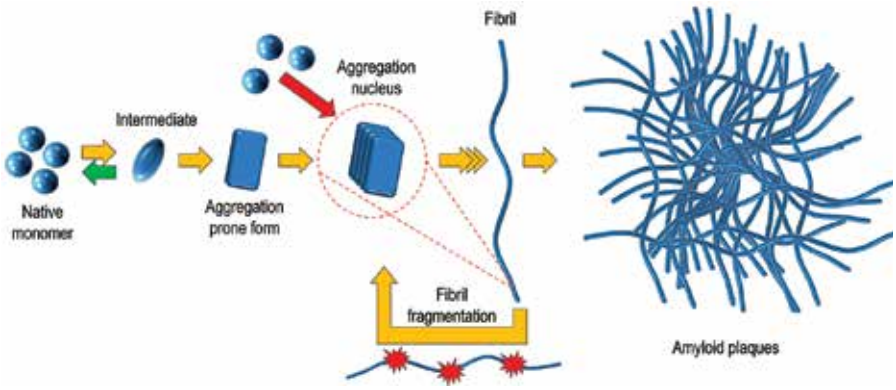
Small molecule inhibitors have a powerful blocking action on viral polymerases. The bioavailability of the inhibitor, nevertheless, often raises a significant selectivity constraint and may substantially limit the efficacy of therapy. Phosphonoacetic acid has long been known to possess a restricted potential to block DNA biosynthesis. In order to achieve a better affinity, this compound has been linked with a natural nucleotide at different positions. The structural context of the resulted conjugates has been found to be crucial for the acquisition by DNA polymerases. We show that a nucleobase-conjugated phosphonoacetic acid is being accepted, but this alters the processivity of DNA polymerases. The data presented here not only provide a mechanistic rationale for a switch in the mode of DNA synthesis but also highlights the nucleobase-targeted nucleotide functionalization as a route for enhancing the specificity of small molecule inhibitors (Mikalkėnas et al. *Journal of Enzyme Inhibition and Medicinal Chemistry*. 2018, 33(1): 384-389).



Multihost dsRNA Viruses

The knowledge of viral diversity is expanding greatly, but many lineages remain underexplored. RNA viruses in 52 cultured monoxenous relatives of the human parasite Leishmania (Crithidia and Leptomonas), as well as plant-infecting Phytomonas, were surveyed. Numerous relatives of trypanosomatid viruses were found in insect metatranscriptomic surveys, which likely arise from the trypanosomatid microbiota. Despite extensive sampling, we have found no relatives of the totivirus Leishmaniavirus (LRV1/2), implying that it was acquired at about the same time the Leishmania became able to parasitize vertebrates. As viruses were found in over a quarter of isolates tested, many more are likely to be found in the >600 unsurveyed trypanosomatid species. These data shed important insights on the emergence of viruses within a trypanosomatid clade relevant to human disease (Grybchuk et al. *Proc Natl Acad Sci U S A*. 2018 Jan 16, 115(3): E506-E515).





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Protein Misfolding and Aggregation

Protein misfolding and their 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 cataracts. There is increasing evidence on 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.

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 and thus proteinaceous infectivity.

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.

The highlight of 2018 is the study on how the salt affects the mechanism of insulin amyloid formation. We found out that the addition of sodium chloride shifts the equilibrium from monomers towards oligomers without affecting the secondary structure of neither native insulin nor its amyloid state. Initial analysis of the aggregation kinetics showed unusual dependence of aggregation half-times on the initial insulin concentration, suggesting the possibility of self-inhibition. Global fitting of the kinetic data revealed possible capping of fibril ends by insulin tetramers, leading to the inhibition of fibril elongation.

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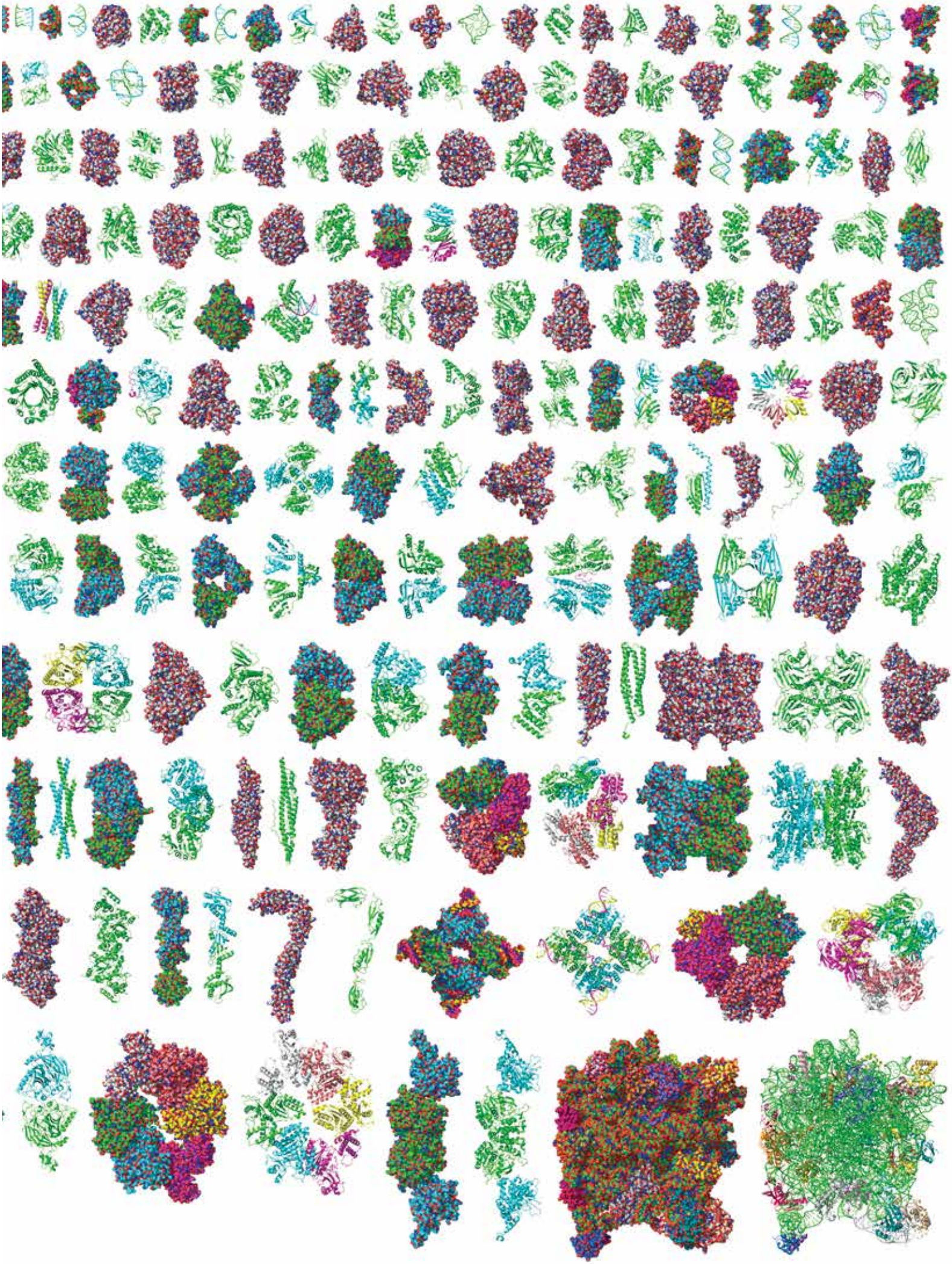

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Electrophysiological Brain States: Modulating Factors and Clinical Applications

Electrophysiological brain responses assessed non-invasively with electroencephalogram (EEG) stand as a widely used cost-effective tool to estimate brain functioning in norm and pathology. Depending on the recoding 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 we have evaluated the promise of electrophysiological resting state activity and auditory evoked brain responses to evaluate the state of the nervous system in various normal conditions (i.e. dependence on subject's sex and the subjective experiences during the experiment [1, 2, 4]) and pathological states (i.e. prolonged disorders of consciousness, schizophrenia and association to clinical symptoms [3, 5]).

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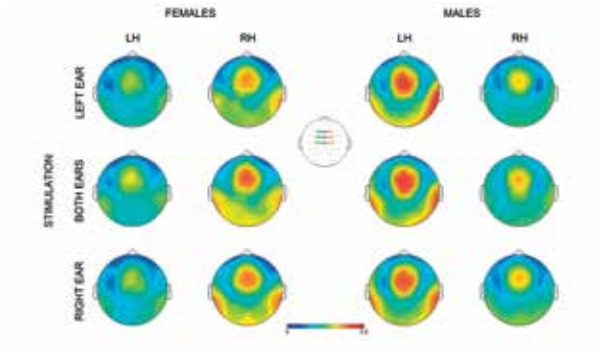
Handedness and Gender

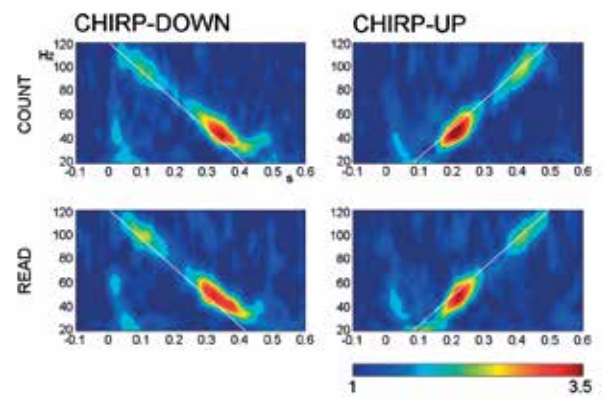
Electrophysiological studies of auditory steady-state response (ASSRs) are mainly dominated by right-handed participants, and the observed findings can only be generalized to the right-handed populations. However, for a potential use of 40 Hz ASSR as a translational biomarker of neuropsychiatric disorders, it is important to investigate the response in association to handedness and gender. We show that the processing of 40 Hz auditory stimulation depends on the subjects' gender and handedness: significantly lower phase-locking and strength of 40 Hz ASSRs were observed in the left-handed females as compared to the left-handed males, while right-handers did not differ in 40 Hz ASSRs. This finding is of particular importance for clinical studies in psychiatry and neurology (Melynyte et al. *Brain Topography*. 2018, 31(3): 419–429).



Phase-locking index of 40Hz ASSRs is higher in left-handed (LH) males as compared to left-handed females. There is no difference between genders in the right-handed (RH) group

Responses at Multiple Frequencies: Attentional Effect

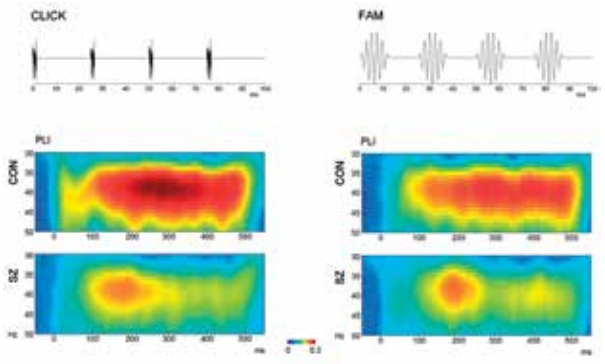
For the neurodiagnostic and in neurotechnological applications of auditory steady-state response (ASSRs) it is important to test responses at different frequencies. A low-frequency carrier tone modulated with 500 ms chirps at 1-120 Hz was tested to evoke the low and high gamma band activity. Results suggest that brief low-frequency tones modulated with chirps can be used to test responses at low and high gamma frequencies, and these responses are not modulated by attention. This makes chirp stimulation suitable for the use in populations with increased perceptual sensitivity to auditory stimuli and reduced ability to control attention, for instance patients with schizophrenia. (Pipinis et al. *Neuroscience Letters*. 2018, 678: 104–109).



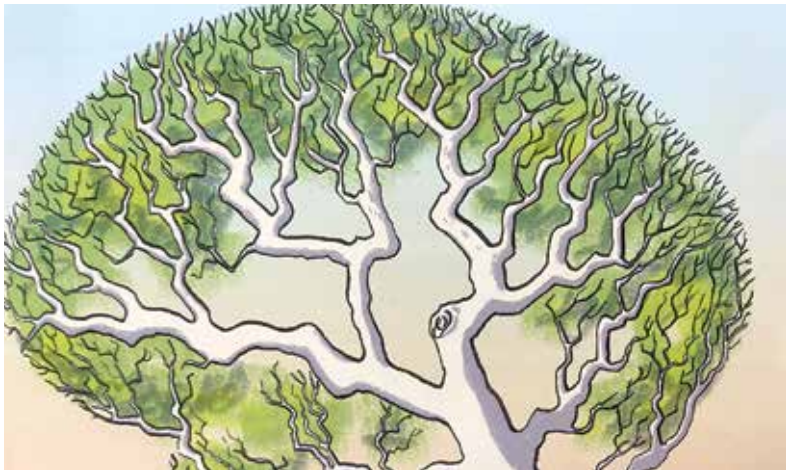
Phase-locking index in response to increasing and decreasing modulation rates of chip stimulations shows no signs of attentional modulation

Responses to Different Stimulation Types: Impairment in Schizophrenia

40Hz auditory steady-state response (ASSR) has been proposed as a potential biomarker for schizophrenia. However, the sensitivity of 40Hz ASSRs to different stimulation types in the same group of patients has not been previously evaluated. Two stimulation types for ASSRs were tested in this study: (1) 40Hz clicks (conventional way, unpleasant) and (2) flutter-amplitude modulated tones (novel stimulation type, pleasant). Responses to both stimulation types were impaired in patients compared to healthy controls, suggesting that FAMs can be used in clinics. Careful consideration and optimization of experimental stimulation settings can contribute to the interpretation of ASSR deficits and utilization as a potential biomarker (Griskova-Bulanova et al. *Neuroscience Letters*. 2018, 662: 152–157).



Phase-locking index (PLI) from healthy controls (CON) and schizophrenia (SZ) patients in response to two distinct stimulation types – clicks and flutter-amplitude modulated tones (FAM). Both responses are reduced in SZ



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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 circuit refinement. Appropriate synaptic pruning appears to be required for the strengthening of remaining synapses and is critical for normal brain development. In animal models, aberrations of synaptic pruning lead to impaired brain circuit maturation and dysfunctional connectivity. In human brain imaging and post-mortem studies, the reduction of brain volume and the reduced density of dendritic spines in schizophrenia is suggestive of over-pruning, whereas an increased brain volume and dendritic spine densities may indicate under-pruning in autism. For a long time, synaptic pruning has been seen as a neuron-autonomous process. However, recent studies have revealed that unnecessary synapses may be phagocytosed by resident immune cells – microglia, but no neuronal molecule has been identified that allows to discriminate between strong synapses that need to be maintained and weak synapses that need to be removed. We aim to define the molecular signalling pathways that drive this highly specific pruning of unnecessary synapses. Our investigations are supported by the European Union's Horizon 2020 Research and Innovation Programme under the Marie Skłodowska-Curie grant agreement No. 705452 (2018–2021) and the International Brain Research Organization Return Home Fellowship (2016–2018).

For microglia to discriminate between subsets of synapses that need to be removed or maintained, there must be molecular signal(s) exposed on the surface of the synapse to trigger or inhibit microglial recognition and engulfment. Therefore, we focus is on the molecular profile of removable synapses in a developing mouse hippocampus. 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 studies (in collaboration with Prof. D. Ragozzino, Sapienza Università di Roma, Italy), functional brain imaging (in collaboration with Dr. A. Gozzi, Istituto Italiano di Tecnologia, Italy) 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.

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State-of-the-Art Imaging of Microglia-Synapse Interactions

Capturing interactions between microglia and synapses during synaptic pruning requires advanced imaging techniques. Recently, together with our collaborators, we have employed light sheet fluorescent microscopy and correlative light and electron microscopy to visualize, for the first time, how the microglia "nibble" synapses and promote their plasticity through the growth of spine. We have demonstrated highly dynamic microglia-synapse interactions on both microglial and neuronal side. We have defined that microglia target only pre-synaptic structures for synaptic phagocytosis, a finding that we are currently further corroborating with molecular methods. In addition, we have shown that microglia induce filopodia on spine heads, which we propose as a mechanism of synaptic plasticity and the development of multisynaptic boutons. Our study provided the first evidence of synaptic remodelling by microglia at ultrastructural level.

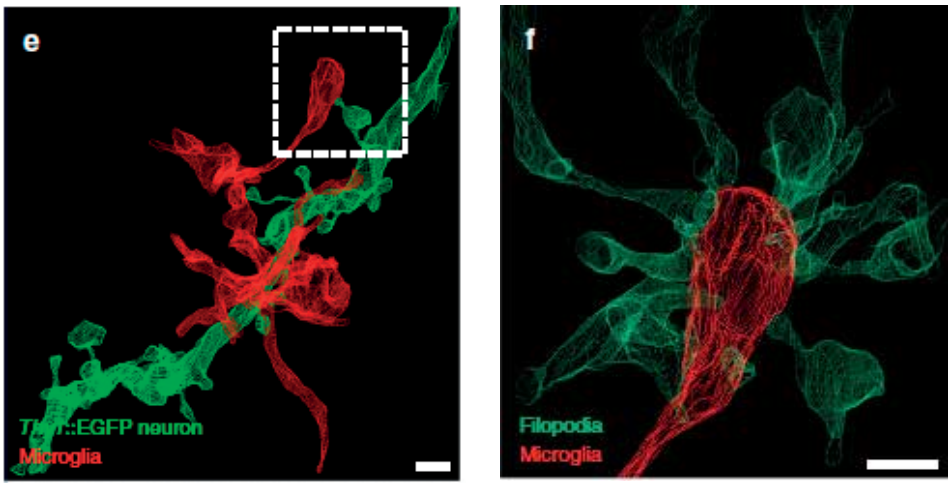

Fig. 1. CLEM reconstruction of microglia cell contacting dendritic spine, which extends multiple filopodia towards microglial process filopodia (Weinhard *et al. Nat Commun*, 2018)

Investigating Microglia in a Developing Brain

Brain macrophages microglia have a highly flexible morphology that is correlated with their functions during development, homeostasis and pathology. Individual microglial cells can cycle reversibly from an amoeboid to a ramified form, and this transition can be either very rapid or can be absent for years in a healthy mature brain. We demonstrated that in the developing hippocampus, microglia undergo waves of activation that are sex-specific and define the course of neural circuit development. We found a sex-dependent shift in microglia volume and phagocytic capacity across the first four postnatal weeks, suggesting a precocious development of both microglia and synapses in the female brain. We hypothesize that this bias may contribute to sex-specific brain wiring.

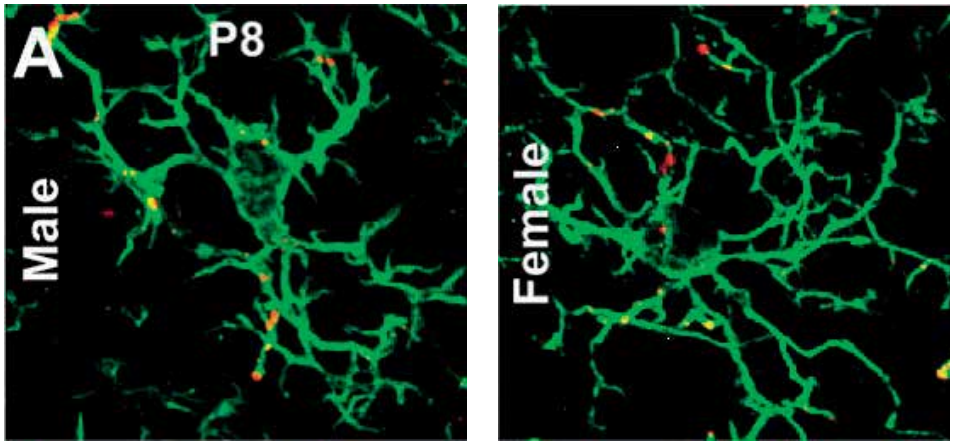

Fig. 2. During the first postnatal week female microglia are significantly more activated than male microglia (Weinhard *et al. Dev Neurobiol*, 2018)

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Neuroscience and Cellular Biophysics

The understanding of the functioning of the most complicated structure – the nervous system – in norm and pathology is one of the most challenging questions of modern science. We investigate mechanisms within the nervous system at different levels – starting with the electrophysiological properties of single neurons and excitable plant cells up to an investigation of the different brain states, the modulatory effects of sex steroids, the pathological mechanisms of depression, schizophrenia and various addictions. We employ various methods – EEG, fNIRS, fMRI, 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, 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 on electrophysiological data analyses' approaches resulted into 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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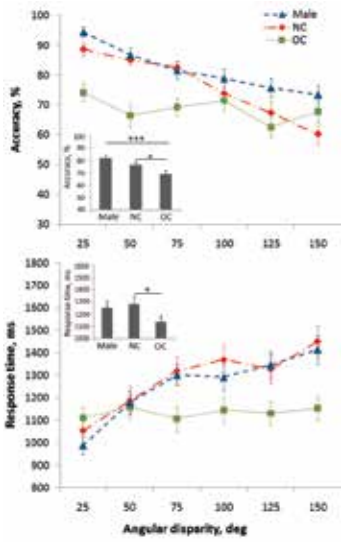
4. Cinciute, S.; Daktariunas, A.; Ruksenas, O. Hemodynamic effects of sex and handedness on the Wisconsin Card Sorting Test: the contradiction between neuroimaging and behavioural results. Peer J. 2018, doi 10.7717/peerj.5890.
5. Katauskis, P.; Ivanauskas, F.; Alaburda, A. The "memory" effect in a chain of biochemical reactions with a positive feedback is enhanced by substrate saturation described by Michaelis-Menten Kinetics. Bull Math Biol. 2018, doi.org/10.1007/s11538-018-00541-5.

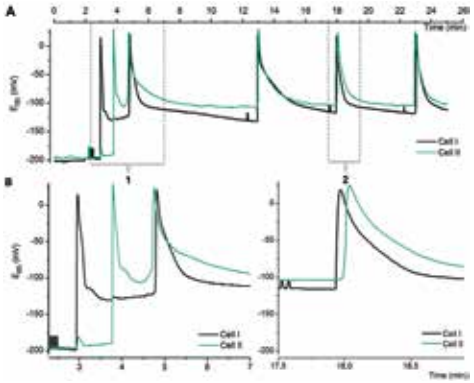
Does the Use of Hormonal Contraceptives Affect the Mental Rotation Performance?

In the present study, mental rotation performance was compared between women using anti-androgenic oral contraceptives (n=35), naturally cycling (NC) women (n=33) and men (n=29). On average, OC users were less accurate than NC women and men. Men performed the task more accurately than NC women, but the difference reached significance only in the highest angular disparity condition (150 deg). The response time was positively related with progesterone level while accuracy was negatively related with 17ß-estradiol level, in NC, but not OC women. We propose that OC users implemented some alternative method(s) instead of using rotation in mind strategy and this defined lower performance accuracy. (Griksiene et al. *Hormones and Behaviour*. 2018, 100: 29–38).



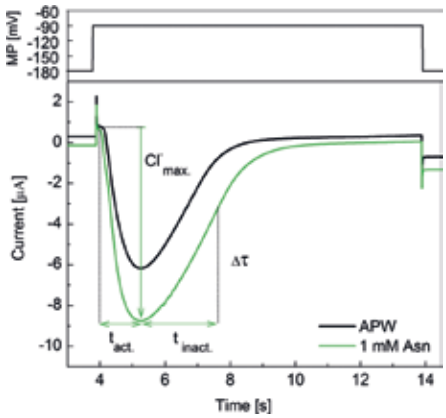
Electrical Signalling in *Nitellopsis obtusa*: Potential Biomarkers of Biologically Active Compounds

Membrane potential alterations and action potential (AP) patterns in response to biologically active compounds (BC) were shown to represent the *Nitellopsis obtusa* cell state. Application of voltage clamp revealed that changes in AP peak value were caused not only by increment in averaged maximum amplitude of the Cl^- current, but in prolonged Cl^- channel opening time also. The cytoplasmic droplet can serve as a model system in which the effects of BC on single tonoplast ion channels can be studied by patch clamping. (Kisnierienė et al. *Funct Plant Biol*. 2018, 45(1—2): 132—142).



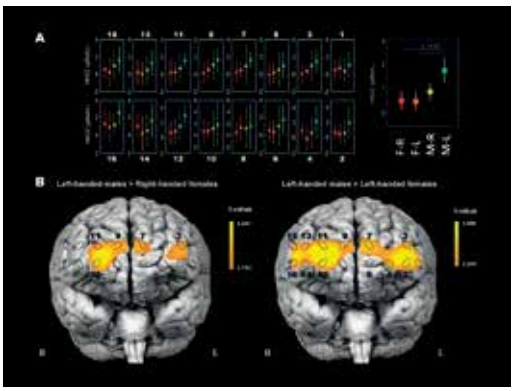
Asparagine Alters Action Potential Parameters in Single Plant Cell

Effect of amino acid L-asparagine on electrical signalling of single *Nitellopsis obtusa* (Characeaen) cell was investigated using glass-microelectrode technique in current-clamp and voltage-clamp modes. Cell exposure for 30 min to 0.1 mM and 1 mM of asparagine resulted in the changes of electrically stimulated action potential (AP) parameters in comparison to standard conditions. Results indicate that asparagine acts in a dose-dependent manner: increasing AP amplitude by hyperpolarizing AP threshold potential (Eth) prolongs action potential repolarization, increases maximum Cl^- efflux amplitude along with the increase of activation and inactivation durations. (Lapeikaite et al. *Protoplasma*. 2018, doi: 10.1007/s00709-018-1315-0).



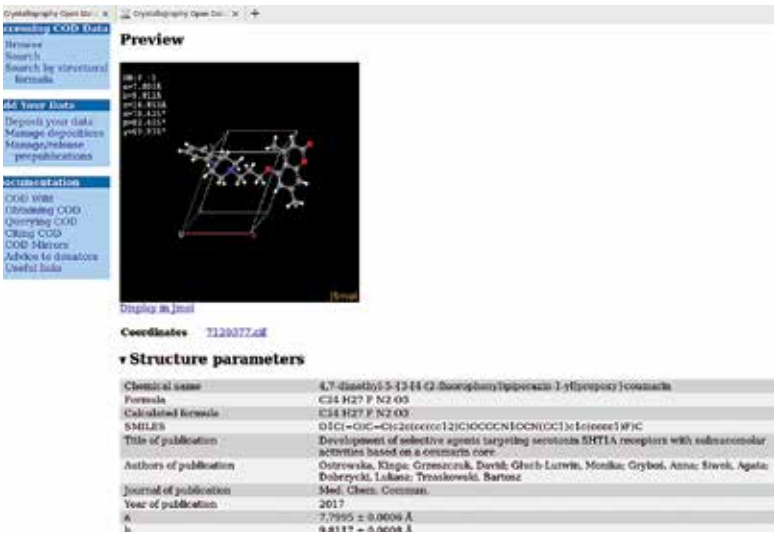
Impact of Intersubject Variability in the Neuroimaging Results through the Translation of Cerebrovascular Regulation

Functional magnetic resonance imaging and functional near-infrared spectroscopy, despite their data acquisition differences, are based on a common underlying phenomenon termed neurovascular coupling (NVC). As a part of a complex cerebrovascular regulation mechanism in the brain, NVC is still not entirely understood. Current research findings support the hypothesis that some contradiction between neuroimaging and behavioural results in sex-related cognitive task performance may be due not to different cognition, but rather caused by differences in cerebrovascular regulation as no supporting behavioural differences were found (Cinciute et al. *PeerJ*. 2018, 6: e5890, doi.org/10.7717/peerj.5890).



OPEN ACCESS CORE FACILITIES

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.

CONTACT INFORMATION:

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DNA Sequencing Centre

The DNA Sequencing Centre (SC), part of the Institute of Biotechnology (IBT) at the Life Sciences Centre of Vilnius University, has been successfully running since March 27, 2003. The SC was founded to help researchers, both at 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 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 the receiving of 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 (.ab1).

CONTACT INFORMATION:

EGLĖ RUDOKIENĖ, M. Sc.
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Phone: + 370 5 223 4370

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 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: the preclinical studies of novel drugs and chemical compounds, acute and repeated dose toxicity tests (oral, dermal, skin irritation, eye irritation, skin sensitization); immunization services etc. The facility provides qualified services to the scientific community of the Life Sciences Centre 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
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Proteomics and Imaging (Confocal Microscopy)

The Proteomics Centre is designated to perform high throughput, differential, quantitative proteome analyses and analyse protein localization and functions in fixed or live cells. The Centre 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) to discover and quantitate various covalent protein modifications; 4) to perform 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 chemotherapic resistance of colon cancer chemotherapy (in collaboration with A. Laurinavicius, 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:

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Chemical Synthesis of Organic Compounds for Industrial and Academic Purposes

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

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

- The development and optimization of technologies for the synthesis of chemical compounds;
- Testing of the scalability of chemical technology designed by the interested developers;
- The investigation of synthesis methods for organic compounds of different classes, the development and design of multi-step synthesis schemes;

A 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 scale equipment includes different volume glasses (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 type and volume 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), Bapeks Ltd. (Latvia), Thermofisher Scientific Baltics UAB (Lithuania), UAB Elymus, (Lithuania), UAB Certumtech (Lithuania), UAB Ekorama (Lithuania), UAB Vilniaus Ventos Puslaidininkiai (Lithuania).

CONTACT INFORMATION:

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X-Ray Diffractometry and Crystal Growth Equipment

Fig. 1. a) Robotic equipment for crystal growth and automatic crystal observations; a crystallization plate preparation robot (in the back).
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 tree-dimensional structures by means of single crystal X-ray crystallography techniques. The current diffractometer (Fig. 1b) comprises the Rigaku MM-007HF rotating anode microfocus generator with a Cu anode, VariMax focusing mirrors and two detectors: the Raxis-IV++ Image Plate detector (for protein crystallography) and the Pilatus 200k direct-conversion detector with a kappa stage (suitable for both small molecule and protein crystals). The Cu K_{α} radiation used in experiments is suitable for most organic crystals with light elements, and it allows to determine 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 are available. Help with data processing and structure solution is offered as well, if necessary.

CONTACT INFORMATION:

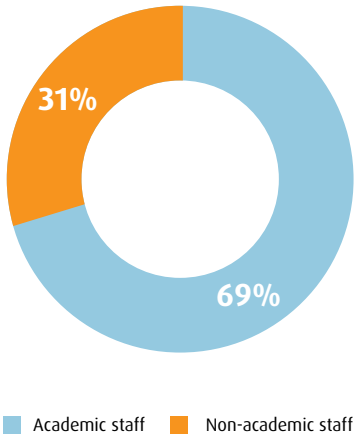
SAULIUS GRAŽULIS
Email: grazulis@ibt.lt
Phone: +370 5 223 4353

FACTS & FIGURES

Staff and Students

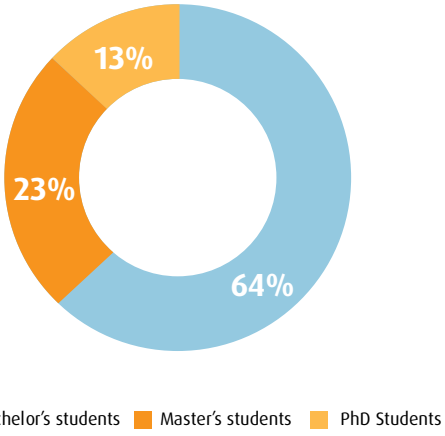
Staff

Academic staff	242
Non-academic staff	111
Total staff	**353**



Students

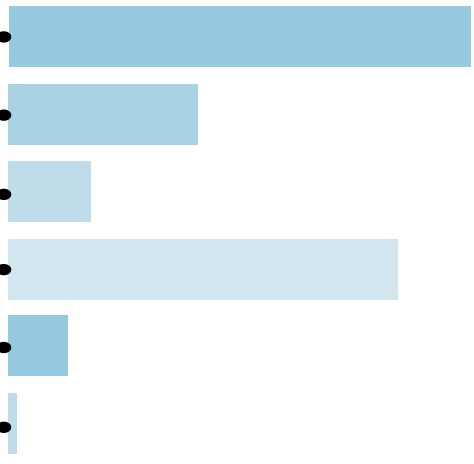
	2018/19
Bachelor's students	591
Master's students	209
PhD students	117
Total students:	**917**



Financing Sources 2018

Total – 9,6 M EUR

State subsidy for research	3 779 498 EUR	39%
State subsidy for studies	1 525 167 EUR	16%
Income from contracts with industry	626 135 EUR	7%
National grants	3 172 597 EUR	33%
International grants	447 048 EUR	5%
Other	66 883 EUR	0,7%



International Advisory Board

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

HEINRICH LEONHARDT
Biozentrum
Ludwig Maximilians
University Munich, Germany

CHRIS LOWE
Cambridge Academy of
Therapeutic Sciences, UK

BARBARA MAZUR
Du Pont Pioneer, USA

ANDRES METSPALU
Estonian Genome Bank
Tartu University, Estonia

TOMMY NYLANDER
Lund University, Sweden

TAINA PIHLAJANIEMI
Oulu University, Finland

SILKE SCHUMACHER
EMBL, Germany

Inaugural International Advisory Board Meeting in 2018

International Grants

Horizon 2020

Title	*Lead scientist*	*Duration*
Single-cell temporal tracking of epigenetic DNA marks (EpiTrack) ERC-2016-ADG: 742654	S. Klimašauskas	2017–2023
High throughput screening of single-cells using droplet microfluidics (Cells-in-drops) MSCA-IF-2015-EF: 705791	L. Mažutis K. Sasnauskas	2016–2018
Eat me microglia: lipid scrambling as a signal for synaptic pruning. MSCA-IF-2015-EF: 705452	U. Neniškytė A. Alaburda	2016–2021
Industrial applications of marine enzymes: innovative screening and expression platforms to discover and use the functional protein diversity from the sea (INMARE) BG-04-2014 : 634486	R. Meškys	2015–2019
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 MSCA-ITN-2018: 813786	L. Mažutis	2018–2022

Lithuania–Latvia–Taiwan Cooperation Programme

Title	*Lead scientist*	*Duration*
Design of anticancer pharmaceutical compounds using structure and energetics of lead – target interaction. No.TAP LLT-1/2015	D. Matulis	2016–2018
Understanding prion peptide fibril-induced aggregation of prion protein. No. TAP LLT-01/2017	V. Smirnovas	2017–2019
Studying of human parvovirus B19, bocavirus and parvovirus 4 involvement in inflammatory neurological diseases using interdisciplinary approach. No. TAP LLT-3/2017	R. Petraitytė-Burneikienė	2017–2019

Lithuania–Japan Research Programme

Title	*Lead scientist*	*Duration*
Research on prediction of environmental change in the Baltic Sea based on comprehensive (meta)genomic analysis of microbial viruses. No. LJB-17-001	G. Gasiūnas E. Šimoliūnas	2017–2019

Lithuania–Poland Cooperation Programme "Daina"

Title	*Lead scientist*	*Duration*
Long-distance electrical signaling systems in plants – adaptation to the change from water to terrestrial environment. No. P-LL-18-47	V. Kisnerienė	2018–2021
CRISPR tools for the study of embryonic development in zebrafish. S-LL-18-7	G. Tamulaitis	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

Other International Collaboration Projects

Title	*Lead scientist*	*Duration*
New EEG clustering methods for pre-clinical and clinical applications" No. 15309442-K in collaboration with Valparaiso University. Funded by Chilean funding agency Comisión Nacional de Investigación Científica y Tecnológica (CONICYT).	I. Griškova-Bulanova	2018–2019
Csm effector complex labelling for single molecule FRET experiments, APP-3-2016. Open Partnership research programme	G. Tamulaitis	2015–2018

COST

Title		*Duration*
Development of a European network for preclinical testing of interventions in mouse models of age and age-related diseases (MouseAGE). No. BM1402	R. Navakauskienė V. Borutinskaitė	2014–2018
Non-globular proteins: from sequence to structure, function and application in molecular physiopathology. No. B1405	V. Smirnovas G. Valinčius	2014–2019
Between atom and cell: integrating molecular biophysics approaches for biology and healthcare (MOBIEU). No. CA15126	D. Matulis A. Zubrienė	2015–2020
Multi-target paradigm for innovative ligand identification in the drug discovery process (Mu TaLig). No. CA15135	A. Zubrienė L. Baranauskienė	2015–2020
European network of multidisciplinary research and translation of autophagy knowledge (TRANSAUTOPHAGY). No. CA15138	V. Borutinskaitė R. Navakauskienė	2015–2020
Personalized nutrition in aging society: redox control of major age-related diseases. No. CA16112	V. Smirnovas L. Baranauskienė	2016–2021
In vitro 3-D total cell guidance and fitness. No. CA16119	D. Baltriukienė V. Bukelskienė	2016–2021
New exploratory phase in research on east European cultures of dissent. No. CA16213	V. Vaitkevičius I. Kelpšienė	2017–2021
Targeted chemotherapy towards diseases caused by endoparasites. No. CM1307	J. Šarlauskas N. Čėnas	2013–2018
Epigenetic Chemical Biology. No. CM1406	S. Jarmalaitė S. Klimašauskas	2014–2019
Challenging organic syntheses inspired by nature: from natural products chemistry to drug discovery. No. CM1407	D. Matulis D. Tauraitė	2014–2019

Graduate School

The aim of the Graduate School of the Life Sciences Centre is to promote innovative graduate education in the life sciences at Vilnius University. Graduate School offers centralized services for both PhD students and PhD Committees enabling individual PhD programs to focus on the education of the graduate students within the respective research fields such as biology, biophysics, ecology and environmental sciences, zoology, biochemistry and biotechnology.

The Graduate School focuses on increasing the visibility and attractiveness of PhD programs world-wide in order to reach those undergraduates, who consider doing a PhD in the life sciences.

For more detailed information, please refer to
http://www.gmc.vu.lt/en/graduate-school
Admission contact: gmc@gmc.vu.lt

Doctoral Theses

Name	*Title*	*Supervisor*
A. Aučynaitė	Metabolic Enzymes of Modified Nucleotides	J. Urbonavičius
M. Baranauskas	Associations of Brain Electrical Activity with Heart Rate Regulation and Body Awareness	I. Griškova-Bulanova R. Stanikūnas
S. Baronaitė	The Studies of Amniotic Fluid and Amniotic Fluid Stem Cells	R. Navakauskienė
R. Grigonis	Response Properties of Motoneurons during Spinal Neural Network Activity	A. Alaburda
R. Jarašienė-Burinskaja	Molecular Mechanisms of Quinone and Polyphenol Induced Mouse Hepatoma Cell Death	N. Čėnas
D. Jurėnas	Activity, neutralization and regulation of Escherichia coli O157:H7 acetyltransferase toxin AtaT	L. Van Melderen A. Garcia-Pino E. Sužiedėlienė
A. Kaunietis	Identification, Heterologous Biosynthesis and Characterization of Novel Bacteriocins from Thermophilic Bacteria	L. Kalėdienė D. J. Čitavičius
M. Kazlauskienė	Target RNA-dependent Catalytic Activities in a type III-A CRISPR-Cas system	G. Tamulaitis
J. Kazokaitė	Investigation of Human Carbonic Anhydrase VI and IX Inhibitor Efficacy and Toxicity	D. Matulis
V. Kiseliovas	Development of Microfluidic Techniques for Cancer and Other Biological Research	L. Mažutis
A. Konovalovas	Molecular Determinants of *Totiviridae* family Viruses of *Saccharomyces sensu stricto* Clade	S. Serva
J. Lazutka	The Development and Evaluation of Schmallenberg Virus Detection Systems	K. Sasnauskas
A. Merkys	Extraction and Usage of Crystallographic Knowledge for Refinement and Validation of Molecular Models	S. Gražulis
A. Mlynska	The Role of Systemic and Local Immunity in Tumour Development and Response to Treatment	V. Pašukonienė
M. Norkienė	Optimization of Polyomavirus VP1 Protein Biosynthesis in *S. cerevisiae* Cells and Application of Recombinant Virus-like Particles for Serology	A. Gedvilaitė
A. Smirnov	Crystallographic Studies of Carbonic Anhydrase Isoforms and their Complexes with Inhibitors	D. Matulis
A. Stumbrytė	Human Papillomavirus and Polymorphism Involved in Tumorogenesis Effect on survival of Laryngeal and Lung Cancer Patients survival	Ž. Gudlevičienė
P. Toliušis	Structure and Function Correlations within the Atypical ATP-Dependent Restriction Endonuclease CglI	M. Zaremba
G. Valiulienė	Epigenetic Regulation and Leukaemia – Research of Novel Biological, Molecular and Therapeutic Aspects	R. Navakauskienė
E. Zagorskaitė	Recognition of Modified Cytosine by Methyl-Directed Restriction Endonucleases	G. Sasnauskas

International Study Programs

The VU Life Sciences Centre (LSC) offers 14 different bachelor's and master's study programs. For international students interested in studying life sciences, the LSC offers six international master's study programs.

Biochemistry

The LSC master's program 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 program, please, refer to https://www.vu.lt/en/studies/graduate-studies/56-studies/studies/4593-biochemistry
Academic contact: Prof. Edita Sužiedėlienė.
Email: edita.suziedeliene@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 program, please, refer to https://www.vu.lt/en/studies/graduate-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 program 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 program, please, refer to https://www.vu.lt/en/studies/graduate-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 program, please, refer to https://www.vu.lt/en/studies/graduate-studies/tuition-fees-graduate/51-aboutus/4596-molecular-biology
Academic contact: Prof. Edita Sužiedėlienė.
Email: edita.suziedeliene@gf.vu.lt
Admission contact: admissions@cr.vu.lt

Molecular Biotechnology

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

For more detailed information regarding the program, please, refer to https://www.vu.lt/en/studies/graduate-studies/tuition-fees-graduate/51-aboutus/4596-molecular-biology
Academic contact: Dr. Inga Matijošytė.
Email: inga.matijosyte@bti.vu.lt
Admission contact: admissions@cr.vu.lt

Neurobiology

The LSC master's program 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 program, please, refer to https://www.vu.lt/en/studies/graduate-studies/56-studies/studies/4598-neurobiology
Academic contact: prof. Osvaldas Rukšėnas.
Email: osvaldas.ruksenas@gf.vu.lt
Admission contact: admissions@cr.vu.lt

International Awards

The 2018 Kavli Prize In Nanoscience and a Gold Medal

The Norwegian Academy of Science and Letters granted its biennial Kavli Prize in Nanoscience for 2018 to three of CRISPR's pioneers – Virginijus Šikšnys at the Vilnius University Life Sciences Centre, Emmanuelle Charpentier at the Max Planck Institute for Infection Biology in Berlin, Germany, and Jennifer A. Doudna at the University of California, Berkeley, USA – "for the invention of CRISPR-Cas9, a precise nanotool for editing DNA, causing a revolution in biology, agriculture, and medicine."

Professor Šikšnys has made a major and sustained contribution to the understanding of the CRISPR-Cas systems. Professor Šikšnys was also awarded the Warren Alpert Prize and the Novozymes Prize.

The Kavli Prize is established in partnership between The Norwegian Academy of Science and Letters, The Kavli Foundation (United States), and The Norwegian Ministry of Education and Research. It recognizes scientific achievements in Astrophysics, Nanoscience and Neuroscience.

Virginijus Šikšnys, Jennifer Doudna, Emmanuelle Charpentier

Virginijus Šikšnys Received an Honorary Degree of the University of Bristol

On July 16, Prof. Virginijus Šikšnys of Vilnius University Life Sciences Centre (VU LSC) received a Doctor of Science accolade of the University of Bristol (JK).

Collaboration between Vilnius University and the University of Bristol continues through the Wellcome Trust and European Commission projects. Three young VU LSC researchers PhD Giedrius Sasnauskas, PhD Mindaugas Zaremba and PhD Tomas Šinkūnas did their internships in Prof. Stephen Halford's laboratory.

Bioinformatics from VU Life Sciences Centre are among the Best in the World

The team of bioinformaticians from VU Life Sciences Centre – Dr. Česlovas Venclovas, Dr. Justas Dapkūnas and Dr. Kliment Olechnovič – excelled in the Critical Assessment of Protein Structure Prediction (CASP) competition. CASP is a biennial worldwide experiment that objectively measures state-of-the-art in computational protein structure modelling and provides unbiased comparison of methods developed by different research groups. According to the independent CASP assessment, the Life Sciences Centre team achieved the best results in modelling protein complexes. In addition, their method for estimation of model accuracy (VoroMQA) was also among the best performing at CASP.

Justas Dapkūnas, Česlovas Venclovas, Kliment Olechnovič

Winners of Gold and Bronze Medals in International Genetically Engineered Machine (iGEM) Competition in 2018

In 2018, two teams of students from Vilnius University – one team comprised of undergraduate and post-graduate students – participated in the Synthetic Biology competition iGEM (International Genetically Engineered Machine) in Boston, MA, USA, and won gold and bronze medals.

The projects of both teams were ranked among the top five breakthrough projects in their age categories.

The overgraduate Vilnius-Lithuania iGEM team (leader – molecular biology student Laurynas Karpus) won a gold medal. This team has developed a completely new approach based on microfluidic technologies and DNA modifications, which allow characterization of enormous amount of biological components and their combinations quite efficiently. In order to protect the invention before the competition, students submitted a patent application.

The undergraduate team (leader – biochemistry student Justas Ritmejeris) developed the SynDrop – synthetic droplets for membrane protein research system and won a bronze medal, as well as was nominated for the Best Foundational Advance Project and Best Model. The SynDrop was designed using innovative microfluidic technologies and cell-free protein expression systems. This allowed utilizing liposomes as nanofactories for the synthesis and various modifications of membrane proteins. The undergraduate team submitted a patent application for this invention as well.

iGEM is the largest and most prestigious international Synthetic Biology competition, held in Boston, Massachusetts, USA, which attracts the best and brightest students from universities around the world, including such prestigious universities, as MIT, Harvard University, Oxford University, Imperial College, Yale University and others.

The Undergraduate Vilnius-Lithuania iGEM team: J. Ritmejeris, T. Venslovas, S. Jasiūnas, V. Brasas, A. Gaižauskaitė, L. Škiudaitė, I. Juškaitė, K. Vitkutė, G. Jakutis, U. Kapočiūtė, H. Yeliseyeva, K. Žukauskaitė, D. Vaitkus

The overgraduate Vilnius-Lithuania iGEM team: I. Mogila, I. Rokaitis, R. Meškys, J. Jakubovska, I. Maželis, L. Karpus

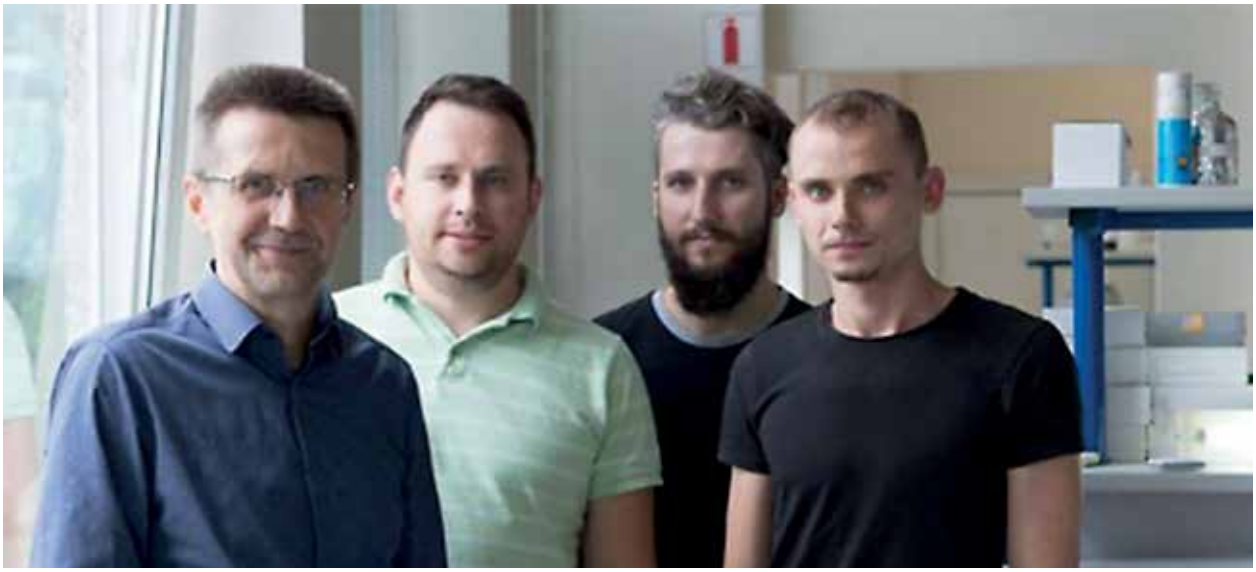
National Awards

The Lithuanian Science Award

At the break of the year, Prof. Saulius Klimašauskas was awarded the Lithuanian Science Prize for the development and applications of novel "Molecular tools for epigenomics and RNomics", which was a major research focus of his team during 2002–w2016. The Lithuanian Science Prize was also granted to Prof. Virginijus Šiksnys, Dr. Giedrius Gasiūnas, Dr. Tomas Šinkūnas and Dr. Tautvydas Karvelis for their "Investigation of CRISPR-Cas systems: from immunity of bacteria to the gene-editing technology". The research series includes their pioneering CRIPSR work performed from 2011 to 2016. These awards will inspire generations of young scientists and provide momentum for the development of scientific excellence at the LSC.

The Lithuanian Science Award is granted every year by the Research Council of Lithuania for research and development projects of national importance.

Prof. Dr. Saulius Klimašauskas



Prof. Dr. Virginijus Šikšnys, Dr. Giedrius Gasiūnas, Dr. Tomas Šinkūnas, Dr. Tautvydas Karvelis

The Global Lithuanian Leaders Award-2018

For bringing scientific innovations to Lithuania, Dr. Artūras Petronis, professor at the University of Toronto, has received the Global Lithuanian Leaders Award, which is given to Lithuanians and Lithuania-related individuals who have been contributing to the prosperity and global standing of Lithuania.

Prof. Dr. Artūras Petronis

Three Scientists Attained Distinguished Professor Status

Three prominent professors of the Life Sciences Centre attained the special status for their outstanding achievements: Saulius Klimašauskas, Rolandas Meškys and Virginijus Šikšnys.

The special status of a Distinguished Professor was approved by the Vilnius University Senate in 2017. This status is given to VU scientists who demonstrate outstanding, internationally and nationally recognized achievements, who help to improve a specific field of science or study areas and who develop their own "schools" in their research fields.

Prof. Dr. Saulius Klimašauskas

Prof. Dr. Rolandas Meškys

Prof. Dr. Virginijus Šikšnys

Scientific Events and Achievements

CRISPR 2018 – International Conference on Genome Editing Technology

International scientific conference *CRISPR 2018*, dedicated to the revolutionary genome editing technology, took place in Vilnius on June 20th. The conference was organized by the Vilnius University Life Sciences Centre professor Virginijus Šikšnys, North Carolina University professor Robert Barrangou, and Philippe Horarth, a scientist working in "DuPont".

More than 250 CRISPR researchers from 24 countries participated in the conference.

Dr. Feng Zhang from Massachusetts Institute of Technology (MIT) delivered the keynote speech. Scientists from Rockefeller, Cornell, Toronto, Zurich universities, University of California, Berkeley, and the Max Planck Institute in Berlin introduced their most recent studies concerning genome editing technology.

Discovery of the New Cell Type – Ionocyte

Vilnius University Life Sciences Centre PhD student is one of the new cell type discoverers

On the 1st of August 2018, the article "A single-cell atlas of the airway epithelium reveals the CFTR-rich pulmonary ionocyte" describing the discovery of a new cell type in the airway was published in the *Nature* journal. The first two authors of the publication are Dr. Lindsey Plasschaert and Rapolas Žilionis, PhD student at the VU Life Sciences Centre. Research was carried out in collaboration between Dr. A. Klein's laboratory at Harvard Medical School and Dr. Arono Jaffe's Laboratory at Novartis Institutes for BioMedical Research.

The discovery is not only important for a better understanding of respiratory biology, but is also of practical value for scientists investigating cystic fibrosis, still an incurable genetic disease.

Rapolas Žilionis

International Conference ProtStab

The 12th International Conference on Protein Stabilization (ProtStab2018) was held on the 16–18 May 2018 at the Life Sciences Centre. The ProStab2018 was organized by the Life Sciences Centre of Vilnius University (VU) and the Lithuanian Biotechnology Association (LBTA) with significant contribution of European Section of Applied Biocatalysis (ESAB) of the European Federation of Biotechnology (EFB) and communication partner *GO Vilnius*. The programme was designed to cover methodologies for increasing protein stability, enzymes from extremophiles and their applications, stabilization of enzymes for industrial processes. The participants from 15 countries attended the conference.

The COINS

The COINS is the 14th international conference of life sciences that has been organized by the students of the Life Sciences Centre. Annually organized event gathers not only students and scholars but various scientists working in the field of life sciences.

During the conference, participants discuss and share their scientific results, learn about scientific innovations and meet key experts in the fields of Biotechnology, Genetics, Biophysics, Biochemistry and Ecology. COINS opens up opportunities for bachelor and master degree-seeking students and doctorates of the Life Sciences Centre to present their research results and benefit from the experience of internationally-known researchers.

Two Nobel Prize winners – biophysicist Erwin Neher and the virologist Harald zur Hausen – gave lectures at the COINS 2018.

Thermo Fisher Scientific "Science Day"

The annual event "Science Day," organized by the Life Sciences Centre's industrial partner Thermo Fisher Scientific and the Life Sciences Centre, was held on October 10 at the Life Sciences Centre. Researchers and students from various Lithuanian research and study institutions had the opportunity to see a broad selection of products used in life sciences, discuss new technologies with representatives from Thermo Fisher as well as participate in seminars given by the LSC researchers.

Topical Meeting of the International Society of Electrochemistry

Life Sciences Centre hosted Topical meeting of International Society of Electrochemistry dedicated to bioelectrochemical systems. Among prominent speakers were C. Amatore, P. Anatassov, W. Schuman and many other world known scientists.

10th Conference of Lithuanian Neuroscience Association and 2nd International Symposium on Visual Physiology, Environment and Perception

On November 30–December 1, the10th conference of the Lithuanian Neuroscience Association (LNA) and 2nd international symposium on visual physiology, environment, and perception *VisPEP 2018* was organized by the Lithuanian Neuroscience Association and Vilnius University. The conference was dedicated to advanced topics in neurosciences, sensory systems, ranging from the visual system to interoception sense. It attracted over 200 neuroscientists from France, Switzerland, USA, UK, Russia, Israel, JAE, Lithuania, Latvia, and Estonia.

Community Events

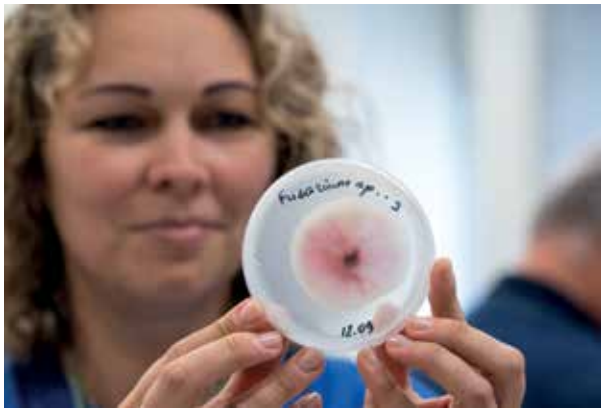
Celebrating the 100th Anniversary of Lithuania

Life Sciences Centre organized a public event "The Road of Sunrise" dedicated to the100th anniversary of restoring the independent state of Lithuania. This initiative united the community of research and study institutions, based in the Sunrise Valley, into a common chain that reminded of and resembled the Baltic Way of 1989, the symbol of unity, solidarity, freedom and love for Lithuania.

More than a thousand people, holding hands and carrying national attributes, stretched over the entire Saulėtekis avenue, from the Centre for Physical Sciences and Technology to Vilnius Gediminas Technical University.

International Microorganism Day

On the 17th of September, researchers of the Life Sciences Centre and members of the Lithuanian Microbiological Society together with partners from the National Public Health Surveillance Laboratory, the Nature Research Centre and the Lithuanian Centre of Non-formal Youth Education organized the First International Microorganism Day in Lithuania. This event aims at raising awareness among the society on the essential role of microorganisms in our everyday life, as well as their biotechnological potential. Interactive exhibitions, seminars were given by LSC scientists during this day. In the interactive exhibition area, there was an opportunity to see and to smell the microorganisms and their aromatic products, to acquire knowledge of cultivating different groups of microorganisms as well as of applying them in biotechnology, biomedicine and food industry. A special lecture was given about the structure of viruses and their research. More than a thousand participants of all age groups came to this event and had a possibility to discover and explore the world of microorganisms.



Mobile Bioclass

The Mobile Bioclass is a mobile laboratory in Lithuania. It aims at promoting biosciences among school children 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, which has been carried out since 2011. During 2018, the Mobile Bioclass visited over 100 schools in 70 cities of Lithuania.

National Science Festival "Spaceship Earth"

The national science festival "Spaceship Earth" is an annual festival organized in two biggest cities of Lithuania – Vilnius and Kaunas. Since 2004, it has become the most important and biggest cluster of popular science events and spread into more than ten cities. During a series of diverse hands-on activities (lectures, demonstrations, excursions, exhibitions, now annually exceeding 300) each September, more than 30 000 participants visit all the main universities, laboratories of the biggest technological companies, other innovative companies and museums.

Night of the Researchers

Life Sciences Centre researchers take an active part in the European research festival "European Night of the Researchers" – an annual scientific event held all over Europe aiming to increase awareness, especially among young people, about the role and impact of research. The LSC laboratories were opened during the day for the society on the occasion of this scientific event. The Museum of Zoology was one of the most attractive places for visitors of all ages.

European Biotech Week

In 2018, eighteen old continental countries joined the annual initiative European Biotech Week (http://www.biotechweek.org/). Lithuania is participating in the event for the sixth time, and the Life Sciences Centre celebrated this occasion by organizing several events. Lectures were delivered by two prominent scientists: the Nobel Prize winner from Stanford University (USA) prof. Brian K. Kobilka, as well as a famous Japanese biochemist, president of the Japanese Medical Research and Development Agency (AMED) prof. Makoto Suematsu. Educational programs were organized for schoolchildren from various schools, who visited the Life Sciences Centre, performed laboratory work in our laboratories and attended lectures given by our researchers. These activities have been coordinated by prof. Gervydas Dienys.

Invited Speakers

Speaker	*Institution*	*Title*
Brian K. Kobilka 2012 Nobel Prize in Chemistry	Stanford University, USA	Structural Insights into G-protein-coupled Receptor Activation
Erwin Neher 1991 Nobel Prize in Physiology or Medicine	The Max Plank Institute for Biophysical Chemistry, Germany	Characterization of Small Bovine Circular Single-stranded DNAs which are Infectious for Human Cells
Harald zur Hausen 2008 Nobel Prize in Physiology or Medicine	German Cancer Research Centre, Germany	The Search for Infectious Agents Linked to Human Cancer
Andreas Vilcinskas	LOEWE Centre for Insect Biotechnology & Bioresources, Germany	Insect Biotechnology
Artūras Petronis	Campbell Family Mental Health Research Institute, Canada	Epigenetics of Human Diseases
Charles H. Matthews	University of Cincinnati, USA	The Art & Science of Entrepreneurship: Past, Present, and Future
Ichizo Kobayashi	Kyorin University, Japan	DNA Modification and Restriction: Epigenomics and Base-excision Restriction Enzymes
Indrė Žliobaitė	University of Helsinki, Finland	Do the Species Get Older?
Makoto Suematsu	Japan Agency for Medical Research and Development, Japan	Deciphering Metabolism of Reactive Sulfur Species in Cancer by Surface-Enhanced Raman Scattering (SERS) Imaging
Manfred Roessle	Luebeck University of Applied Sciences, Germany	Investigations on Structure and Dynamics of Proteins by SAXS
Norman J. Maitland	The University of York, UK	Modelling Epigenetic Control of Stem Cell Fate and Differentiation Using Tissue-derived Human Prostate Epithelial Cells
Rugilė Stanytė	IMBA, Institute of Molecular Biotechnology, Austria	Spatiotemporal Dynamics of Sister Chromatid Resolution
Rotem Sorek	Weizmann Institute of Science, Israel	The Immune System of Bacteria: beyond CRISPR
Rytis Prekeris	University of Colorado, USA	How to Make Invadopodia: the Role of Polarized Membrane Transport and Cytoskeleton Dynamics during Cancer Cell Migration and Metastasis
Tatsuhiro Shibata	National Cancer Centre, Japan	Mutational Processes in Hepatobiliary Cancers

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Patents

GRANTED US PATENTS

1. New s-adenosyl-L methionine analogues with extended activated groups for transfer by methyltransferases (US 8822146 B2)
2. Process for the production of monoclonal antibodies using chimeric VLPS (US7919314 B2)
3. 5-Aryl-4(5-substituted 2,4-dihydraxyphenyl)-1,2,3-thiadiazoles as inhibitors of HSP90 chaperone and the intermediates for production thereof (US8314132 B2)
4. Conversion of alpha-hydroxyalkylated residues in biomolecules using methyltransferases (US8889352 B2)
5. Derivatization of biomolecules by covalent coupling of non-cofactor compounds using methyltransferases (US 8822146 B2)
6. Nucleic acid production and sequence analysis (US9347093 B2)
7. Fluorinated benzenesulfonamides as inhibitors of carbonic anhydrase (US9725467 B2)
8. RNA-directed DNA cleavage by the CAS9-CRRNA complex (US 9637739 B2)

GRANTED EU PATENTS

1. 5-Aryl-4(5-substituted 2,4-dihydraxyphenyl)-1,2,3-thiadiazoles as inhibitors of HSP90 chaperone and the intermediates for production thereof (EP2268626B1)
2. New s-adenosyl-L methionine analogues with extended activated groups for transfer by methyltransferases (EP1874790B1)
3. Benzimidazo [1,2-C][1,2,3] thiadiazol-7-sulfonamides as inhibitors of carbonic anhydrase and the intermediates for production thereof (EP2054420B1)
4. Derivatization of biomolecules by covalent coupling of non-cofactor compounds using methyltransferases (EP2414528B1)
5. Conversion of alpha-hydroxyalkylated residues in biomolecules using methyltransferases (EP2414527B1)
6. Nucleic acid production and sequence analysis (EP2776575B1)
7. Production of selenoproteins EP3019194 (B1)

PATENT APPLICATIONS

1. System and method for a biomimetic fluid processing (US20150336095A1; EP2941642A1; CA2896997A1; Nr. CN105308452A1)*
2. System and method for synthesis of DNA particles and use thereof (US20190002943A1)
3. Systems and methods for barcoding nucleic acids* US2018304222 (A1) US2015298091 (A1) US2018071705 (A1)
3. Fluorinated benzenesulfonamides as inhibitors of carbonic anhydrase (EP2914583A1)
4. Analysis of methylation sites (EP2594651A1)*
5. RNA-directed DNA cleavage by the Cas9-crRNA complex (US2018187195 (A1) CA2867849 (A1) EA201491728 (A1) EP2828386 (A1) HK1206392 (A1) US2015045546 (A1) US2015050699 (A1) US2015240261 (A1) US2015291961 (A1) WO2013142578 (A1)
6. Analysis of single-stranded RNA US2018251814 (A1) EP3271478 (A1) WO2016148556 (A1)
7. Selective inhibitors of carbonic anhydrase US2018222856 (A1) EP3328833 (A1) WO2017017505 (A1)

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

Key Performance Indicators	2018
Total number of patent applications	21
New patent applications filed in 2018	2
Total number of patents	15
US Patents Granted in 2018	1
EU Patents Granted in 2018	1
Licenses	11

Collaboration

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Aarhus University (Denmark)
Auckland University of Technology (New Zealand)
August Kirchenstein Institute of Microbiology and Virology (Latvia)
Australian Animal Health Laboratory - CSIRO (Australia)
Bangor University (UK)
Barcelona Supercomputing Center (Spain)
Bern University (Switzerland)
Bordeaux University (France)
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INTERNATIONAL INDUSTRY

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NATIONAL INDUSTRY

Amilina, Baltymas, Bioanalizės sistemos, Bioenergy LT, CasZyme, Imunodiagnostika, Naujoji Ringuva, Nomads, Pienas LT, Profarma, ThermoFisher Scientific Baltic, 3D Creative, Valentis.

COMPANIES FOUNDED BY LSC RESEARCHERS

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