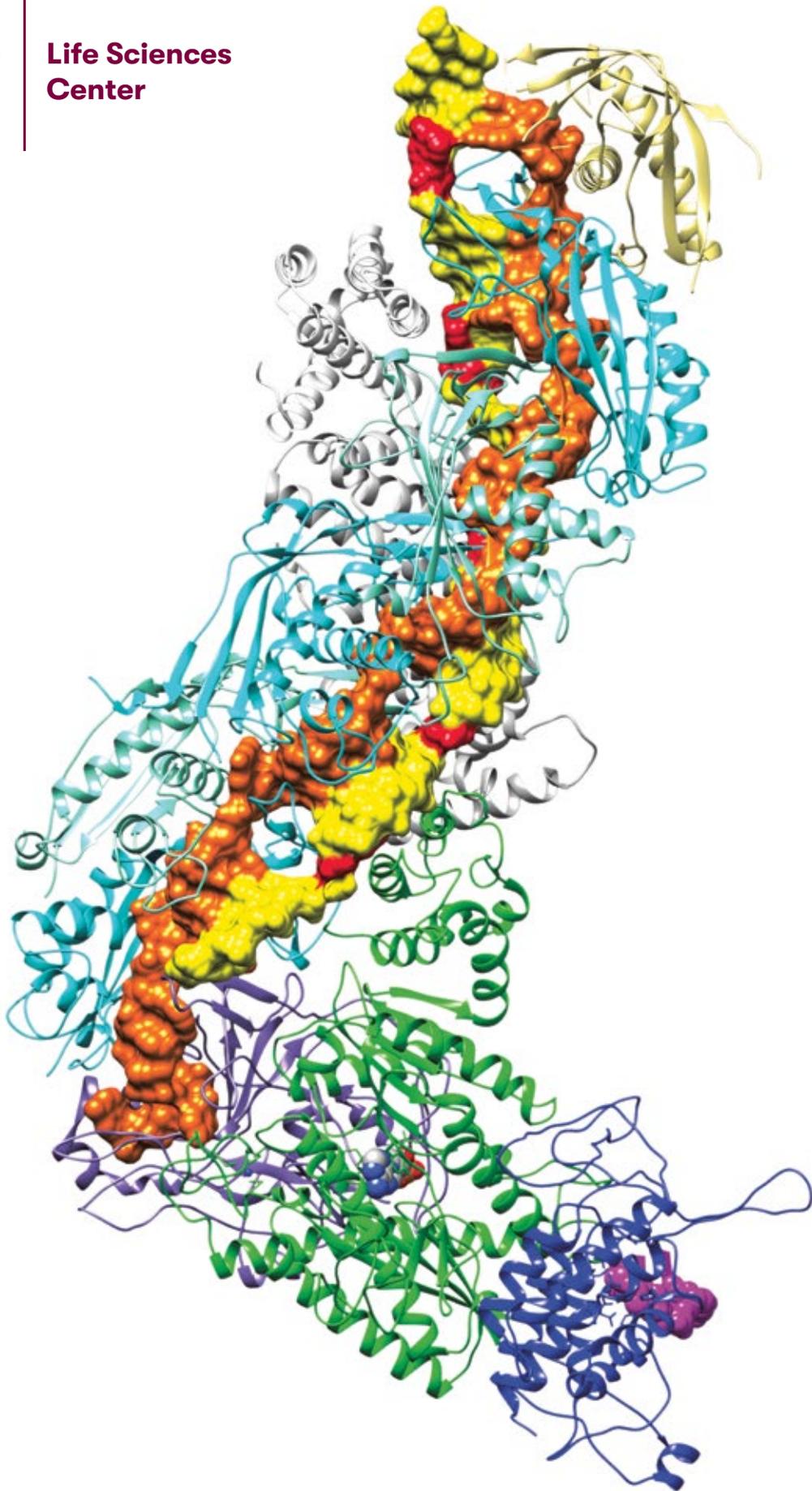




**Life Sciences
Center**



Annual Report 2017

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FOREWORD

The Life Sciences Center entered 2017 as a newly established academic unit of Vilnius University. For the Center, it was an election year. Our academic community elected the strategic management body – the Council of LSC, which includes 8 members of the research community, 3 student representatives, 2 social partners from businesses and 1 member from the support staff of the LSC. The Council elected a director of the LSC, who joined the Council as an ex officio member. Two new deputy directors stepped into their positions: Ingrida Prigodina-Lukošienė took over the organization and management of the studies, and Rokas Abraitis accepted the responsibilities for general operations at the LSC. During the year, the academic subunits of LSC – the Institute of Biochemistry, the Institute of Biosciences and the Institute of Biotechnology – also elected their directors, thus finalizing the reorganization process. LSC became the first academic unit that had fully implemented the management reorganization processes outlined in the 2015–2017 Strategic Plan of Vilnius University.

The year 2017 was exceptional in many aspects. For the first time, the European Research Council grant was awarded to a Lithuanian research group. This group is one of the leading teams at the LSC, led by Saulius Klimašauskas, who also stepped into the position of the Director of the Institute of Biotechnology this year. I am confident that Saulius's passion for science and academic success will inspire whole generations of young scientists and provide momentum for the development of scientific excellence at the LSC. Another prominent achievement was the publication of a research article in *Science* by the group led by Virginijus Šikšnys. The article was prepared solely on the experimental data obtained in our brand-new facility in Saulėtekis Valley, demonstrating both the brilliant talents of our researchers and the highest technological capabilities of our research infrastructure. It is worth mentioning that at the beginning of 2017, Virginijus was elected chairman of the LSC Council. I believe his election will promote excellence in scientific activity, modern studies and trigger discussions in our community about the strategic development plans of the Center.

A whole set of new initiatives were started in 2017, including the establishment of the Graduate school for Life Sciences, the



drafting of a major instrumentation upgrade of the CossyBio Project, the initiation of the Medical Epigenetics laboratory project, the drafting of brand new, student-centered, project-based Biotechnology study programs and the establishment of the International Advisory Board. I believe that we will continue working on these projects next year, so the goals set therein will be implemented in the nearest future.

The LSC was highly visible nationally and internationally in 2017. Research papers in top-tier international journals, new discoveries and technology developments at the LSC, exhibitions, vibrant seminars and talks at the LSC presented by prominent scientists, including Nobel laureates Rober Huber and Rober J. Shiller, marked an intellectually vibrant year and made Life Sciences one of the major news topics in the national media. A number of prestigious awards – which include the Lithuanian Science Prize, the L'Oréal Women in Science Prize, the VU Rectors Awards for Excellence in Science and Teaching, as well as a historic victory of the VU iGEM team in the Synthetic Biology contest in Boston and them bringing the Grand Prix of the contest home to the LSC – all these recognitions demonstrate the strength and dynamism of the Life Sciences community in Vilnius. 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.



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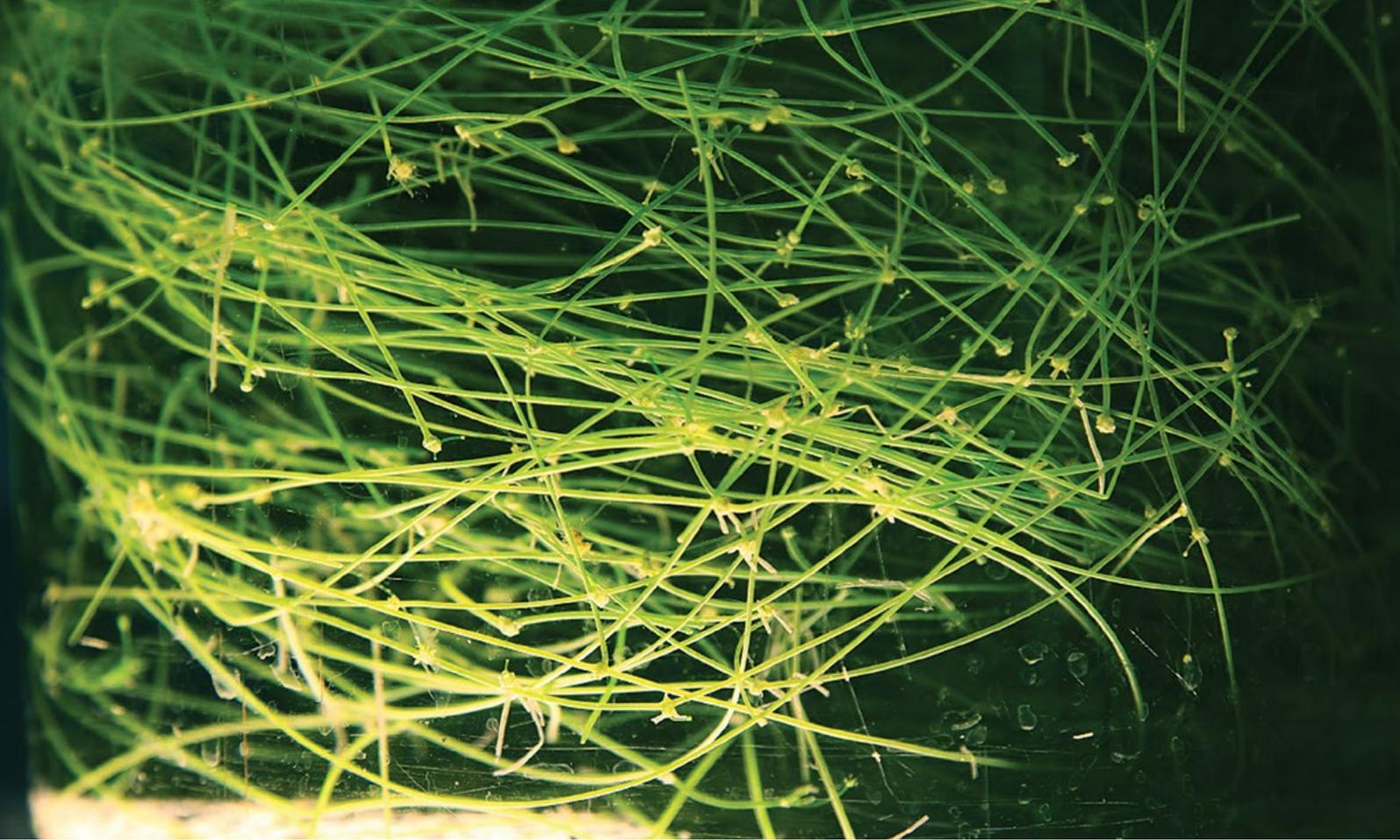
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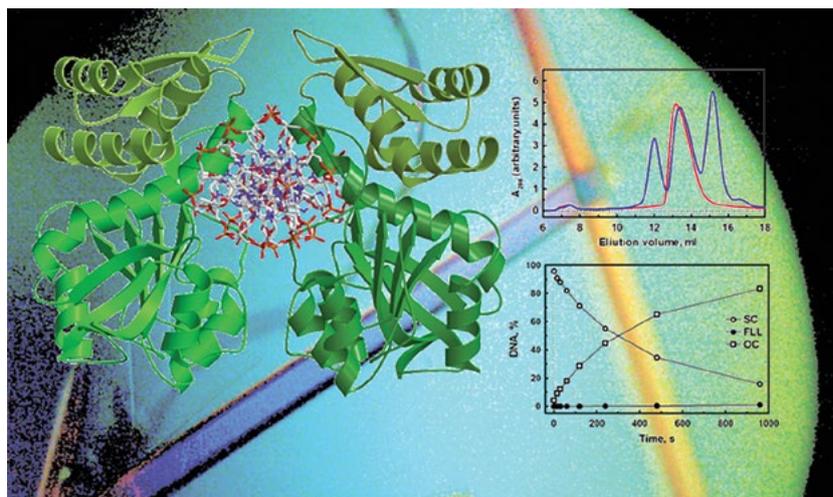
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Antiviral Defense Systems in Bacteria

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 defense 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 defense systems that target invading nucleic acids. We are in particular 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 defense line and act as sentries that guard bacterial cells against invasions by bacteriophages. R-M systems typically 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 recently discovered prokaryotic antiviral defense 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 the 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 synthesizes a novel signaling molecule in response to target RNA binding [3, 4]. To explore type IIIA CRISPR-Cas systems as molecular tools initiated *in vivo*, we've 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).

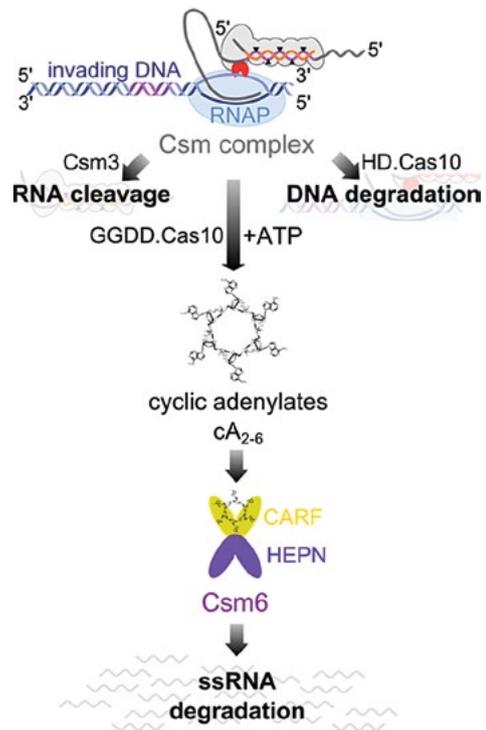
SELECTED PUBLICATIONS



1. Tamulaitis et al. Functional significance of protein assemblies predicted by the crystal structure of the restriction endonuclease BsaWI. *Nucleic Acids Research*. 2015, 43, 8100-8110.
2. Kostiuik et al. The dynamics of the monomeric restriction endonuclease BcnI during its interaction with DNA. *Nucleic Acids Research*. 2017, 45, 5968-5979.
3. Kazlauskienė et al. Spatiotemporal Control of Type III-A CRISPR-Cas Immunity: Coupling DNA Degradation with the Target RNA Recognition. *Molecular Cell*. 2016, 62, 295-306.
4. Kazlauskienė et al. A cyclic oligonucleotide signaling pathway in type III CRISPR-Cas systems. *Science*. 2017, 357, 605-609.

The Structural and Molecular Mechanisms of CRISPR-Cas Systems

Type IIIA CRISPR-Cas systems in prokaryotes provide immunity against invading nucleic acids through the coordinated degradation of transcriptionally active DNA and its transcripts by the Csm effector complex (Tamulaitis et al. *Trends Microbiol.* 2017, 25, 49-61). The Cas10 subunit of the complex contains an HD nuclease domain that is responsible for DNA degradation and two Palm domains with elusive functions. In addition, Csm6, a ribonuclease that is not part of the complex, is also required to provide full immunity. We show that target RNA binding by the Csm effector complex of *S. thermophilus* triggers Cas10 to synthesize cyclic oligoadenylates (cAn; $n = 2$ to 6) by means of the Palm domains. Acting as signaling molecules, cAn bind Csm6 to activate its nonspecific RNA degradation. This cAn-based signaling pathway coordinates the different components of CRISPR-Cas to prevent phage infection and propagation (Kazlauskienė et al. *Science.* 2017, 357, 605-609).

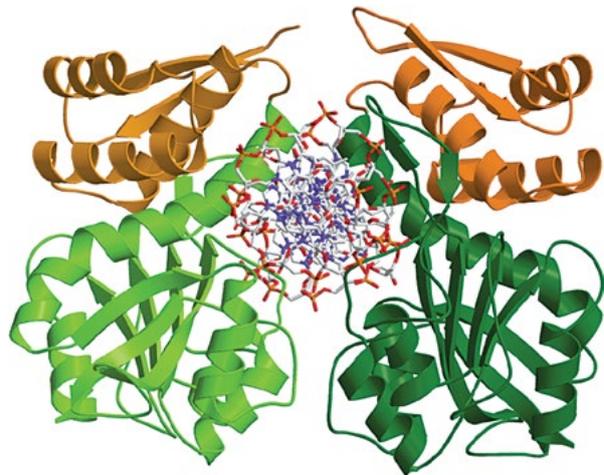


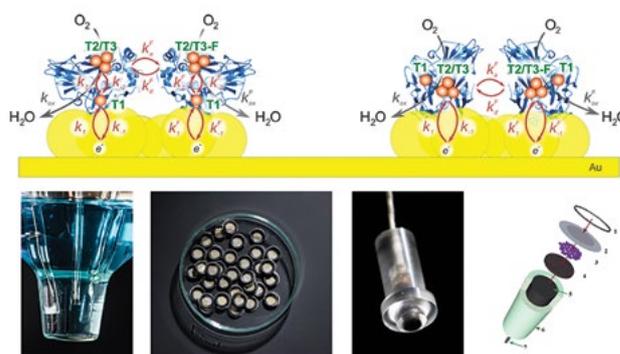
The Structure and Function of Restriction Endonucleases

We solved crystal structures of Type IIP REase AgeI (A/CCGGT) in apo- and DNA-bound forms and demonstrated a novel DNA cleavage mechanism for AgeI (Tamulaitienė et al. *Nucleic Acids Res.* 2017, 45, 3547-3558).

We used single-molecule techniques and bulk kinetic analysis to study the intricate dynamics of DNA interactions of the monomeric REase. We found that BcnI adopts either an “open” or “closed” conformation in solution. Next, we directly demonstrated that BcnI slides over long distances on DNA using 1D diffusion and showed that sliding is accompanied by occasional jumping events, where the enzyme leaves the DNA and rebinds immediately at a distant site (Kostiuk et al. *Nucleic Acids Res.* 2017, 45, 5968-5979).

We used a set of different methods to evaluate the DNA cleavage mechanism of ATP-dependent heterotetrameric REase CgII. Based on experimental data, we proposed that ATP hydrolysis by CgII triggers a translocation on DNA, preferentially in a downstream direction from the target, although upstream translocation is also possible (Toliusis et al. *Nucleic Acids Res.* 2017, 45, 8435-8447).





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Bioelectrochemical Systems and Application in Biosensors

A 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 the direct measurement of a single compound in the presence of interfering materials in complex media, such as blood. On the other hand, provided the power density generated by an enzyme-based electrode is high enough, biofuel cells can be constructed, where bioelectrodes selectively oxidize 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 the device. The shortcoming could be avoided whatsoever by adsorbing live, whole cells on electrodes at the expense of the reduced power density. Currently, the most efficient bioelectrocatalytic systems are based on direct ET, where an enzyme exchanges the electrons directly with the electrode surface without any 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 cores of which were based on either specially modified graphite or gold surfaces. For the most part, the biosensors were produced by an immobilization 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 the designing of prototypes of an analyzer, which was applied for the detection of urea in dialysate and other biologic liquids. As a future step in the whole-cell biosensors field, a self-organization of *E. coli* in nutrient-rich microtiter wells is analyzed and modelled [4]. On an international level, we collaborate with scientists from institutions such as Malmö University (Sweden), the Institute of Molecular Biology and Genetics of the National Academy of Sciences of Ukraine and the Moscow Kurchatov NBICS Center (Russia).

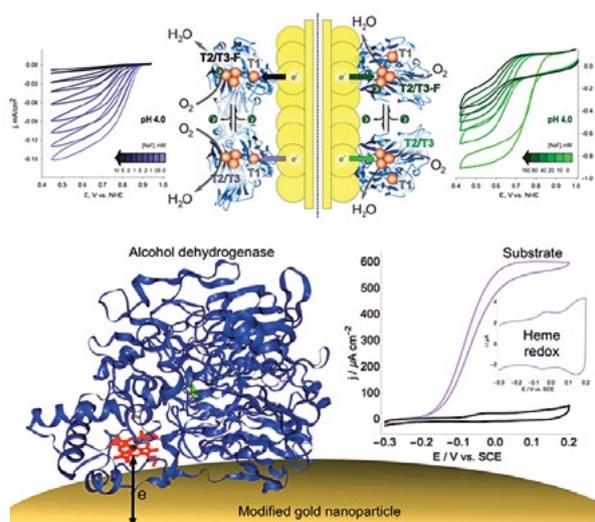
SELECTED PUBLICATIONS



1. M. Dagys, et al. Oxygen electroreduction catalysed by laccase wired to gold nanoparticle via the trinuclear copper cluster. *Energy & Environmental Science* 2017, 10, 498-502.
2. J. Gaidukevič, J. Razumiene, et al. Study on the structure and electrocatalytic activity of graphene-based nanocomposite materials containing (SCN)_n. *Carbon* 2017, 118, 156-167.
3. D. Ratautas, et al. Bioanode with alcohol dehydrogenase undergoing a direct electron transfer on functionalized gold nanoparticles for an application in biofuel cells for glycerol conversion. *Biosensors Bioelectronics* 2017, 98, 215-221.
4. R. Šimkus, et al. Microtiter plate tests for segregation of bioluminescent bacteria. *Luminescence* 2016, 31, 127-134.

Bioelectrocatalysis on Nanostructured Gold Surfaces

The ability to employ techniques for optimal enzyme adsorption on nanostructured gold surfaces for obtaining an efficient bioelectrocatalysis is a prominent skill of our team. Recently, we demonstrated an electroreduction of oxygen catalyzed by a laccase wired to gold nanoparticles through a trinuclear copper cluster [1]. The direct electron transfer catalysis mechanism has been mathematically proven by using the results of spectral and electrochemical measurements. The bypass of the laccase's intramolecular electron transfer has resulted in the fabrication of a high-current-density biocathode, able to operate in solutions of a broad pH range in the presence of high inhibitor concentrations. A similar approach has led to the creation of a bioanode suitable for fast glycerol/glyceraldehyde oxidation [3]. The techniques are currently used in our project for creating a bioreactor for the oxidation of non-starch poly/oligosaccharides (RCL grant No. 01.2.2-LMT-K-718-01-0019).



Biosensors 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 the Faculty of Chemistry and Geosciences, graphene/graphite-based materials were synthesized and characterized by the SEM, XRD, TGA analysis, Raman spectroscopy and BET measurements. The investigations of the synthesized composites also revealed remarkable catalytic performance toward oxygen or hydrogen peroxide reduction reactions, which opens new opportunities for the creation of new technologies consuming oxygen or hydrogen peroxide [2]. The materials are involved in the development of analyzers commercialized by our start-up company UAB Bioanalizės Sistemas; also, they saw use in other recent projects for creating biosensors for the early diagnosis of acute pancreatitis (RCL grant No. 01.2.2-LMT-K-718-01-0025) and an urea analyzer for fertilizer media (MITA grant No. TPP-01-054).

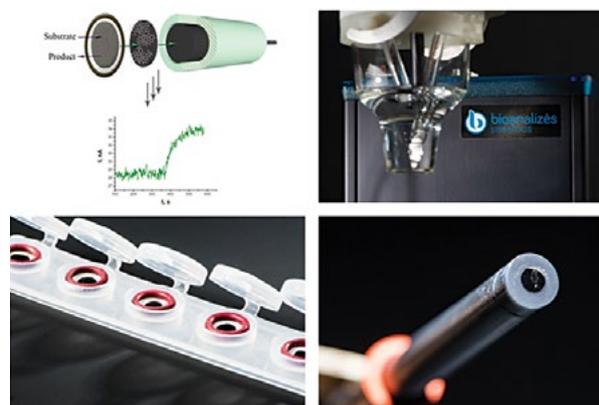
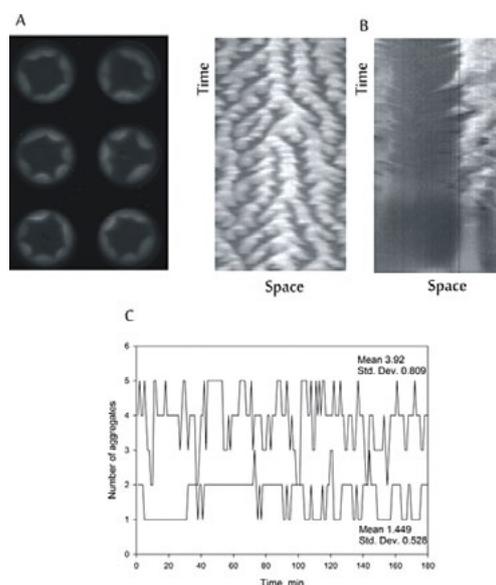


Image courtesy: Marius Jovaiša, photo album "Unseen Lithuania."

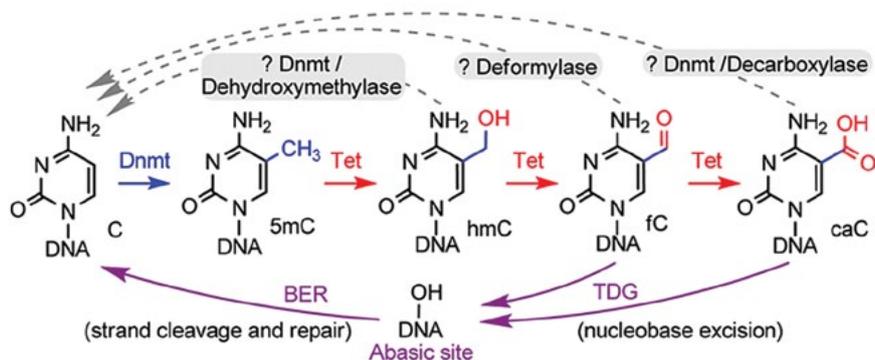
The Self-Organization of Bacteria

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. The obtained bioluminescence imaging data has been interpreted by employing the Keller-Segel-Fisher model of chemotaxis and logistic growth, adapted to systems of metabolically flexible (two-state) bacteria. The results of the plate wells' tests and their simulations indicate that the segregation of bacteria with different activities proceeds in the three-phase contact line region [4]. Currently, we are analyzing the mutation effects on the self-organization of bacteria. These studies were partly funded by the RCL grant No. S-MIP-17-98.





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Biological DNA Modification

Epigenetic Regulation in Mammals

In multicellular organisms, cells share identical genomes but can undergo differentiation to a vast range of lineages. This variability derives from changes in their epigenome – heritable but reversible changes in DNA modification, chromatin structure and miRNA pools. Over the last decade, epigenetic phenomena have taken the center stage in studies of embryonic development, genomic imprinting and chromosome stability and were shown to play significant roles in various human diseases. One of the best understood epigenetic mechanisms is enzymatic DNA methylation. In the mammalian genome, cytosines are often methylated to 5-methylcytosine (5mC), which is largely confined to CpG dinucleotides. DNA methylation profiles are highly variable across different genetic loci, cell types and organisms, and are dependent on age, sex, diet and disease. Besides 5mC, 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 5mC residues by TET oxygenases. However, research into the epigenetic regulation is hampered by the limitations of available analytical techniques.

Molecular Tools for Biopolymer Labeling and Epigenome Profiling

Following our long-standing interest in the mechanistic studies of DNA MTases, we turned to redesigning the methyltransferase reactions for targeted covalent deposition of desired functional or reporter groups onto biopolymer molecules, such as DNA and RNA [2]. The newly developed cofactor analogs carry activated linear side chains, permitting a targeted transfer of these side chains (named mTAG) on a target biomolecule/site. Using the mTAG labeling technology, we went on to develop new experimental approaches for profiling DNA methylation for epigenome studies and improved diagnostics. Since unmodified cytosines represent a smaller proportion of all genomic CpG sites, we mapped the unmodified fraction of the genome – the so-called DNA “unmethylome” (the inverse signal to methylome) [1]. Another unique direction of our research was the discovery of atypical chemo-enzymatic reactions catalyzed by DNA C5-MTases. We found that bacterial C5 MTases can use other substrates, such as small aliphatic aldehydes and thiols, thereby catalyzing their addition to the target C or hmC residues in DNA. Most recently, we were surprised to observe a MTase-directed C-C bond cleavage in hmC and caC yielding unmodified C in DNA. These atypical reactions demonstrate a surprising catalytic versatility of C5-MTases and provide a plausible precedent for the direct formation and reversal of hmC and caC modifications to the unmodified state in genomic DNA (bypassing the known TDG/BER dependent pathway) [2].

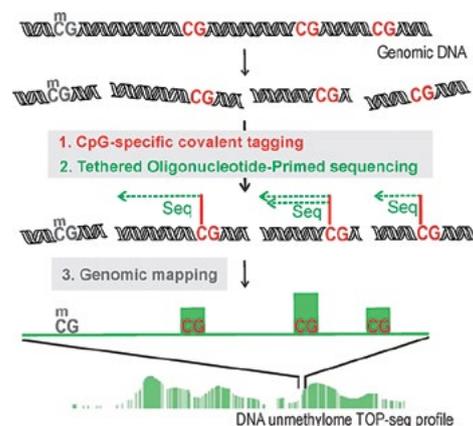
SELECTED PUBLICATIONS



1. Kriukienė *et al.*, DNA unmethylome profiling by covalent capture of CpG sites. *Nat. Commun.*, 2013, 4: 2190.
2. Plotnikova *et al.*, Selective covalent labeling of miRNA and siRNA duplexes using HEN1 methyltransferase. *J. Am. Chem. Soc.*, 2014, 136: 13550–13553.
3. Liutkevičiūtė *et al.* Direct decarboxylation of 5-carboxylcytosine by DNA C5-methyl-transferases. *J. Am. Chem. Soc.*, 2014, 136: 5884–5887.
4. Granted patents: EP2414528 (B1), US8822146 (B2), EP2414527 (B1), US9505797 (B2), US9347093 (B2).

Molecular Tools for Epigenome Analysis

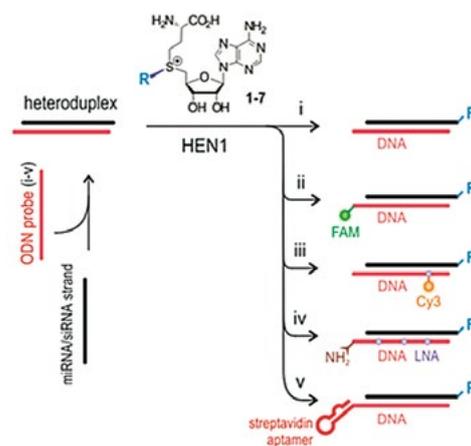
In our quest to expand the analytical toolbox of epigenomic techniques, we proposed a novel concept in analysis of DNA modification patterns that bridges the existing economy-versus-resolution gap. Our newly developed approach, named Tethered Oligonucleotide-Primed Sequencing (TOP-seq), is based on combining the covalent tagging of individual unmodified CpG sites [1] with non-homologous priming of the DNA polymerase action at these sites to directly produce the adjoining regions for their sequencing and precise genomic mapping. Pilot studies of bacterial and human genomes showed a better agreement of TOP-seq with published bisulfite sequencing maps as compared to the widely used enrichment-based techniques and permitted the identification of long-range and gene-level differential methylation among human tissues and neuroblastoma cell types (Staševskij et al., *Mol. Cell*, 2017, 65: 554-56;). Altogether, we propose an affordable single CpG-resolution technique that is well-suited for large-scale



epigenome studies (Kliškauskas et al., EU patent EP2776575; US Patent App. US2017016055).

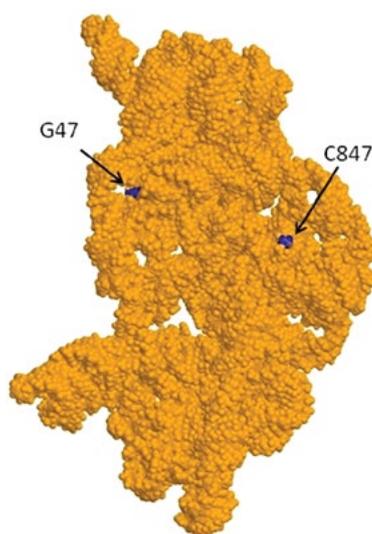
Addressable Covalent Derivatization of Small RNAs

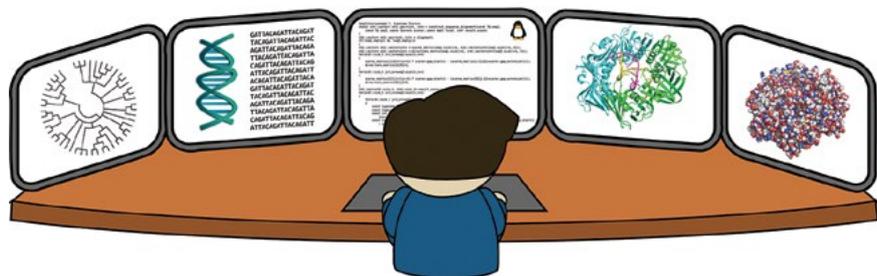
The HEN1 RNA 2'-O-methyltransferase plays important roles in the biogenesis of small non-coding RNAs in plants and proved to be a valuable tool for a selective transfer of functional groups from cofactor analogues onto miRNA and siRNA duplexes *in vitro* [2]. We demonstrate the versatile HEN1-mediated methylation and alkylation of small RNA strands in heteroduplexes with a range of complementary synthetic DNA oligonucleotides carrying user-defined moieties, such as internal or 3'-terminal extensions or chemical reporter groups. This novel DNA-guided covalent functionalization of RNA broadens our understanding of the substrate specificity of HEN1 and paves the way for the development of novel chemo-enzymatic tools with potential applications in miRNomics, synthetic biology and nanomedicine (Osipenko et al., *Angew. Chem. Int. Ed.*, 2017, 56: 6507-6510).



Stand-Alone Activity of Archaeal Fibrillarin

Archaeal fibrillarin (aFib) is a well-characterized AdoMet-dependent RNA 2'-O-methyltransferase that is known to act in a large C/D ribonucleoprotein (RNP) complex together with Nop5 and L7Ae proteins and a box C/D guide RNA. In the reaction, the guide RNA serves to direct the methylation reaction to a specific site in tRNA or rRNA by sequence complementarity. Here we show that a *Pyrococcus abyssi* aFib-Nop5 heterodimer can alone perform SAM-dependent 2'-O-methylation of 16S and 23S ribosomal RNAs *in vitro* and independently of the L7Ae and C/D guide RNAs. Using tritium-labeling, mass spectrometry and a reverse transcription analysis, we identified three *in vitro* 2'-O-methylated positions in the 16S rRNA of *P. abyssi*, all of which lie outside of the previously reported pyrococcal C/D RNP methylation sites. This newly discovered stand-alone activity of aFib-Nop5 provides an example of an ancestral activity retained in enzymes that were recruited to larger complexes in the course of evolution (Tomkuvienė et al., *RNA*, 2017, 23: 1329-1337)





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Computational Analysis of Proteins

Proteins typically function as three-dimensional (3D) structures, often through interaction with each other and/or with other macromolecules. The 3D structure of proteins 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 power of computers and the flood of biological data make the 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-centered research topics that can be collectively described as Computational Studies of Protein Structure, Function and Evolution. We've established two main research directions:

1) The development of computational methods intended for detecting protein homology (common evolutionary origin) from sequence data, comparative modeling of protein structures, analyzing and evaluating 3D structures of proteins and protein complexes. In recent years, our team has developed several new methods for addressing these research topics. All of the software packages implementing these methods are freely available at our website (<http://bioinformatics.lt/software>);

2) The application of computational methods to biological problems. In this research direction, we have been using computational methods for discovering general patterns in biological data, conducting structural/functional characterizations of proteins and their complexes and designing novel proteins and mutants with desired properties. Although we have addressed a variety of biological problems, our major focus has been on the 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 the structural and mechanistic properties of CRISPR-Cas systems.

SELECTED PUBLICATIONS



1. Dapkūnas J, Olechnovič K, & Venclovas Č. (2018) Modeling of protein complexes in CAPRI Round 37 using template-based approach combined with model selection, *Proteins* 86 Suppl 1:292-301.
2. Olechnovič K, & Venclovas Č. (2017) VoroMQA: Assessment of protein structure quality using interatomic contact areas, *Proteins* 85:1131-1145.
3. Kazlauskienė M, et al. (2017) A cyclic oligonucleotide signaling pathway in type III CRISPR-Cas systems, *Science* 357:605-609.
4. Dapkūnas J, et al. (2017) The PPI3D web server for searching, analyzing and modeling protein-protein interactions in the context of 3D structures, *Bioinformatics* 33:935-937.

The Analysis and Modeling of Protein-Protein Complexes

At present, it is relatively easy to find out whether some proteins interact. However, these data have only limited value without knowing how the proteins interact. The interaction details can be obtained from the three-dimensional (3D) structures of protein complexes. As the structures of protein complexes tend to be conserved, the information about protein interactions can often be inferred using homologous structures. The overall number of the determined structures of protein complexes is steadily growing, but the interaction data is highly redundant and difficult to analyze. To facilitate this task, we have developed PPI3D, a webserver for searching, analyzing and modeling protein interactions in the context of 3D structures. We tested PPI3D combined with VoroMQA (see below) in modeling protein-protein complexes in CAPRI, the latest worldwide competition in modeling protein-protein



PPI3D

Protein-Protein Interactions in Three Dimensions

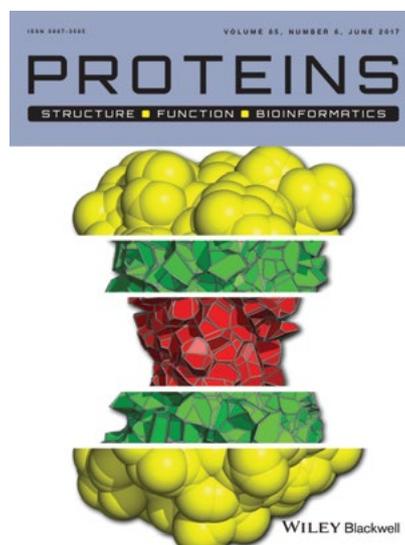
<http://bioinformatics.lt/ppi3d>

Dapkūnas et al. (2017) *Bioinformatics* 33:935-937.

interactions. Both methods turned out to be effective, since an independent assessment deemed our results to be the best (Lensink et al. (2018) *Proteins*; 86:257-273).

A New Method for the Assessment of Protein Structural Models

The utility of protein structural models depends on their quality. Therefore, the estimation of the predicted structures' quality is an important problem. We developed a new method for the estimation of protein structure quality - VoroMQA (Voronoi tessellation-based Model Quality Assessment). VoroMQA combines the idea of statistical potentials with the use of interatomic contact areas instead of distances. VoroMQA produces scores at atomic, residue and global levels, all in the fixed range from 0 to 1. We tested VoroMQA along with other methods during the most recent worldwide competition of protein structure prediction methods (CASP12). VoroMQA demonstrated a very strong performance, especially in selecting the best models. An independent assessment of the obtained results placed our method ("VoroMQA-select") among the top two (*Proteins* 2018; 86:321-334), suggesting that the use of interatomic contact areas might be very fruitful in structural studies.

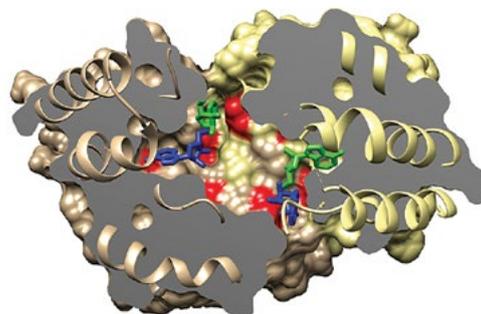


<http://bioinformatics.lt/wtsam/voromqa>

Olechnovič & Venclovas (2017) *Proteins* 85:1131-1145 (featured on the cover).

Computational Methods in the Studies of CRISPR-Cas Systems

Type III CRISPR-Cas systems in prokaryotes provide immunity against invading nucleic acids through the coordinated degradation of transcriptionally active DNA and its transcripts by the Csm effector complex. The Cas10 subunit of the complex contains an HD nuclease domain, which is responsible for DNA degradation, and two Palm domains with elusive functions. In addition, Csm6, a ribonuclease that is not part of the complex, is also required to provide full immunity. Our collaborators (the Siksnys Lab) discovered that Cas10 synthesizes cyclic oligoadenylates (oAs) acting as signaling molecules that activate a nonspecific RNA degradation by Csm6. In turn, our computational modeling and docking results were instrumental in identifying the oA binding site in Csm6. This



Kazlauskienė et al. (2017) *Science* 357:605-609.

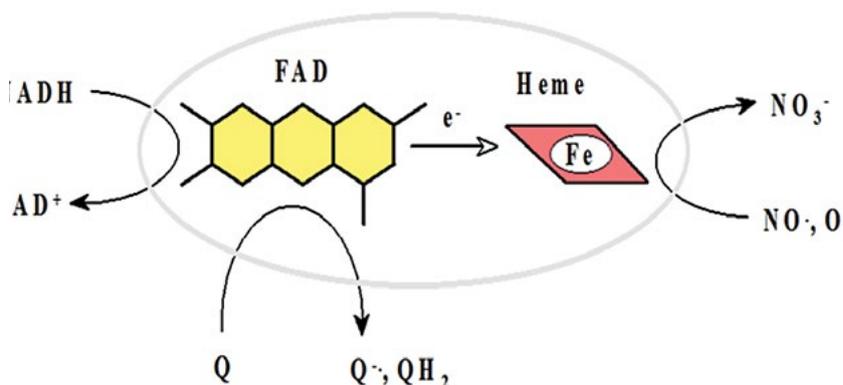
discovery of the new signaling pathway and its mechanism has been published in *Science* and has immediately generated a strong interest in the field.


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Mechanisms of Flavoenzyme Redox Reactions

Flavoenzymes contain flavinmononucleotide (FMN) and flavinadeninucleotide (FAD) in their active centers. The distinctive feature of flavoenzymes is their ability to transform a single-electron transfer into a two-electron one. They play important roles in biological oxidation-reduction, hydroxylation, transhydrogenation, antioxidant protection, redox signaling and other processes. Flavoenzymes also participate in the biodegradation of toxic environmental pollutants and the 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/hydrate transfer in the catalysis of flavoenzyme electrontransferases and transhydrogenases; 2) A single- and two-electron reduction of quinoidal and nitroaromatic compounds by mammalian or microbial flavoenzymes and their impact on their cytotoxicity; 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 during 2014–2017 include the following: a) A characterization of intraprotein electron transfers and the mechanism of the reduction of quinones and nitroaromatics by the *S. aureus* flavohemoglobin as well as a discovery of the activation of quinone reduction byazole antibacterial drugs that bind to the heme of flavohemoglobin (in collaboration with Drs. L. Baciou and F. Lederer, Universite Paris-Sud, France); b) A characterization of intraprotein electron transfers in flavocytochromes b₂, their reduction mechanisms of quinones and their application in L-lactate biosensors (in collaboration with Dr. M. Gonchar, Institute of Cell Biology of UAS, Lviv, Ukraine); c) A characterization of the mechanisms of two- and mixed single- and two-electron reductions of quinones and nitroaromatic compounds by the *E. coli* nitroreductase A and *Thermotoga maritima* thioredoxin reductase (in collaboration with Dr. D. F. Ackerley, Wellington University, New Zealand, and Dr. J.-P. Jacquot, Universite de Lorraine, France); d) An evaluation of nitroaromatic compounds as inhibitors and subversive substrates for the glutathione reductase of various origin in the context of developing antiparasitics (collaboration with dr. E. Davioud-Charvet, Universite de Strasbourg, France); e) A continuation of synthesis and studies of enzymatic single- and two-electron reductions as well as mammalian cell culture cytotoxicity studies of new quinones (European Social Fund, Global Grant Measure, Grant No. VP1-3.1-ŠMM-07-K-01-103, 2011–2015).

SELECTED PUBLICATIONS

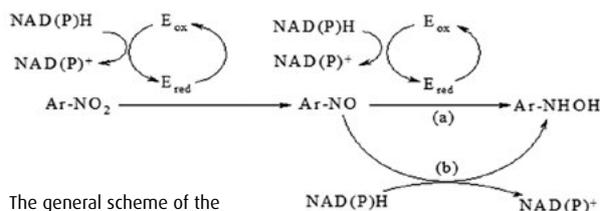
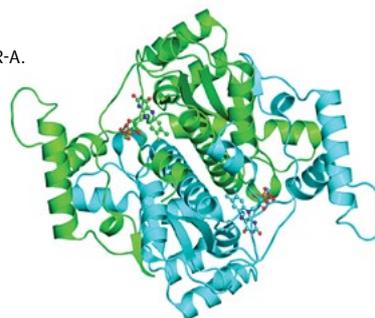


1. B. Valiauga et al. Reduction of quinones and nitroaromatic compounds by *Escherichia coli* nitroreductase A (NfsA): Characterization of kinetics and substrate specificity. *Arch. Biochem. Biophys.* 2017, 614, 14-22.
2. J. Šarlauskas et al. Naphtho[1,2':4,5]imidazo[1,2-a]pyridine-5,6-diones: Synthesis, enzymatic reduction and cytotoxic activity. *Bioorg. & Med. Chem. Lett.* 2016, 26, 512-517.
3. B. Valiauga et al. Quinone- and nitroreductase reactions of *Thermotoga maritima* thioredoxin reductase. *Acta Biochim. Polon.* 2015, 62, 303-309.
4. J. Šarlauskas et al. The study of NADPH-dependent flavoenzyme-catalyzed reduction of benzo[1,2-c]1,2,5-oxodiazole N-oxides (benzofuroxans). *Int. J. Molec. Sci.* 2014, 15, 23307-23331.

The Reactions of Nitroaromatic Compounds and Quinones with Bacterial Nitroreductases

Bacterial nitroreductases (NR) reduce quinones and nitroaromatic compounds in a two-electron way. The transfection of cancer cells by NRs enhances the cytotoxicity of nitroaromatics and may improve tumor imaging. We characterized the mechanism of the reduction of quinones and nitroaromatics by *E. coli* NR-A: a) The reaction proceeds based on the ping-pong mechanism; b) The rate-limiting step is an oxidative half-reaction; c) Except for the 2-hydroxy-naphthoquinones, quinones are less efficient oxidants of NR-A than nitroaromatics; d) The midpoint potential of NR-A is close to -200 mV; e) The intensity of the fluorescence of FMN and its affinity for dicumarol indicate that the active center of NR-A possesses a larger conformational freedom than in NR-B; f) In a net four-electron reduction of the formation of nitroaromatics, hydroxylamines are formed mainly in a direct reduction of nitroso compounds by NADPH (B. Valiauga et al. *Arch. Biochem. Biophys.* 2017, 614, 14-22).

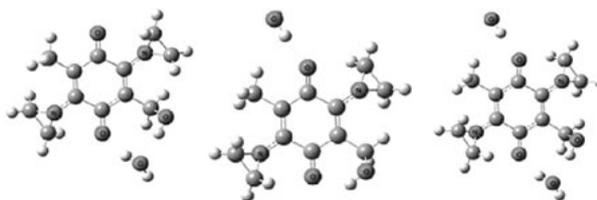
The structure of *E. coli* NR-A.



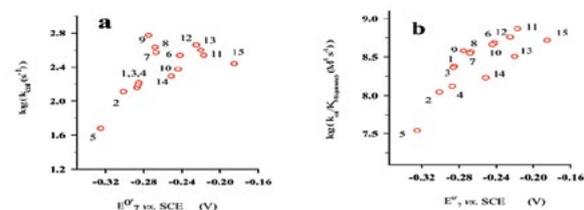
The general scheme of the two(four)-electron reduction of nitroaromatics by nitroreductases.

The Synthesis, Enzymatic, Physicochemical and Cytotoxicity Studies of Quinones

Using quantum mechanical calculations and an X-ray analysis, we characterized the solvent influence on the torsion angle of the aziridine group and the spectral characteristics of several synthesized antitumor diaziridinyl-1,4-benzoquinones (J. Šarlauskas et al. *Spectrochim Acta A.* 2017, 178, 136-141). We synthesized a series of *N*-tetracyclic-*o*-quinones and characterized their enzymatic reactivity and cytotoxicity: (1) their single-electron reduction by NADPH:cytochrome P-450 reductase proceeded at close to diffusion-limited rates, (2) Their two-electron reduction by DT-diaphorase was sensitive to their reduction potential, and (3) these compounds were active against A-549 and MCF-7 cancer cells at micromolar and submicromolar concentrations (M. Pečiukaiytė-Alksnė et al. *EXCLI J.* 2017, 16, 663-678).



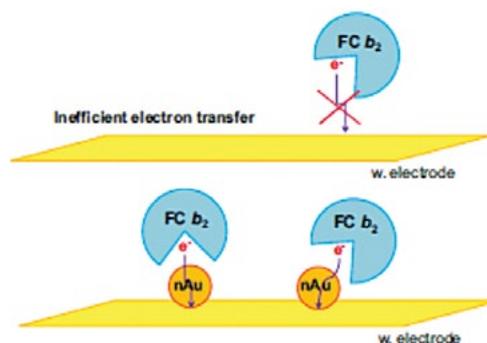
The interaction of aziridinyl-1,4-benzoquinones with solvent molecules.



The reactivity of new *N*-tetracyclic-*o*-quinones in DT-diaphorase-catalyzed reactions.

The Electron Transfer Reactions of Flavocytochrome b_2 from the *Hansenula Polymorpha*

Earlier, we characterized the catalysis mechanism of flavocytochrome b_2 (L-lactate: cytochrome *c* reductase) from thermotolerant yeast *H. polymorpha* (M. Lesananavičius et al. *Chemija* 2016, 27, 123-127). Later, we found that this enzyme is electrochemically inactive at conventional Au electrodes. However, the electrode modification by Au nanoparticles resulted in the efficient electrooxidation of the enzyme-active center, which enabled the construction of a relatively stable amperometric biosensor for L-lactate in human liquids (O. Smutok et al. *Sensors and Actuators B.* 2017, 250, 469-475).



The flavocytochrome b_2 -mediated electrooxidation of the L-lactate on an Au-nanoparticle modified electrode.

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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 N-heterocycles-utilizing or producing microorganisms, and the investigation of metabolic pathways of said compounds in individual bacteria, or Archaea, are some of the aims of our group [1, 3]. More than 50 bacterial strains belonging to *Rhodococcus*, *Arthrobacter*, *Pusillimonas*, genera capable of degrading 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. A four-component dioxygenase from *Rhodococcus* sp. PY11 is the first enzyme shown to catalyze dioxygenase-mediated hydroxylation, where the pyridine ring is a primary substrate [1]. The pyridine derivatives are in great demand as synthons for pharmaceutical products. The application of enzymes or whole cells is an attractive strategy for the preparation of hydroxylated pyridines, since the methods for the chemical synthesis of pyridinols, particularly aminopyridinols, are usually limited or inefficient. Hence, a regioselective oxyfunctionalization of pyridine derivatives using *Burkholderia* sp. MAK1 has been established as a promising method for the preparation of various pyridin-5-ols and pyridin-N-oxides [2].

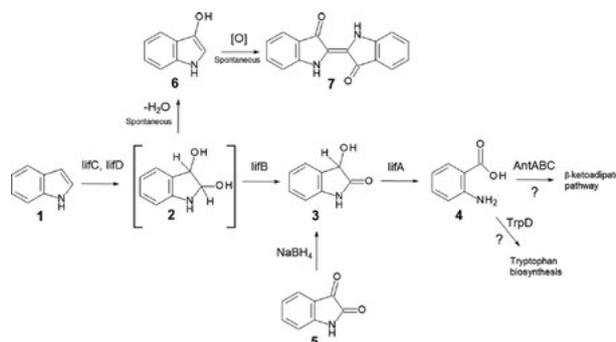
SELECTED PUBLICATIONS



1. Vaitekūnas et al. A 2-hydroxypyridine catabolism pathway in *Rhodococcus rhodochrous* strain PY11. *Appl. Environ. Microbiol.* 2016 82: 1264.
2. Stankevičiūtė et al. Oxyfunctionalization of pyridine derivatives using whole cells of *Burkholderia* sp. MAK1. *Sci. Rep.* 2016 6: 39129.
3. Urbonavičius et al. Evolution of tRNAPhe:imG2 methyltransferases involved in the biosynthesis of wyosine derivatives in Archaea. *RNA* 2016 22: 1871.

Indole Biodegradation in *Acinetobacter* sp. Strain 0153: Genetic and Biochemical Characterization

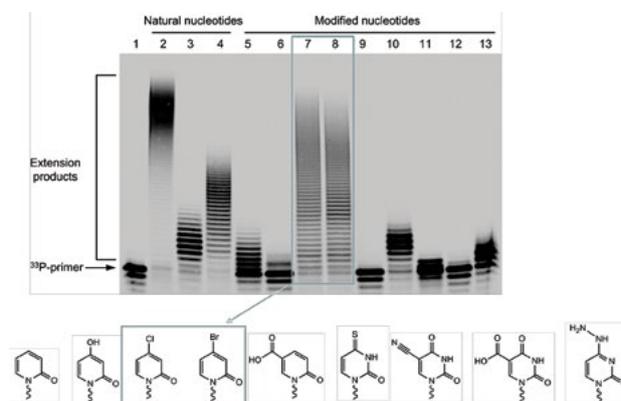
Indole is a molecule of considerable biochemical significance, acting as both an interspecies signal molecule and a building block for biological elements. Bacterial indole degradation has been demonstrated for a number of cases; however, very little is known about genes and proteins involved in this process. This study reports the cloning and initial functional characterization of genes responsible for the indole biodegradation in the *Acinetobacter* sp. strain 0153. The degradation starts with oxidation, mediated by *lifC* and *lifD*. The final product – anthranilic acid – is formed by *lifA*, an enzyme that is both structurally and functionally comparable to cofactor-independent oxygenases. Moreover, the *lif* cluster was identified in the genomes of a wide range of bacteria, suggesting



the potential widespread of *lif*-mediated indole degradation (Sadauskas M, Vaitekūnas J, Gasparavičiūtė R, Meškys R. *Appl. Environ. Microbiol.* 2017 83: e01453-17).

Modified Nucleotides as Substrates of Terminal Deoxynucleotidyl Transferase

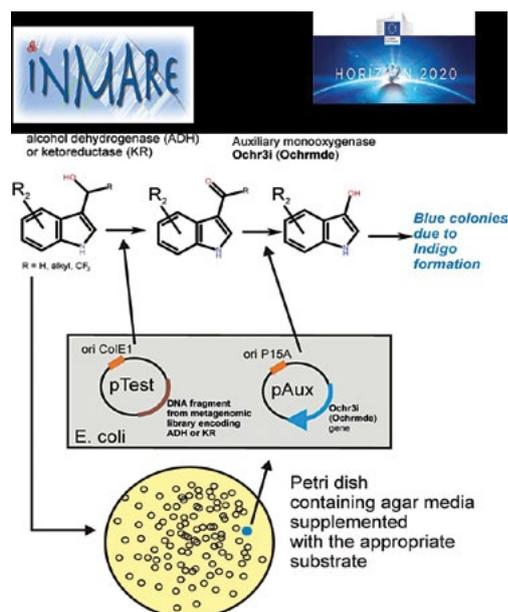
The synthesis of novel modified nucleotides and the incorporation of them into a DNA sequence open many possibilities for changing the chemical properties of oligonucleotides (ONs) and, therefore, broaden the field of the practical applications of modified DNA. The chemical synthesis of nucleotide derivatives, including the ones bearing a thio-, hydrazine-, cyano- and carboxy-group, as well as a 2-pyridone nucleobase containing nucleotides, was carried out. The prepared compounds were tested as substrates of terminal deoxynucleotidyl transferase (TdT). The nucleotides containing N4-aminocytosine, 4-thiouracil, as well as 2-pyridone, 4-chloro- and 4-bromo-2-pyridone as a nucleobase, were accepted by TdT, thus allowing the enzymatic synthesis of 3'-terminally modified ONs.



(Tauraitė D., Jakubovska J., Dabužinskaitė J., Bratčikov M., Meškys R. *Molecules* 2017 22: 672).

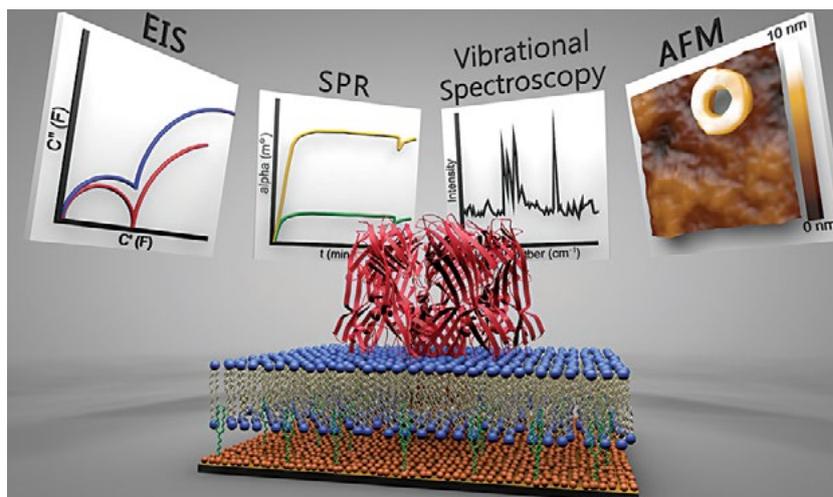
An Efficient Screening, Development and Application of Novel Biocatalysts

We are experts in discovering new enzymes, including hydrolases, oxidoreductases and transaminases. Metagenomic libraries combined with the “smart” substrates that are developed and synthesized by our group is a technology that offers an efficient screening and selection of novel biocatalysts applicable in bioconversion, organic syntheses and the development of prodrugs, biosensors as well as biofuel cells (EU Horizon2020, INMARE Project; Tetianec L, Chaleckaja A, Kulys J, Janciene R, Marcinkeviciene L, Meskiene R, Stankeviciute J, Meskys R. *Process Biochem.* 2017 54, 41–48; Dagys M, Laurynėnas A, Ratautas D, Kulys J, Vidžiūnaitė R, Talaikis M, Niaura G, Marcinkevicienė L, Meškys R, Shleev S. *Energy Environ. Sci.* 2017, 10: 498–502; Meškys R, Jakubovska J, Tauraitė D. LT patent application (patent pending) LT2017 523; Urbonavičius J, Tauraitė D, Aučynaitė A, Rutkienė R, Meškys R. LT patent application (patent pending) LT2017 533.





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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 proteins of known structure are that of MPs, and, unfortunately, such a lack of knowledge seriously affects the 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 MPs arises from their instability outside a lipid bilayer environment, where specific hydrophobic and other molecular forces keep the protein in its native and active conformational state. Therefore, considerable efforts are directed toward the development of simplified but biologically relevant model membrane systems for studying molecular processes in membranes.

Our group is specializing in the development of tethered bilayer membrane (tBLM) systems. tBLMs are surface supported bilayers anchored to a metal film via hydrophobic interactions between the molecular anchors and hydrophobic sheet of the bilayer. The molecular anchors are synthetic thiolipid molecules, which covalently attach to a surface. The anchor molecules contain oligoethylene fragments separating the thiol group and the glycerol backbone of the lipid, thus ensuring a 1-2 nm thick water-reservoir between the tethered bilayer and solid support. Recently, we developed an affordable and reproducible methodology based 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 surface-specific techniques, including surface plasmon resonance, vibrational spectroscopies and atomic force microscopy. Fine structural details revealing the molecular geometry of tBLMs are evaluated using neutron reflectometry. The functional properties of both membranes and reconstituted protein complexes are accessible by 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.

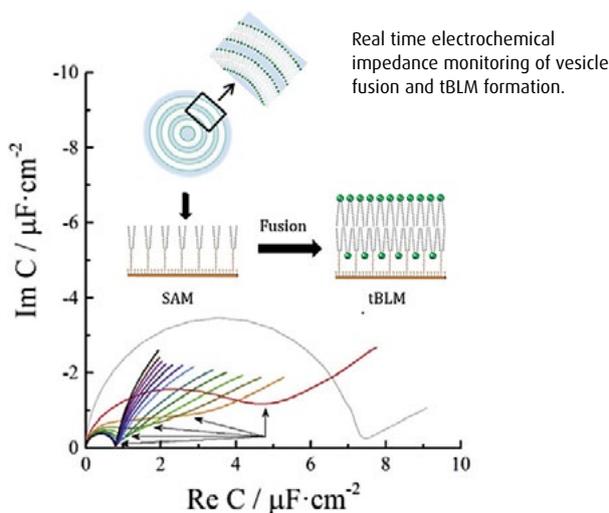
SELECTED PUBLICATIONS



1. Ragaliauskas, et al. Fast formation of low-defect-density tethered bilayers by fusion of multilamellar vesicles. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1859(5), pp.669-678 (2017).
2. G. Preta, et al. Tethered bilayer membranes as a complementary tool for functional and structural studies: The pyolysin case. *Biochimica et Biophysica Acta-Biomembranes*. 1858(9), 2070-2080 (2016).
3. G. Valincius, M. Mickevicius, Tethered phospholipid bilayer membranes: An Interpretation of the Electrochemical Impedance response, in: A. Iglíč (Ed.) *Advances in Planar Lipid Bilayers and Liposomes*, Elsevier, 2015, pp. 27-61.

New Tethered Bilayer Membrane Technology

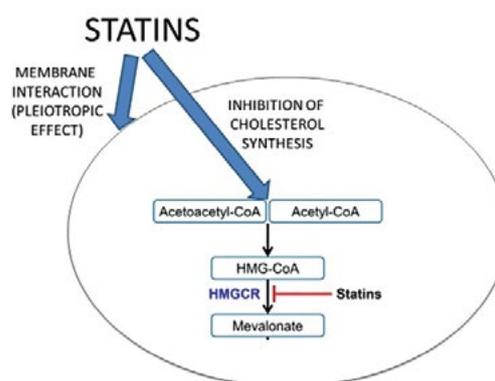
We demonstrate that multilamellar vesicles fuse to molecular-anchor-grafted surfaces yielding low-defect-density, tethered bilayer membranes. Continuous bilayers are formed within 10 min, while the electrically insulating bilayers with less than $0.1 \mu\text{m}^{-2}$ defect density can be accomplished within 60 min. The process of the bilayer's formation may be monitored in real-time by electrochemical impedance spectroscopy. Along with the surface plasmon resonance, it attests for the formation of intact bilayers independent of the tethering agent density. Neutron reflectometry (NR) revealed the atomic level structural details of the tethered bilayer, showing, among other things, that the total thickness of the hydrophobic slab of the construct was 3.2 nm and that the molar fraction of cholesterol in the lipid content is essentially the same as the molar fraction of cholesterol in the multilamellar liposomes. Fast assembly and low residual defect density, achievable within an hour of fusion, make our tethered bilayer methodology an attractive



platform for the biosensing of membrane damaging agents, such as pore forming toxins. Ragaliauskas, et al. *Biochimica et Biophysica Acta (BBA)-Biomembranes*, 1859(5), pp.669-678 (2017).

Statins and cell toxicity

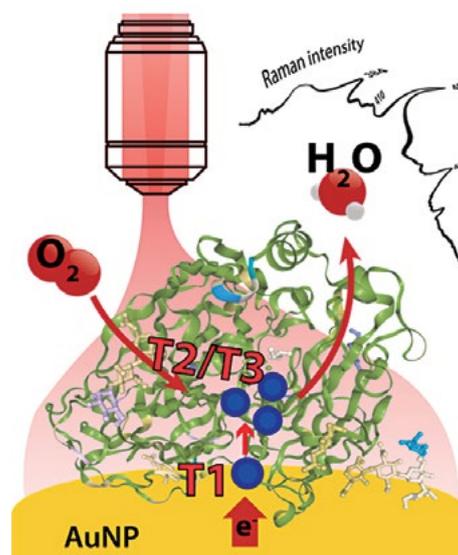
Statins are effective inhibitors of cholesterol biosynthesis and they protect cells against the damage induced by cholesterol dependent cytotoxins. However, their use in clinical trials of cardiovascular diseases clearly indicates that the general benefits observed with statins appear to be greater than what might be expected from changes in lipid levels alone, suggesting effects beyond cholesterol lowering (S. Griffin et al. *Sci Rep.* 2017 Dec 6;7(1):17050). Recent studies indicate that some of the cholesterol-independent or "pleiotropic" effects of statins involve the modification of the nanomechanical stability of the bilayers and the increase of their elastic moduli depending on the lipid bilayer order. Using a combination of biological (hemolysis and MTT assays on cancer cell lines) and biophysical (tethered bilayer membranes) methods, we



aim to elucidate the nature of the interaction between the statins and the cell membrane.

Vibrational Spectroscopy of Proteins at Interfaces

The understanding of the structure and interaction of proteins with a solid surface is of fundamental importance in order to control their biological function and to construct bioelectrochemical devices. Surface-enhanced Raman spectroscopy (SERS) was demonstrated as a powerful experimental technique providing molecular level information on molecular bonding, subtle protein conformational changes upon interactions at interfaces and the laccase catalyze reduction of oxygen to water via the direct electron transfer (DET) process. The attractiveness of DET in biodevices lies in the conceptual simplicity of their operational mechanism. In order to ensure a strong SERS signal, a high protein load on the surface and to establish an interface between the electrode and the LAC, gold nanoparticles (AuNP/LAC) were designed. The advantage of SERS was taken to investigate the possible spectral features of specific amino acids and/or the catalytic center of LAC and to provide insight into the effect of electrode potential during adsorption on the structure of the surface-adsorbed enzyme (Dagys, M. et al. *Energy Environ. Sci.* **10**, 498-502 (2017)).



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Applications of the Molecular Microbiology of Prokaryotes

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.

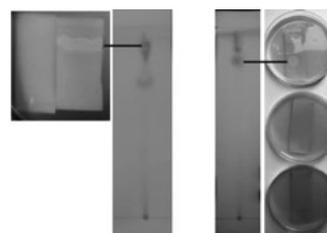
SELECTED PUBLICATIONS



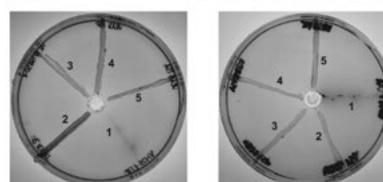
1. S. Ghosh et al. The cave microbiome as a source for drug discovery: reality or pipe dream? *Biochem. Pharmacol.* 2017 134, 18-34.
2. A. Klusaite et al. Characterization of antimicrobial activity of culturable bacteria isolated from Krubera-Voronja cave. *Int. J. Speleol.* 2016 45, 275-287.
3. R. Pranckutė et al. Combining prebiotics with probiotic bacteria can enhance bacterial growth and secretion of bacteriocins. *Int. J. Biol. Macromol.* 2016 89, 669-676.
4. A. Jasilionis & N.Kuisiene. Characterization of a novel thermostable oligopeptidase from *Geobacillus thermoleovorans* DSM 15325. *J. Microbiol. Biotechnol.* 2015 25, 1070-1083.

Volatile Bioactive Compounds with Antimicrobial Activity

Volatile and semivolatile bacterial compounds have been shown to possess antimicrobial activity. A detailed analysis of volatile compounds produced by two Krubera-Voronja Cave bacterial isolates revealed two different mixtures of these compounds. The antibacterial mixture of the first strain was composed of 1,2-benzenedicarboxylic acid, bis(2-methylpropyl) ester; 1,2-benzenedicarboxylic acid, diisooctyl ester; dibutyl phthalate, and 2,4-ditert-butylphenol, and that of the second strain was composed of gancidin W; cyclo(L-prolyl-D-phenylalanyl); 1,2-benzenedicarboxylic acid, diisooctyl ester, and 1,3-dimethylbenzene. The differences in the mixture composition were reflected in the bioactivity against Gram-positive pathogenic bacteria as well as against other strains from the Krubera-Voronja Cave (S.Ghosh et al. The cave microbiome as a source for drug discovery: reality or pipe dream? *Biochem. Pharmacol.* 2017 134, 18-34).



Pathogen	The concentration of the antimicrobial compounds in ethyl acetate, mg/mL					
	4.660	2.330	1.165	0.583	0.291	0.146
<i>Bacillus cereus</i> ATCC 10876	6.5	6.0	5.0	3.0	2.0	0
<i>Enterococcus faecalis</i> ATCC 29212	10.0	7.0	5.0	4.0	2.0	0
<i>Listeria monocytogenes</i> ATCC 7644	7.0	6.5	5.0	3.5	3.0	0
<i>Staphylococcus aureus</i> subsp. <i>aureus</i> ATCC 25923	3.5	1.5	0.5	0.5	0	0
<i>Rhodococcus equi</i> ATCC 6939	12.0	8.0	5.5	5.0	3.0	0



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: an amount of these compounds can be under the detection limits; certain experimental conditions can be inappropriate for the induction of the biosynthesis

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).

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 through an 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 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

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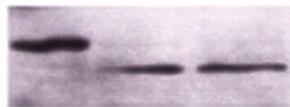
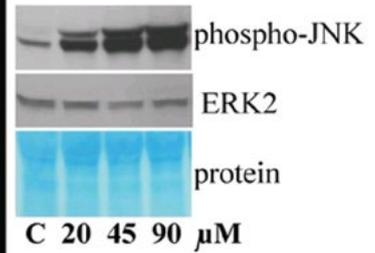
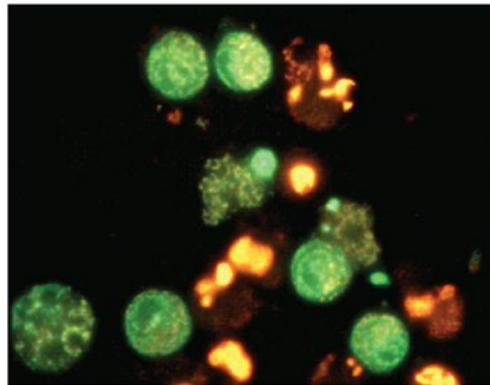
proteins with the desirable characteristics can also be carried out. Therefore, bacterial collagen-like proteins represent an excellent source for the design of new biomaterials with the desirable structural properties and functions. The identification, expression and characterization of bacterial collagen-like proteins represent a highly attractive and important area of research work in the fields of regenerative medicine and biotechnology.


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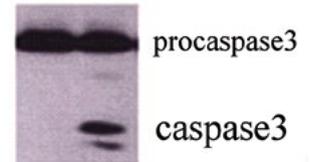
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PARP1



Cell-Specific Death/Survival Signaling in Cancer Treatment

Regulated cell death is a vital component of various processes including normal cell turnover, immunity and embryonic development. Inappropriate cell death is accountable for many human diseases. 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. The control of cell death is increasingly becoming understood. This research is focused on signal transduction mechanisms involved in the initiation, execution and propagation of cell death as well as on their dysregulation in pathophysiological conditions.

Understanding cell death signaling networks upon anticancer drug responses is a promising approach for identifying new drug targets and biomarker profiles in cancer therapy. Manipulating the molecules in a chemotherapeutic drug-induced signaling pathway provides a promising strategy for targeted cancer treatment. At the same time, cytoprotective strategies may be used to avoid unwanted cell death in the cases of stroke, infarction or neurodegenerative disorders.

Stem cells in an organism are also subjected to the toxic effect of antineoplastic drugs. The identification of signaling events leading adult stem cells to apoptosis should uncover new ways for regulating their survival *in vivo*. Moreover, stem cell survival/death pathways are the potential targets for improving the efficiency of cellular therapy.

The sequence of events and relations between signaling molecules, leading cancer cells to apoptosis or protecting normal cells during anticancer drug treatments are studied. Chemotherapeutic drug-induced death signaling has been evaluated in lung cancer and muscle-derived stem cells as well as their differentiated progenies (Kalvelyte et al., 2013; Stulpinas et al., 2012; Ref. 1 and 2).

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. Daunorubicin-induced stem cell death, alongside with the induction of proapoptotic MAP kinase JNK, involved the downregulation of antiapoptotic protein kinase AKT pathway molecules. The induction of different and opposite cell signaling pathways, which may counteract one another, were shown in lung cancer cells during chemotherapeutic drug RH-1 treatment (Ref. 1). Mitochondrial proteins, proapoptotic Bax and antiapoptotic Bcl-2 were regulated in a JNK-dependent manner in the direction that correlated with the role of JNK in adipocytic cell apoptosis induced by cisplatin (Ref. 2). As a result of cooperation with Prof. Eltyeb Abdelwahid and other co-authors, we have published a review article (Ref. 3). 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.

SELECTED PUBLICATIONS

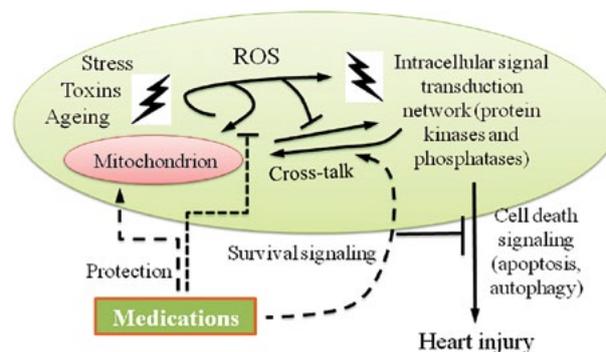


1. Stulpinas A et al. Study of Bioreductive Anticancer Agent RH-1-Induced Signals Leading the Wild-Type p53-Bearing Lung Cancer A549 Cells to Apoptosis. *Chem Res Toxicol*, 2016, 29(1): 26-39.
2. Krestnikova N et al. JNK implication in adipocyte-like cell death induced by chemotherapeutic drug cisplatin. *J Toxicol Sci*, 2015, 40(1): 21-32.
3. Abdelwahid E et al. Stem cell death and survival in heart regeneration and repair. *Apoptosis*, 2016, 21(3): 252-68.
4. Kalvelytė AV et al. Adult stem cells and anticancer therapy. *Advances in Molecular Toxicology*, 2017, 11 (2017): 123-202. Elsevier.



Approaches for Protecting Cardiac Cells in a Diseased Heart

The current understanding of signaling pathways, involved in a broad range of pharmacological activities that contribute to cardiomyocyte protection against death in patients with heart diseases, was reviewed (Abdelwahid et al. 2017). The article focuses on the protective roles of various agents, mostly natural compounds, which express beneficial effects on mitochondrial function and suspend the apoptotic signaling mechanisms by modulating the activity of mitogen-activated protein kinases (MAPKs). The data presented provide evidence of mitochondrial signaling pathways as targets to downregulate cardiac cell apoptosis and thus to prevent and treat cardiovascular diseases. In concordance, the manipulation of MAPK pathways to prevent cardiac cell death may be a promising approach in cardiovascular medicine.

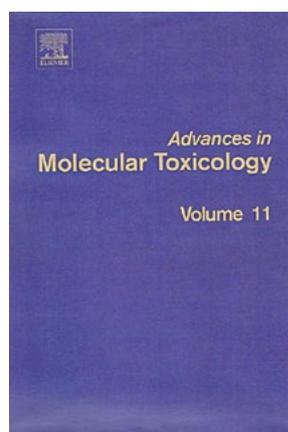


Abdelwahid E, Stulpinas A, Kalvelyte A. Effective Agents Targeting the Mitochondria and Apoptosis to Protect the Heart. *Curr Pharm Des*, 2017. 23(8): 1153-1166. ISSN 1873-4286.

Adult Stem Cells and Anticancer Therapy

The current knowledge about the impact of cancer treatment on normal stem cells, as well as stem cell input into modern toxicology, was presented. Anticancer drugs (both conventional and targeted) and their molecular targets were described with advances and challenges in their use.

Differences in intrinsic molecular resistance mechanisms have been reported between malignant and normal stem cells during cancer therapy. The need of developing new therapeutic strategies, experimental approaches, innovative patient- and organ-specific models for improving the treatment of cancer patients was emphasized. The necessity to develop combination therapies with the application of multiple drugs with different modes of action, targeting different pathways in cancer and normal cells as well as the microenvironment, was suggested. The models that involve a



Audrone V. Kalvelyte, Aušra Imbrasaitė, Natalija Krestnikova, Aurimas Stulpinas. Adult Stem Cells and Anticancer Therapy. *Advances in Molecular Toxicology*, 1st Edition, Volume 11, Chapter Four. 123-202. Edited by J.C. Fishbein and J.M. Heilman, Elsevier. ISBN: 9780128125229.

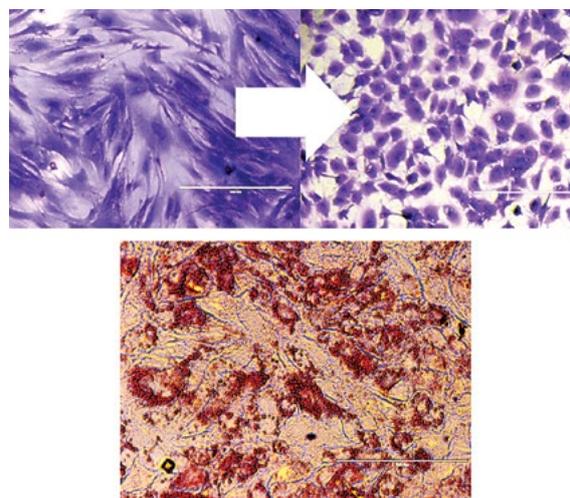
simultaneous evaluation of an anticancer treatment impact on the both kinds of stem cells *in vitro* were proposed.

“Designing of the Patient-Specific, Heterogeneous Lung Cell Ex Vivo Model System for Drug Efficiency Prediction in Personalized Oncotherapy” (SMART Project, Dr. A. Kalvelyte)

It should be noted that despite the advancements, the existing cancer cell biomarker-based approach is not effective enough. It indicates the need for new tools, research systems and treatment innovations.

We aim to develop a new technology for solving the problem of cancer resistance to therapies. The project is based on the idea that phenotypic, not only genetic, heterogeneity of cells determines the resistance to selected drugs.

The effectiveness of conventional and targeted drugs, selected to target the intracellular signal transducing protein kinases, is studied by using patient-derived sets of heterogeneous lung cancer cell lines as a model system. Phenotype switching, cell dynamic state, extracellular contacts, along with the identification of molecular mechanisms, are assessed during the evaluation of drug effectiveness.



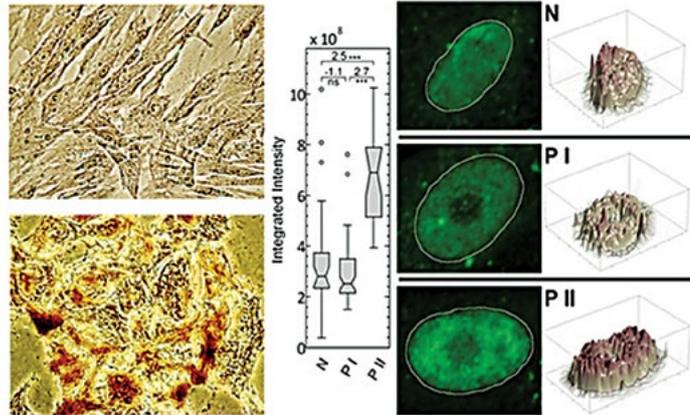
Therefore, our proposed model combines cancer cell heterogeneity with cancer resistance and involves the identification of the best cell-type-specific treatments together with responsible signaling mechanisms.


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Epigenetic Regulation of Stem and Cancer Cell

Epigenetic regulation, when influenced by DNA and histone modifications as well as microRNA expressions, cause variances in gene expression and cell phenotype. They have a great influence on the development and functioning of stem cells as well as epigenetic alterations. These changes could cause cancer and other diseases. An understanding of regulatory and epigenetic molecular mechanisms of stem and cancer cell functioning are 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 signaling 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 from healthy and pathological pregnancies by determining histone modification patterns associated with a 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.

We investigated acute myeloid leukemia cell modulations using various epigenetic agents. Our main objects were acute promyelocytic leukemia and chronic myeloid leukemia cell lines and the bone marrow samples of patients with acute myeloid leukemia. We used natural and synthetic epigenetic modulators to induce cell cycle arrest, apoptosis, cellular senescence, differentiation and metabolic activity. Apart from these cell fate changes, we evaluated the epigenetic changes such as DNA methylation and histone modification patterns.

SELECTED PUBLICATIONS

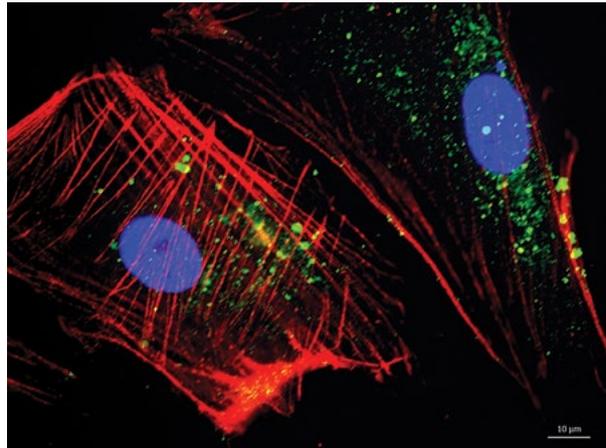


1. Navakauskienė R. Combination Epigenetic Therapy. Handbook of Epigenetics. The new molecular and medical genetics. 2nd Edition, Edited by T.O.Tollefsbol, 2017, Chapter 41, p. 623-632, Elsevier ISBN: 978-0-12-805388-1.
2. Savickienė J., Matuzevičius D., Baronaite S., Treigytė G., Krasovskaja N., Zaikova I., Navakauskas D., Utkus A., Navakauskienė R. Histone Modifications Pattern Associated With a State of Mesenchymal Stem Cell Cultures Derived From Amniotic Fluid of Normal and Fetus-Affected Gestations. *Journal of Cellular Biochemistry*. 2017,118(11):3744-3755.
3. Glemžaitė M. and Navakauskienė R. Osteogenic differentiation of human amniotic fluid mesenchymal stem cells is determined by epigenetic changes. *Stem Cells International*. 2016. Article ID 6465307.
4. Navakauskienė R., Mori M., Christodoulou M. S., Zentelytė A., Botta B., Dalla Via L., Ricci F., Damia G., Passarella D., Zilio C. and Martinet N. Histone demethylating agents as potential S-adenosyl-L-methionine-competitors. *Medicinal Chemical Communications*. 2016, 7 (6):1245-1255.



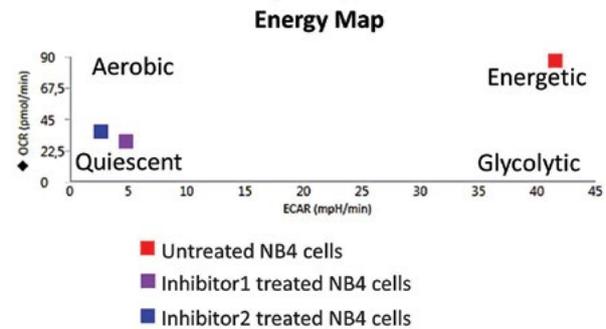
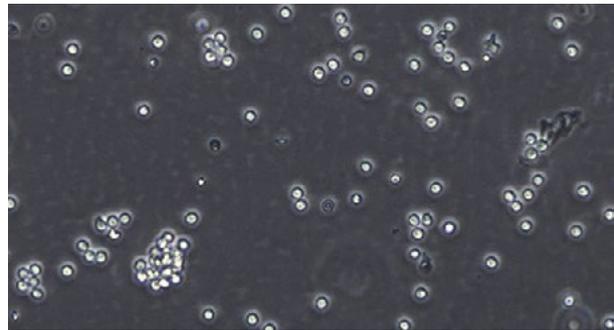
The Cardiomyogenic Differentiation of Human AF-MSCs

The cardiomyogenic differentiation of AF-MSCs was induced using DNMT and p53 inhibitors, such as Decitabine, Zebularine, RG108 and Pifithrin- α . The formation of cardiomyocyte-like cells was determined by morphological changes, an upregulation of the main cardiac-genes (*TNNT2*, *MYH6* etc.) and cardiac ion channel genes together with an increase in the expression of Connexin43, the main gap junction component. The studied differentiation agents enhanced the metabolic potential, arrested the cell cycle at the G0/G1 phase and upregulated the expression of cell cycle regulators p53 and p21 in treated cells. Epigenetic changes during the differentiation were detected by alterations in chromatin remodeling proteins EZH2, SUZ12, DNMT1, HDAC1, HDAC2 and HP1 α as well as by activated histone modifications. In conclusion, all explored DNMT and p53 inhibitors induced cardiomyogenesis-related alterations of AF-MSCs to a different extent and caused global rearrangements in the chromatin state.



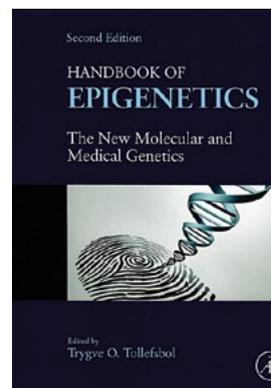
The Epigenetic Regulation of Myeloid Leukemia Cells

We investigated the potential of epigenetic modulators EGCG and BIX-01294 for altering the epigenetic state and causing cellular senescence in acute and chronic myeloid leukemia NB4 and K562 cells. We showed that after leukemia cell treatment with EGCG and BIX-01294, the proliferation and survival of both cell lines were inhibited; however, only NB4 cells underwent apoptosis. Both epigenetic modulators caused a cell cycle arrest in the G0/G1 phase. We also demonstrated that the EGCG had caused cellular senescence, whereas BIX-01294 did not. Epigenetic players such as DNMT1, HP1 α , H3K9me3, EZH2, and SUZ12 demonstrate beneficial epigenetic modulation by both agents, with the exception of mainly no epigenetic changes caused in K562 cells by EGCG. Therefore, we suggest EGCG as a promising epigenetic modulator for acute promyelocytic leukemia therapy and as a potential cellular senescence inducer in both acute and chronic myeloid leukemia treatment, whereas BIX-01294 could be beneficial as an epigenetic modifier for the treatment of both myeloid leukemias.



Combined Treatment and Targeted Therapy

Epigenetic therapy is a promising strategy in oncology, and it is based on returning transformed cells to a normal way of differentiation using a variety of chemical and bio-agents. In the “Combination Epigenetic Therapy” Chapter of *Handbook of Epigenetics*, the combined use of epigenetic modifying drugs, such as the inhibitors of DNA and histone methyltransferases and histone deacetylases, is reviewed by R. Navakauskiene. The therapeutic potential of DNMT1, HMT1 and HDAC1 alone and in combination as evaluated on cell lines and clinical trials plays a key role in the development of targeted epigenetic therapy.



CHAPTER 41
Combination Epigenetic Therapy
Rita Navakauskiene
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INTRODUCTION
The importance of chromatin structure for regulating gene expression regulation are recognized over the last few years. In eukaryotes, chromatin is organized from DNA and protein factors provides and maintains functional systems. This complex provides a three-dimensional genome organization. Chromatin is a dynamic constantly visible structure because of various DNA and histone modifications chromatin serves from one state to another, which leads to the important cell division and development processes. Chromatin is a dynamic macromolecular structure can be investigated according to the various structural and certain modifications is known as an epigenetic regulation. For example, several chemical agents may be classified by histone-hypomethylation. On the contrary, hypermethylation is associated as the methylated chromatin with modification of CpG islands of DNA. In progression and inheritance chromatin structure can lead to cell malignancy and various forms of cancer development. Chromatin modifications can change structure and influence other actions between on within the nucleosomes, and serve as the effects of chromatin remodeling [1]. In our days researchers widely work in the field of chemotherapy and gene therapy, attention for the finding of natural and synthetic compounds to construct on-



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

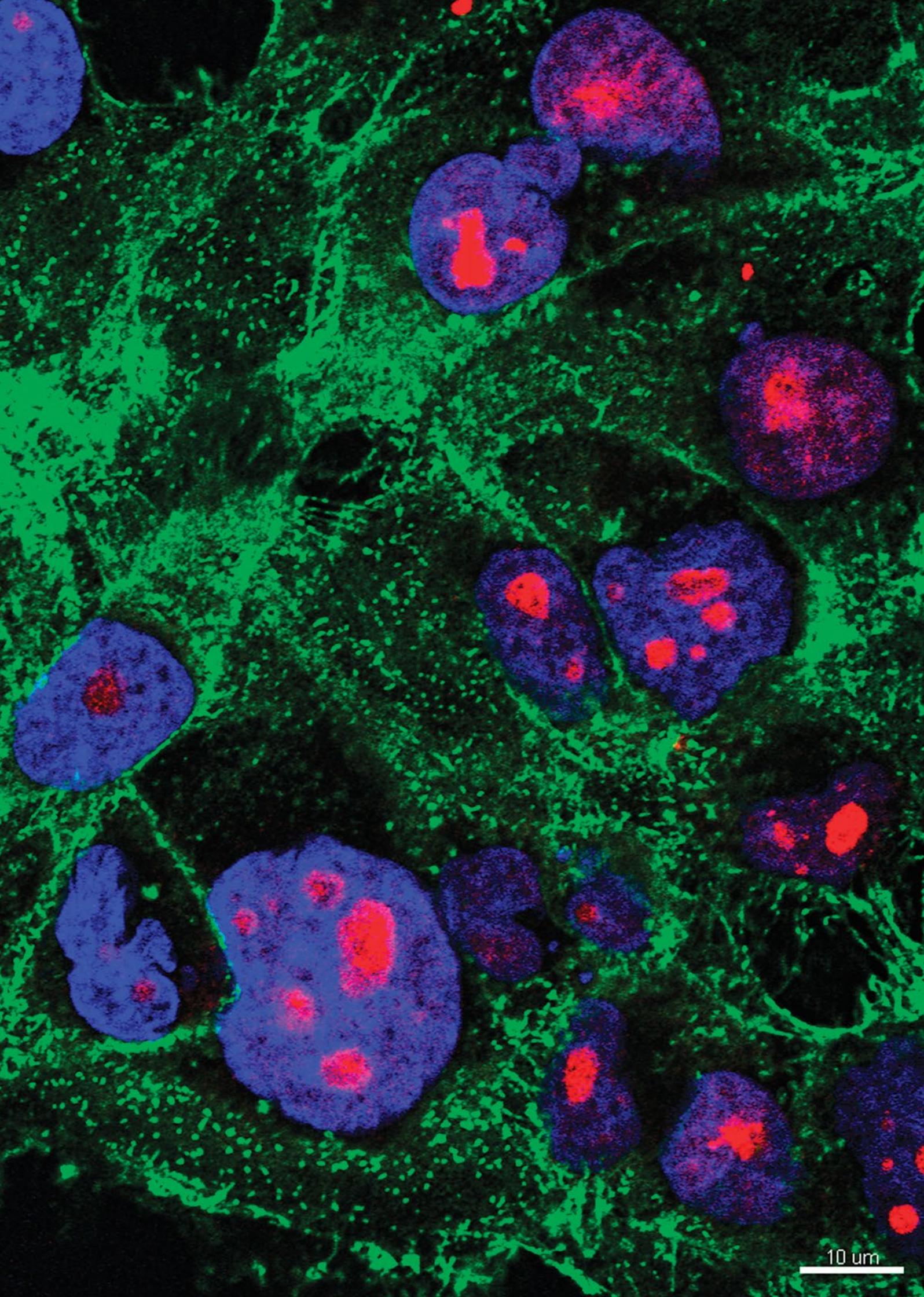
UV lasers have provided completely new possibilities for surgery and therapeutic treatments and are increasingly applied in medicine. A number of studies performed in the field of laser treatment and surgery have proved that there are femtosecond laser pulses that have advantages as compared with the longer duration pulses. Although the employment of femtosecond lasers as medical tools opens new possibilities for eye and skin treatment and surgery, the impact of their use on genetic material is not yet fully understood. 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. We investigated the genotoxicity of different plant products and showed that some natural compounds from *Agrimonia* and *Filipendula* species are relatively safe and have good potential for use in the food industry. 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., JSC Akiu Gydytoju 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.

SELECTED PUBLICATIONS



1. Pukalskienė, M., Slapšytė, G., Dedonytė, V., Lazutka, J.R., Mierauskienė, J., Venskutonis, P.R. *Food Chem. Toxicol.* 2018, 113, 303-313.
2. Fucic, A., Bonassi, S., Gundy, S., Lazutka, J., Sram, R., Ceppi, M., Lucas, J.N. *Anticancer Res.* 2016, 36, 2451-2458.
3. Danieliene E., Gabryte E., Vengris M., Ruksenas O., Gutauskas A., Morkunas V., Danielius R. J. *Biomed. Optics.* 2015, 20, 1-9.
4. Gricienė B., Slapšytė G., Mierauskienė J. *Radiat. Protect. Dosim.* 2014, 159, 10-19.



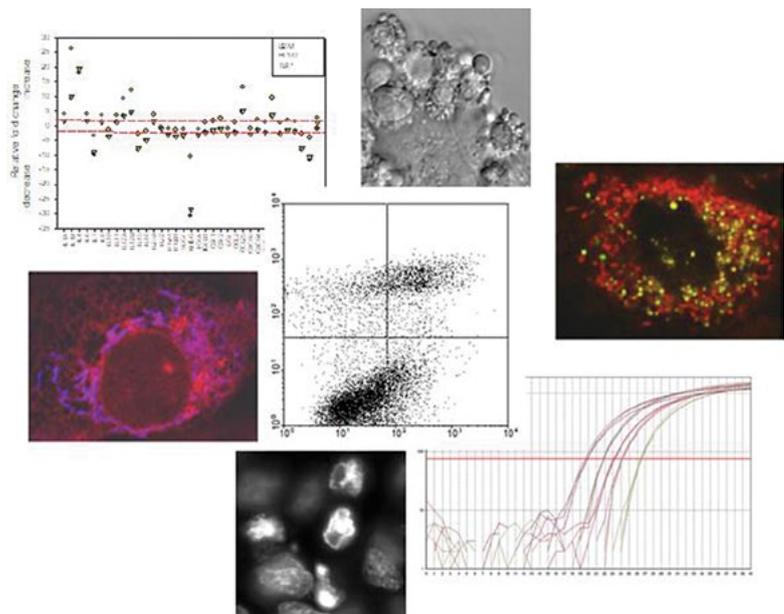
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Molecular Mechanisms of Cell Death and Survival

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. Chemoresistance is caused by various mechanisms, including alterations in drug transport and metabolism, the modification of drug targets, the activation of DNA repair, changes in cell death initiation or survival signaling. We focus our research on molecular mechanisms responsible for the cellular chemoresistance of colorectal cancer cells, in particular on the alterations of growth factor expression, the role of autophagy and the importance of Notch and Wnt signaling. Cancer cells often sustain their proliferation by overproducing growth factors. Autophagy is critically important for cell survival and the ability to overcome different stress conditions, mediating cytoprotective and rarely cytotoxic effects. Notch and Wnt signals control colon cell proliferation and stemness. The uncovering of molecular mechanisms maintaining the drug resistance of cancer cells would provide new targets for successful anticancer therapy.

The studies during the 2014–2017 period were performed by researchers D. Dabkevičienė, V. Jonušienė, A. Sasnauskienė and students E. Kukcinavičiūtė, V. Žitkutė, E. Žalytė, P. Grigaitis, E. Eidėnaitė, A. O. Jančauskaitė. 5-fluorouracil (5-FU) and oxaliplatin (OxaPt) chemoresistant sublines HCT116/FU and HCT/OXA were derived from human colorectal cancer cells HCT116 by V. Jonušienė. These cells displayed an altered length of cell cycle and response to chemotherapeutic drugs. In collaboration with the Proteomic Center (M. Valius), it was determined that chemoresistant cells display altered expression levels of dozens of proteins related to cell death, signaling and autophagy. One of our goals was to evaluate the changes in different cytokine expression levels after the acquisition of chemoresistance. Few dozens of cytokines and their receptors were quantified at the transcriptional level and the most pronounced changes were confirmed by a protein analysis. We have characterized the autophagic response after 5-FU treatment and compared the role of autophagy for the survival of chemoresistant and sensitive cells. The activity and functional importance of Notch and Wnt signalling pathways was compared in chemoresistant and sensitive cells.

SELECTED PUBLICATIONS

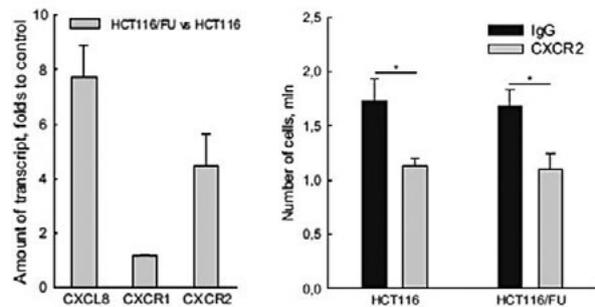


1. Effect of mTHPC-mediated photodynamic therapy on 5-fluorouracil resistant human colorectal cancer cells. Kukcinavičiūtė E, et al. *Photochem Photobiol Sci.* 2017;16(7):1063-1070.
2. The role of interleukin-8 (CXCL8) and CXCR2 in acquired chemoresistance of human colorectal carcinoma cells HCT116. Dabkevičienė D, et al. *Med Oncol.* 2015;32(12):258.
3. Differential expression of VEGF and IL-1α after photodynamic treatment in combination with doxorubicin or taxotere. Dabkevičienė D, et al. *Anticancer Res.* 2014;34(10):5295-302.

The Role of Interleukin-8 and Its Receptors

We have analyzed the expression of interleukin-8 (CXCL8) and its receptors CXCR1 and CXCR2 in chemoresistant HCT116/FU and 5-FU or OxaPt sensitive human colorectal carcinoma HCT116 cells. Interleukin-8 and its receptors were found to be upregulated in the chemoresistant subline HCT116/FU. Chemoresistant cells remained sensitive to the blockade of the CXCR2 pathway, as it reduced the cell number by approx. 30%.

Interleukin-1 alpha was found to stimulate the production of interleukin-8.

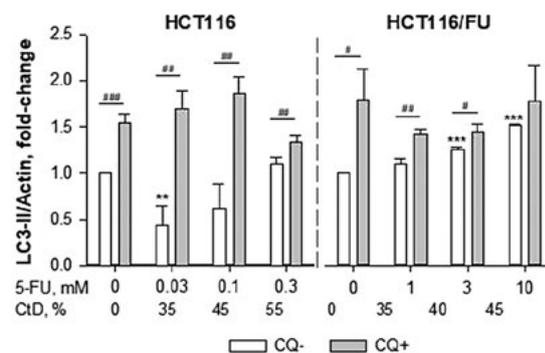


Dabkeviciene, et al. *Med Oncol.* 2015;32(12):258

The Autophagic Response after Chemotherapeutic and Photodynamic Treatment

We have determined the amount of autophagosomes and autophagic flux according to changes of LC3-II protein levels. Our data indicated that 5-FU increased the autophagic flux in HCT116 cells, but the opposite tendency was observed in HCT116/FU cells. A photodynamic treatment (PDT) mediated by mTHPC (meso-tetrahydroxyphenylchlorine) raised the amount of autophagosomes but not the autophagic flux. In the case of the combined treatment of 5-FU and PDT, the amount of autophagosomes increased in both cell lines, supposedly due to the PDT component. An upregulated autophagic flux was observed after a combined PDT and 5-FU treatment in HCT116, not in the HCT116/FU cells.

Our results demonstrate that mTHPC-PDT overcomes 5-FU resistance and is effective against chemoresistant colorectal carcinoma cells.

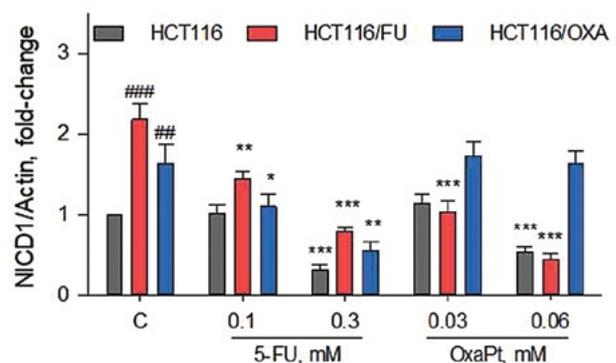


ukcinaviciute, et al. *Photochem Photobiol Sci.* 2017;16(7):1063-1070

The Activity of Notch and Wnt Signaling Pathways

We have found that Notch and Wnt signaling is upregulated in untreated 5-FU or OxaPt chemoresistant HCT116 cells. A treatment with either 5-FU or OxaPt reduced the Notch and Wnt signaling. The roles of Notch and Wnt pathways for cell survival 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.

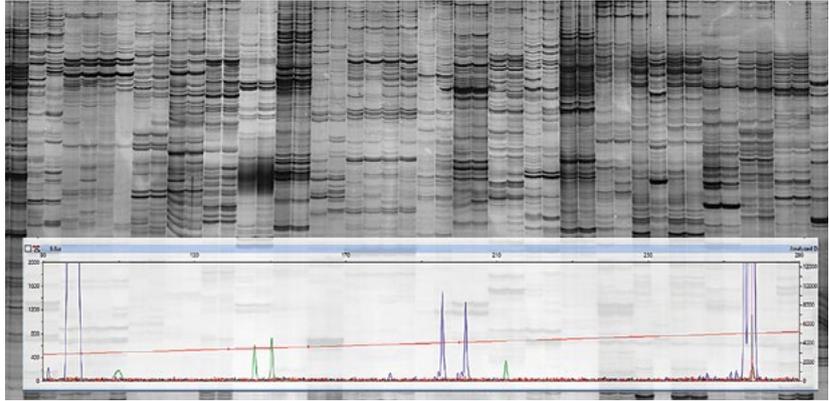
A silencing of HES1, a Notch and Wnt effector, increased the chemoresistance to 5-FU and OxaPt.



Ukcinaviciute, et al. *J Cell Biochem.* 2018 Jul;119(7):5913-5920.



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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 signaling 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. A 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 particular interest was in inflorescence/flower development and the phenomenon of inherited phenotype instability in homeotic barley mutants. The effects of auxin inhibitors suggest that ectopic auxin maxima or deficiencies arise in various regions of the inflorescence/flower primordia [1]. 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 [2, 3, 4] were carried out in collaboration with colleagues from the Nature Research Center. 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 [4].

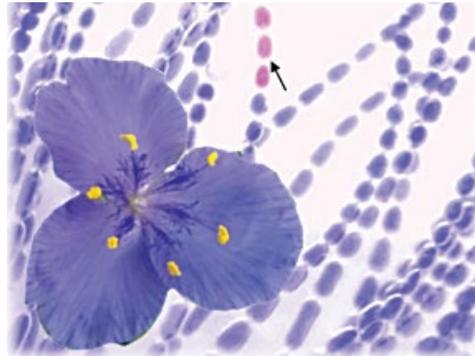
SELECTED PUBLICATIONS



1. Šiukšta, et al. Inherited phenotype instability of inflorescence and floral organ development in homeotic barley double mutants and its specific modification by auxin inhibitors and 2,4-D. *Annals of Botany* 2015, 115, 651-663.
2. Vyšniauskienė, et al. ISSR and chloroplast DNA analyses indicate frequent hybridization of alien *Medicago sativa* subsp. *sativa* and native *M. sativa* subsp. *falcata*. *Plant Systematics and Evolution* 2015, 301, 2341-2350.
3. Butkuvienė, et al. Genetic diversity of *Batrachium* (Ranunculaceae) species reveals the necessity of their protection in Lithuanian rivers. *Aquatic Botany* 2017, 142, 61-70.
4. Čėsniienė, et al. Metal bioaccumulation and mutagenesis in a *Tradescantia* clone following long-term exposure to soils from urban industrial areas and closed landfills. *Mutation Research - Genetic Toxicology and Environmental Mutagenesis* 2017, 823, 65-72.

Metal Bioaccumulation and Mutagenesis in a *Tradescantia* Clone Following Long-Term Exposure to Soils from Urban Industrial Areas and Closed Landfills

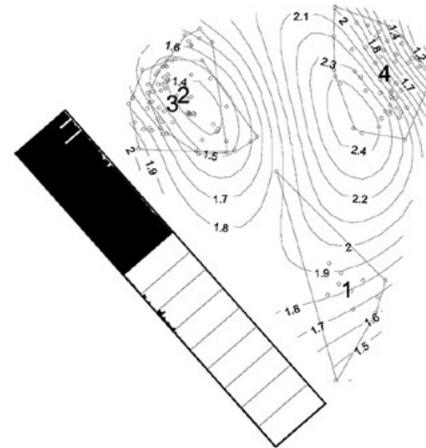
Consider that soil mutagens, particularly metals, may persist long after the source of pollution has been removed, representing a hazard to organisms, we exposed *Tradescantia* clone #4430 plants to soil from industrial areas (with different former uses) and urban waste landfills in the city of Vilnius, all of which were long disused. By using different modes of exposure of the growing plants in test soils, we have observed an increased frequency of micronuclei with both modes of exposure. The concentrations of 24 metals and other elements were analyzed in the test soils and in above-ground plant parts, and the concentration coefficients for various elements, the total contamination index for soils and plants and the bioaccumulation factor for plants were calculated. Metal accumulation levels in plants and soils showed significant



differences, providing a better understanding of the genotoxicity of soils from closed landfills and highlighting the need to determine the concentrations of metals and other genotoxicants in plants in relation to genotoxicity. Čėsniėnė et al. *Mutat Res Gen Tox En* 2017, 823, 65-72.

The Genetic Structure and Diversity of *Batrachium* Populations

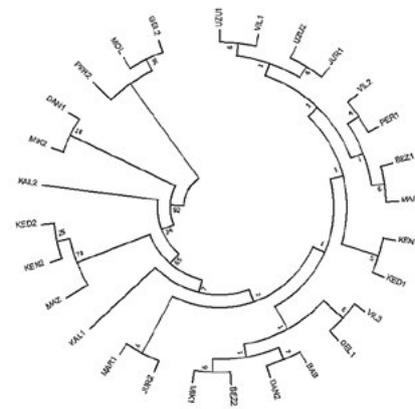
Though *Batrachium* species are an indicator of the sustainability of riverine ecosystems, studies on the genetic variation of *Batrachium* populations are rather scarce. Therefore, we studied *Batrachium* species' diversity and population structures to provide measures for evaluating the future risk of diversity loss. Sixteen *Batrachium* populations were analyzed using morphological characters and molecular markers (ISSRs and trnH-psbA sequences). The study revealed an agreement between these types of data in distinguishing separate groups of species found in Lithuanian rivers. Our study revealed a clonal structure and a low level of genetic diversity in most of the studied *Batrachium* populations. The low degree of genetic diversity, the high level of genetic differentiation and the low level of gene flow between populations of riverine *Batrachium* species in Lithuania indicated that a strategy of conservation should be



adopted. We recommend that certain anthropogenic activities should be regulated by legislation in particular river stretches. Butkuviėnė et al. *Aquat Bot* 2017, 142, 61-70.

Genetic and Allelopathic Differences between Populations

We investigated whether habitat changes during the invasion process are related to variations in the physiological traits (allelopathic properties) and genetic differentiation of *Erigeron annuus*. Genetic and genotypic diversity analyses were performed for 37 populations based on ISSR polymorphisms. The genetic differences among the populations from the different habitats were studied using a Bayesian cluster analysis and AMOVA and by calculating the genetic and genotypic diversity parameters. A Bayesian cluster analysis, AMOVA and allelopathic effects evaluation revealed differences in the allelopathic potential and genetic structure of the *E. annuus* populations from disturbed and stable habitats. Tunaitienė et al. *Biochem Syst Ecol* 2017, 70, 294-303.




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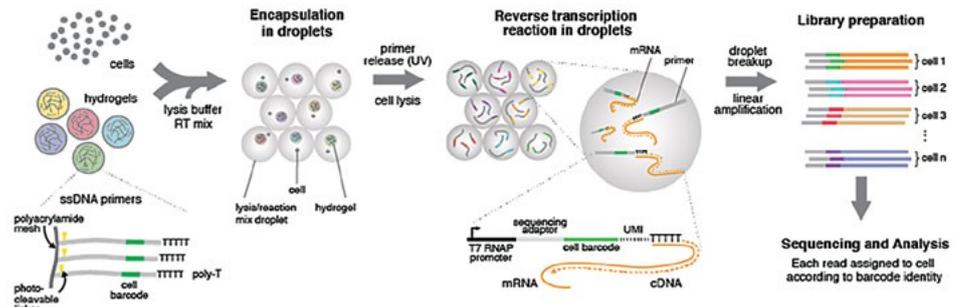
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 Figure 1. The schematics of the *inDrops* technique.

Single-Cell Transcriptomics and Genomics

The advances in DNA sequencing technologies over the last decade have fundamentally transformed our understanding of disease progression, cancer, the immune system, embryo development and many other biological systems. However, the most common techniques harvest genomic DNA (or RNA) from a mixture of cells, thus giving an “average genome” that does not represent the important differences between individual organs and diseased tissues or the diversity of cells. Cellular heterogeneity is particularly relevant in cancer research, since any biopsy or clinical sample will likely represent a mixture of cells from both tumor and healthy tissues. Single-cell sequencing can delineate the differences between individual cells and identify the biological mechanisms that remain obscured in bulk genomic studies. Besides the genomic alterations, individual cells show profound differences at transcriptome and epigenome levels that cannot be discerned in bulk studies. Single-cell transcriptomics and epigenomics technologies have the potential to unlock the fundamental features of seemingly uniform cell populations, ultimately leading to a deeper understanding of biology and disease systems. Our group is pursuing research in cancer and immune system biology, aiming at better understanding the genetic programs that drive tumor heterogeneity and immune response.

Our group has pioneered the droplet microfluidics technique *inDrops* (*indexing Drops*) for barcoding the transcriptome of single-cells (Klein, Mazutis, *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., tumors) and how the immune system responds. In collaboration with the Harvard Medical School (Prof. Allon Klein), we have studied the pluripotency of mouse embryonic cells (1), the T-cell activation in tumors (2) and the osteoblast role in lung adenocarcinoma (3), all single-cell level. In collaboration with Memorial Sloan Kettering Cancer Center and Columbia University (Prof. Dana 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).

SELECTED PUBLICATIONS



1. Klein M*, Mazutis L*, Akartuna I*, Tallapragada N, Veres A, Li V, Peshkin L, Weitz D and Kirschner M, (2015) Droplet barcoding for single cell transcriptomics applied to embryonic stem cells, *Cell*, 161(5): 1187-1201.
2. Zemmour D, Zilionis R, Kiner E, Klein AM, Mathis D, Benoist C. Single-cell gene expression reveals a landscape of regulatory T cell phenotypes shaped by the TCR. *Nat Immunol*. 2018 Mar; 19(3):291-301.
3. Engblom C, Pfirschke C, Zilionis R, Da Silva Martins J, Bos SA, Courties G, Rickelt S, Severe N, Baryawno N, Faget J, Savova V, Zemmour D, Kline J, Siwicki M, Garric C, Pucci F, Liao HW, Lin YJ, Newton A, Yaghi OK, Iwamoto Y, Tricot B, Wojtkiewicz GR, Nahrendorf M, Cortez-Retamozo V, Meylan E, Hynes RO, Demay M, Klein A, Bredella MA, Scadden DT, Weissleder R, Pittet MJ. Osteoblasts remotely supply lung tumors with cancer-promoting Siglec-high neutrophils. *Science*. 2017 12 01; 358(6367).
4. Azizi E, Carr JA, Plitas G, Cornish A, Konopački C, Prabhakaran S, Nainys J, Kenmin Wu, Kiseliovas V, Setty M, Choi K, Dao P, Mazutis L, Rudensky YA, Pe'er D. Single-Cell Immune Map of Breast Carcinoma Reveals Diverse Phenotypic States Driven by the Tumor Microenvironment, *BiorXiv* doi: <https://doi.org/10.1101/221994>.
5. Dijk vD, Sharma R, Nainys J, Kathail P, Carr JA, Moon RK, Mazutis L, Wolf G, Krishnaswamy S, Pe'er D. MAGIC: A diffusion-based imputation method reveals gene-gene interactions in single-cell RNA-sequencing data, *BiorXiv* doi: <https://doi.org/10.1101/111591>.



Embryonic Stem Cell Development Studies

It is postulated that embryonic stem (ES) cells are characterized by a promiscuous gene expression that becomes refined upon differentiation. We have evaluated the distribution of ES cell transcriptional states during the development and found that the intrinsic dimensionality of gene expression space decreases during differentiation. We have identified rare subpopulations expressing markers of distinct lineages and showed that key pluripotency factors fluctuate in a correlated manner across the entire embryonic stem cell population. Upon differentiation, we

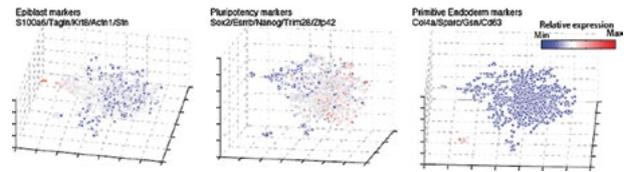


Figure 2. The transcriptional space of mouse embryonic stem cells. The relative expressions of epiblast, pluripotency and primitive endoderm markers are shown.

observe dramatic changes in the gene expression profile, resulting from an asynchronous inactivation of pluripotency factors, and the emergence of novel cell states.

Computational Tools for Single-Cell RNA-Seq Data

Single-cell RNA-seq (scRNA-seq) is a powerful method that enables learning gene-gene relationships in a system-wide scale, based on naturally occurring variation. However, the data generated during scRNA-seq captures only a small fraction, typically 5–15%, of the transcriptome, an issue known as a “dropout.” This problem obscures gene-gene interactions and thus the interpretation of biological information retrieved from transcriptomics studies. In collaboration with Prof. Dana Peer (Columbia University) and Prof. Smita Krishnaswamy (Yale University), we developed an imputation method that we call MAGIC (Markov Affinity-based Graph Imputation of Cells). We show that MAGIC 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

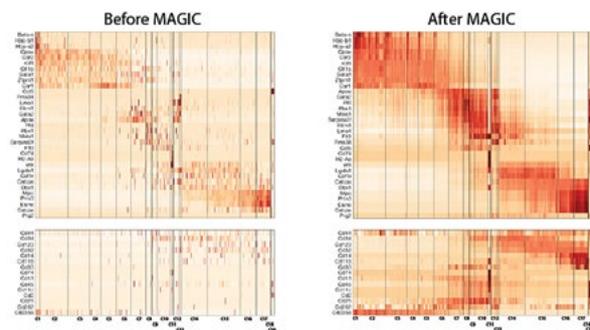


Figure 3. Th Markov Affinity-based Graph Imputation of Cells (MAGIC), a computational approach that shares information across similar cells to recover the information of missing transcripts.

explored the EMT and revealed a continuum of states, where the majority of cells reside in intermediate states that display stem-like signatures.

Transcriptional Profiling of Tumor-Infiltrating Lymphocytes

It may seem surprising that given the wealth of data available, we still have no consensus regarding the complex cellular mechanisms that mediate tumor recognition, tolerance and clearance. We know that the anti-tumor 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. Therefore, a better understanding of the transcriptional landscape of tumor infiltrating lymphocytes (TIL) has important implications for developing new immunotherapy treatment strategies. In this project, we are undertaking a large-scale, high-dimensional, single-cell analysis of cells of hematopoietic origin in human breast tumors of various types – as well as paired normal breast tissue, peripheral blood, and a lymph node – using single-cell RNA-seq. Our preliminary analyses revealed a remarkably increased heterogeneity of intratumoral cells of both lymphoid and myeloid

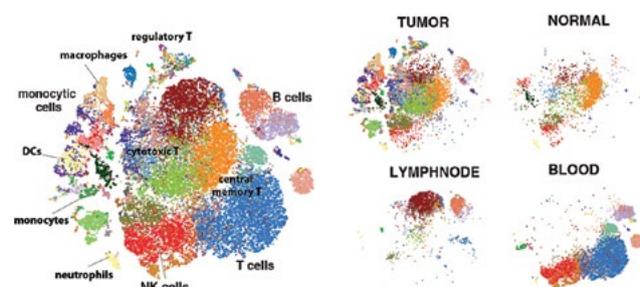


Figure 4. The transcriptional atlas of breast tumor infiltrating immune cells.

cell lineages, which occupy a markedly expanded contiguous phenotypic space in comparison to normal breast tissue. The observed continuum of cell states likely reflects their progressive cellular activation and differentiation and argues strongly against the notion of a few discrete states of differentiation or the activation of individual cell types shaping the tumor microenvironment.

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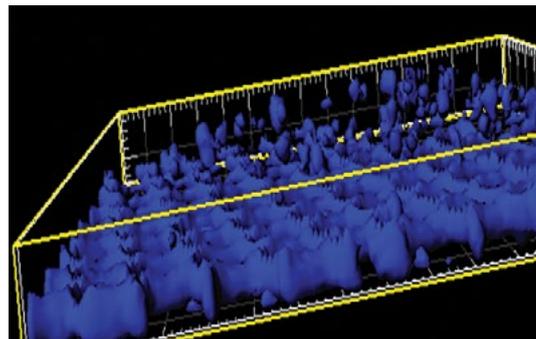
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Stem Cell Technologies for Tissue Engineering

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 behavior, 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: (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 four years, we have developed strategies for creating chemically modified materials with improved physical properties and studied their ability to modulate cellular properties. 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. Such co-culture systems are being increasingly used in biomedical research. These complex systems mimicking the interactions of the native tissues combined with 3D scaffolds are a favorable strategy for tissue engineering.

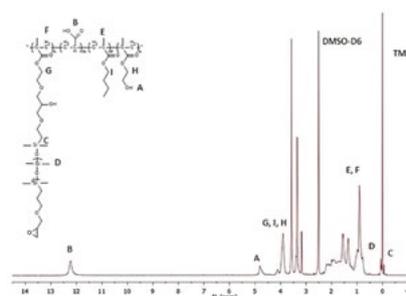
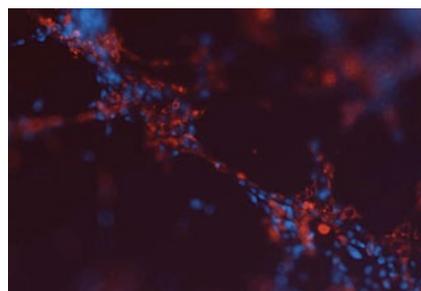
SELECTED PUBLICATIONS



1. Trumpaitė-Vanagiene R., et al. Cytotoxicity of commonly used luting cements - an *in vitro* study. *Dent Mater J.* 2015, 34(3):294-301.
2. Rutkunas V., et al. Assessment of human gingival fibroblast interaction with dental implant abutment materials. *J Mater Sci Mater Med.* 2015, 26(4):169.
3. Malinauskas M., et al. 3D microporous scaffolds manufactured via a combination of fused filament fabrication and direct laser writing ablation. *Micromachines.* 2014, 5(4):839-58.
4. Baltriukienė D., et al. The effect of laser-treated titanium surface on human gingival fibroblast behavior. *J Biomed Mater Res A.* 2014, 102(3):713-20.

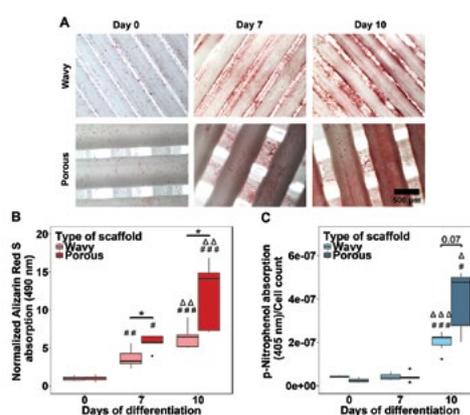
Soft Tissue Engineering: From Cell to Artificial Tissue

The aim of this study is to create a fragment of a functional artificial tissue based on a co-culture of two different cell types and a chemically modified 3D elastomer – poly(dimethylsiloxane) (PDMS) – surface. PDMS has long been used as a flexible, biocompatible substrate for cell culture with tunable mechanical characteristics. However, its fragility and hydrophobicity are still considered as challenges for tissue engineering. Therefore, we have developed a new strategy of a one-pot, three-step synthesis of novel, UV curable, hydrophilic copolymers containing siloxane moieties. These copolymers exhibit good wettability due to hydrophilic fragments, good biocompatibility and elasticity due to the introduction of the siloxane units and good mechanical properties mainly due to a cross-linked structure of the films. Next, the best conditions required for the co-culture of two different types of cells – endothelial (EPCs) and myogenic (MPCs) – were chosen. We have found that the co-culture of MPCs and EPCs is mutually beneficial to each other.



The Effect of Scaffold Topography in Stem Cell Differentiation

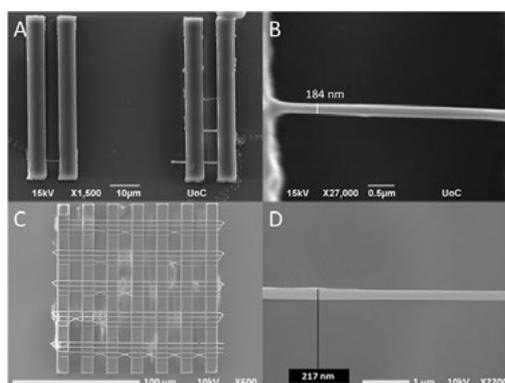
This study is focused on the impact of biodegradable polylactic acid (PLA) scaffold's surface topography on the adhesion of rat's dental pulp stem cells (DPSC), their proliferation and osteogenic differentiation. PLA scaffolds with two different (wavy and porous) topographies were created using 3D printing. The results showed that the surface topographies did not have an impact on the adhesion of DPSC. However, both types of the PLA scaffolds enhanced the proliferation of DPSC. Osteogenic differentiation results demonstrated that porous PLA scaffolds were more suitable for matrix mineralization as compared to the wavy ones; however, an increase in osteogenesis-related gene expression and alkaline phosphatase activity were registered in the cells grown on both types of scaffolds. Finally, it was shown that both types



of PLA topographies were sufficient for inducing spontaneous DPSC differentiation toward an osteogenic lineage.

A Hybrid Organometallic Aluminium-Containing Polymer Biomaterial

The Al-incorporating polymer was prepared by the photopolymerization of a metal complex (the product of the reaction of aluminium isopropoxide, methacrylic acid and 3-(trihydroxysilyl) propyl methacrylate) in the presence of a photoinitiator 4,4'-bis(diethylamino)benzophenone. 3D structures were fabricated using two different laser two-photon polymerization systems and characterized by SEM. Different organometallic polymers containing Al, Ti, V and Zr were then subjected to an energy dispersive X-ray analysis and surface contact angle measurements. Their biocompatibilities were tested by culturing NIH/3T3 fibroblasts on spin-coated films and using an MTT assay for cell viability and an acridine orange/ethidium bromide staining for determining the mechanisms of cell death. Organometallic polymers containing Al,



Ti and Zr supported cell adhesion and proliferation, and showed low toxicity *in vitro*, whereas the organometallic polymer incorporating V was shown to be cytotoxic with the majority of cells dying via necrosis.

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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 *Candida* genera yeast are capable of undergoing morphology switches and form pseudohyphae structures with highly increased resistance to the antifungal compounds. We discovered that after growth in a rotary cell cultivation system (RCCS), a new, super-resistant and morphology-switching-unrelated phenotype of *Candida* is formed. RCCS is changing the pattern of the antibiotic resistance of *Pseudomonas aeruginosa* and *Staphylococcus aureus* as well. The discovery of the natural antifungal and antibacterial substances, synthesized by microorganisms, is promising alternative for the antibiotics. 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.

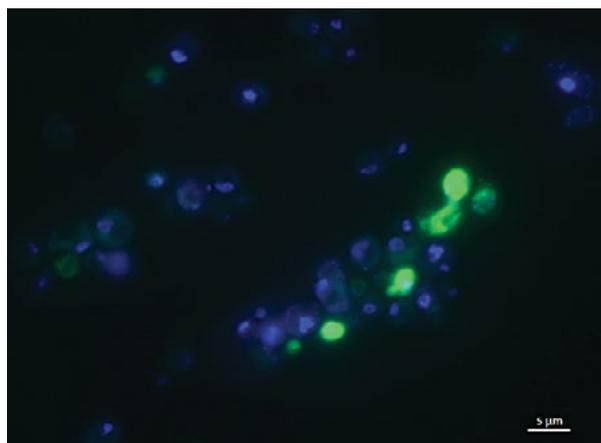
Our team first showed that *Candida guilliermondii* can undergo morphology switching and form pseudohyphae structures with an extremely increased resistance to antifungal treatment [1]. Together with the group of V. Novickij (Vilnius Gediminas Technical University), we successfully applied the PEF and PEMF technologies for the biocontrol of the skin diseases causing microorganisms and optimized the pathogen inactivation parameters [2]. We discovered that yeast and bacteria cultivation in the rotary cell culture system greatly change the physiology and resistance pattern of all the microorganisms. Another powerful tool for developing new antibiotics or biocatalysts for industrial and pharmaceutical applications is protein engineering. Protein engineering allows us to overcome the limitations of natural enzymes and holds 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.

SELECTED PUBLICATIONS

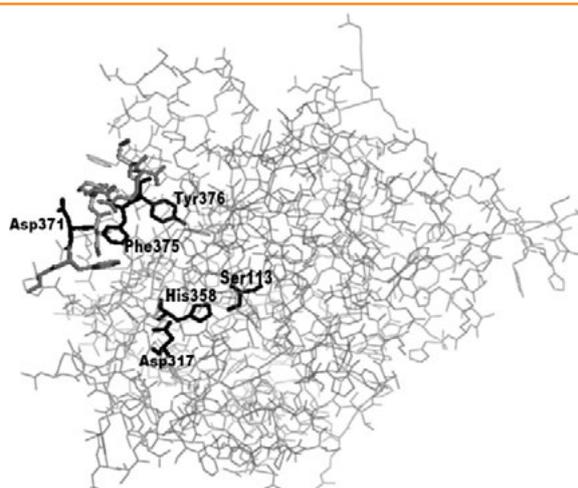


1. Lastauskienė E., et al. Phenotypic switching of *Candida guilliermondii* is associated with pseudohyphae formation and antifungal resistance. *Mycopathologia*. 2015. 179:205-211.
2. Novickij V. et al. Pulsed Electromagnetic Field Assisted *in vitro* Electroporation: A Pilot Study. *Scientific Reports*. 2016. 6:33537.
3. Gudiukaitė R., et al. Influence of N- and/or C-terminal regions on activity, expression, characteristics and structure of lipase from *Geobacillus* sp. 95. *Extremophiles*. 2014. 18: 131-145
4. Gudiukaite R., et al. Construction of a novel lipolytic fusion biocatalyst GDEst-lip for industrial application. *J Ind Microbial Biotechnol*. 2017. 44(6):799-815.

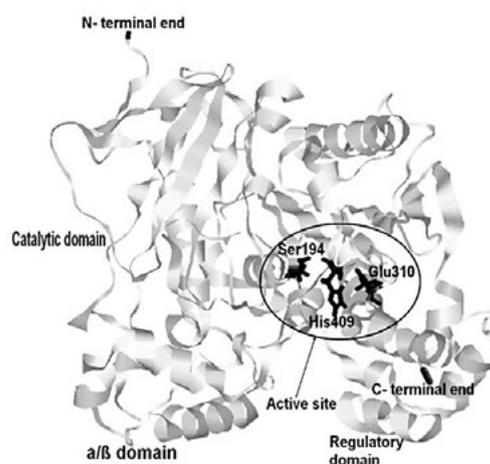
The *Candida* genera yeast-caused infections are frequent and difficult to treat, as the physiology and metabolisms of yeast are similar to the host. The discovery of the PEF and PEMF parameters suitable for inducing apoptosis in *Candida* cells is of great medical relevance, since during the apoptosis, peptides, amino acids and etc. are released into the surrounding medium and can contribute to the regeneration of human tissues. The success of the PEF and PEMF on the inactivation of the microorganisms depends on the growth conditions, growth phase and microorganisms itself. PEF and PEMF can be successfully combined with antifungal compounds and help reduce the effective doses of the therapeutic agents. We optimized the PEF parameters for the apoptosis induction in *Candida*. For the apoptotic phenotype detection, we performed active caspase staining, a TUNEL reaction and a phosphatidylserine externalization analysis in the *Candida* yeast. Apoptotic phenotypes were detected by using fluorescent microscopy and flow cytometry.



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 at extreme conditions. The structure-function relationships studies of these biocatalysts can help to design new beneficial biocatalysts. Our analysis showed that the N- and C-terminal regions are very significant on the activity and functionality for GD-95 lipase. It was detected that at the C-terminal end, there were located three conservative amino acids Asp371, Phe375 and Tyr376, and that they play a significant role on the functionality of this enzyme (Gudiukaitė et al. *Extremophiles*, 2014, 18:131-145; Gudiukaite et al. *App Biochem Biotechnol*, 2016, 178:654-669).



Because of the regio- and stereospecificity and substantial activity in organic solvents, lipases and esterases have been recognized as very useful biocatalysts in industrial applications, such as the production of pharmaceuticals, detergents, medical diagnostics etc. The GDEst-95 esterase was the first carboxylesterase produced by *Geobacillus* bacteria with 55 kDa molecular size analyzed in-depth and used in protein engineering experiments. This esterase, together with the GD-95 lipase, were used for the construction of the new fused lipolytic chimeric biocatalyst GDEst-lip. This new enzyme demonstrated improved lipolytic activity and physicochemical characteristics as compared to parental enzymes, and it is a promising biocatalyst for applications in various industrial areas, including the synthesis of precursors of active compounds against microorganisms caused by skin infections (Gudiukaite et al. *J Ind Microbial Biotechnol*, 2017, 44(6):799-815).



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Animal Biodiversity, The 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 catalogization 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, Sternorrhyncha: Aphididoidea and Adelgoidea), spiders, slugs (Mollusca: Gastropoda), freshwater fishes, birds of prey and owls, black storks. Research topics include taxonomy and systematics (including morphological and molecular methods based on the material from different parts of the world), distribution, ecology and economic importance and the monitoring of local faunas in the protected areas of Lithuania (the Cepkeliai, Kamanos and Viesvile State Strict Nature Reserves; the Aukštaitija, Dzūkija, the Curonian Spit and Žemaitija National Parks; the Zuvintas Biosphere Reserve and other protected areas).

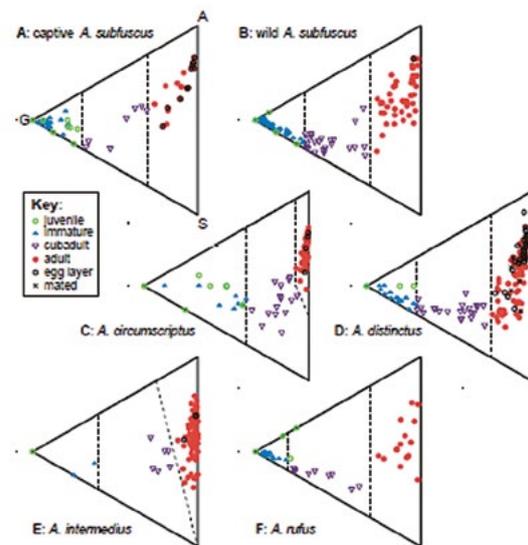
SELECTED PUBLICATIONS



1. Hutchinson, J. M.C.; Reise, H., Skujienė, Grita. Life cycles and adult sizes of five co-occurring species of Arion slugs // *Journal of molluscan studies*. Oxford: Oxford University Press. ISSN 0260-1230. eISSN 1464-3766. 2017, Vol. 83, No. 1, p. 88-105.
2. Petrašiūnas, A., Podėnas, S. New data on winter crane flies (Diptera: Trichoceridae) of Korea with description of a new species // *Zootaxa*. Auckland: Zootaxa. ISSN 1175-5326. eISSN 1175-5334. 2017, Vol. 4311, Iss. 4, p. 561-575.
3. Rakauskas, V., Masiulytė, R., Pikūnienė, A. Predator-prey interactions between a recent invader, the Chinese sleeper (*Percottus glenii*) and the European pond turtle (*Emys orbicularis*): a case study from Lithuania // *Acta Herpetologica*. Firenze: Firenze University Press. ISSN 1827-9635. eISSN 1827-9643. 2016, Vol. 11, No. 2, p. 101-109.
4. Rakauskas, R., Havelka, J., Bernotienė, R. *Rhopalosiphum rufiabdominale*: first records from winter host plants in Europe // *Bullettin of insectology*. Bologna: Università di Bologna. ISSN 1721-8861. 2015, Vol. 68, no 1, p. 73-81.

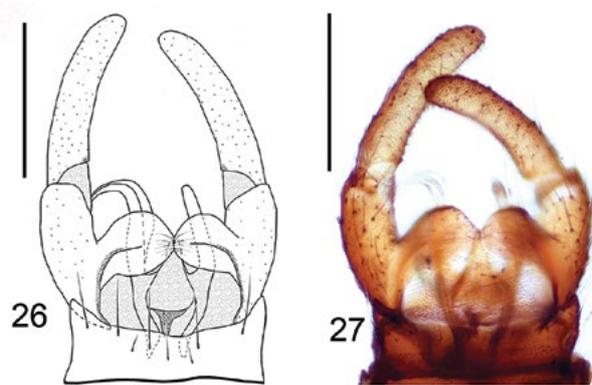
Life Cycles and Adult Sizes of Five Co-occurring Species of Arion Slugs

Arion slugs were collected repeatedly at a woodland site in southern England. The relative weights of the gonad, spermatiduct and albumen gland provided the basis to categorize it into adult, subadult or as juvenile. The time of year at which individuals matured into adults varied between species: *A. intermedius* in August and September, *A. distinctus* in December and January, *A. circumscriptus* from January to April, *A. subfuscus* from April to October and *A. rufus* from July to September. The largest two species thus dominated during summer, but at other times, the species overlapped considerably in size. In four species, individuals maturing later in the season did so at a smaller size; the exception was *A. intermedius*, the maturation of which was highly synchronized. The coefficients of variation in adult size were compared against a collection of such data from other terrestrial molluscs. *A. intermedius* had disproportionately large hatchlings (Hutchinson et al. 2017).



New Data on the Winter Crane Flies (Diptera: Trichoceridae) of Korea with a Description of a New Species

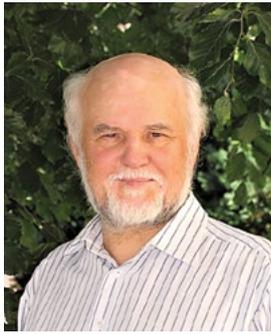
A long-term project of the diversity of the Tipulomorpha of Korea is carried out in cooperation with research institutions from Korea, the US, Hungary and other countries. One of the outcomes of the project was a review of the Trichoceridae family. Eight of the species of this family have been recorded in Korea for the first time. Two taxa are proposed as synonyms, one is transferred to a subspecific rank, and a new species – *Trichocera (Saltrichocera) latipons* sp. nov. is described as new to science (Petrašūnas, A. and Podėnas, S. 2017).



The Distribution, Host Specificity and Molecular Diversity of Exotic Aphid Species *Brachycaudus divaricatae* in Central Europe

The Aphid species *Brachycaudus divaricatae* Shaposhnikov, 1956, originally described as appearing in Turkmenistan and earlier known from the Middle East and Eastern Europe only, is a recent invader in Central Europe. It is today the most common pest on the cherry plum in the Eastern Baltic region of Europe, including also Belarus and Northern Ukraine. *B. divaricatae* is closely related to *Brachycaudus lychnidis*, which is native to Europe. The present study provided new information on the distribution of *B. divaricatae* in Europe and to its genetic variability together with that of the closely related species *B. lychnidis* using two molecular markers, mitochondrial COI and nuclear EF-1 α (Havelka et al. 2017).





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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 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 the members of several lineages within plants, algae and fungi. We use up-to-date approaches to study the species' diversity and the population structure of terrestrial and aquatic plants, algae, lichens and fungi. 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. Sites with emerging juvenile club moss populations in dry pine forests are characterized by vegetation composition stability, an absence of tree canopy shading and a presence of the herbaceous plant *Deschampsia flexuosa* [1]. The life, scientific activities and discoveries of the botanist and social figure Dr. Abromas Kisinias (1899–1945) were investigated by the analysis of the historical herbarium collections and a biographical approach [2]. In collaboration with our Italian colleagues from the Institute of Ecosystems Studies in Florence, we discovered that lipid production for biodiesel conversion can be successfully accomplished by culturing *Chlorella vulgaris* under repetitive batch growth regimen conditions, with the replacement of fresh culture broths deprived of nitrogen. The high pH-stressed conditions imposed at the end of the growth cycle are a suitable technique for reducing the high cost of harvesting the *Chlorella* biomass. For the laboratory experiment, cylindrical and flat glass photobioreactors were used [3]. 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 [4].

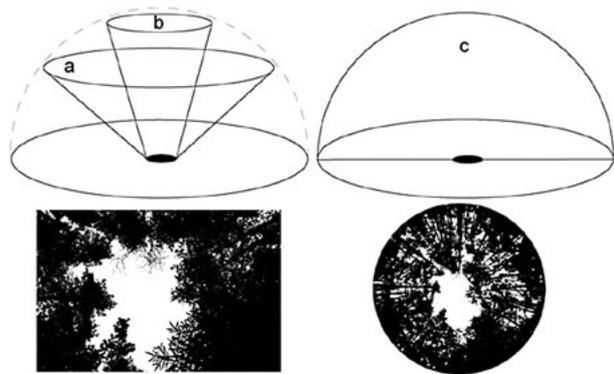
SELECTED PUBLICATIONS



1. R. Rimgailė-Voicik, J.R. Naujalis. Presence of juvenile club moss (*Lycopodiaceae*) sporophytes and gametophytes in relation to vegetation cover in dry pine forests. *Am Fern J.* 2016, 106(4), 242-257.
2. J.R. Naujalis, R. Rimgailė-Voicik. Plant community associations and complexes of associations in the Lithuanian seashore: retrospective on the studies and tragic fate of the botanist Dr Abromas Kisinias (1899-1945). *Israel J Plant Sci.* 2016, 63 (3), 167-175.
3. S. Vaiciulyte, et al. Batch Growth of *Chlorella vulgaris* CICALA 896 versus Semi-Continuous Regimen for Enhancing Oil-Rich Biomass Productivity. *Energies* 2014, 7 (6), 3840-3857.
4. J. Motiejūnaitė, et al. Early fungal community succession following crown fire in *Pinus mugo* stands and surface fire in *Pinus sylvestris* stands. *Eur J For Res.* 2014, 133, 745-756.

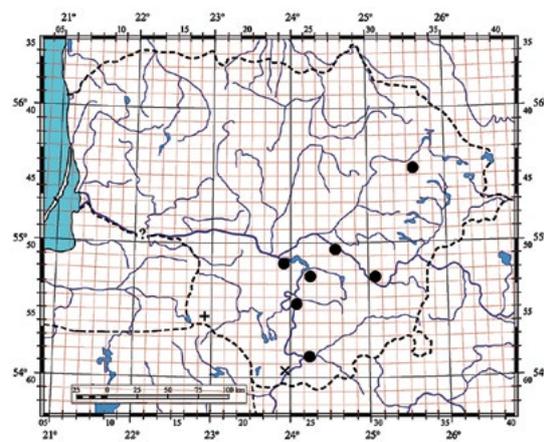
The Impact of Clear Cuttings on the Transformation of Biodiversity in Forest Ecosystems

The task of our group in the research project, in collaboration with researchers from Vytautas Magnus University and the Lithuanian Research Centre for Agriculture and Forestry, is to determine the response of forest ecosystem components to drastic changes of vegetation in forest ecosystems affected by clear cuttings. Our studies have shown that the canopy closure calculated from hemispheric photographs correlates with the horizontal structure characteristics of *Lycopodium annotinum* and *L. clavatum* clones.



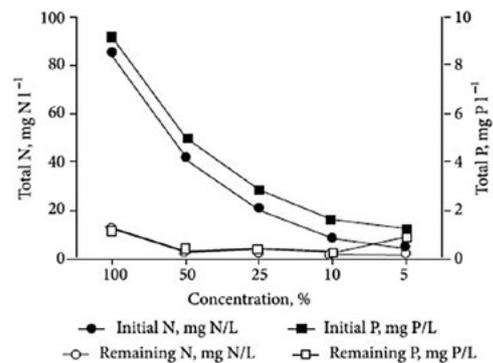
The Distribution, State and Conservation of *Equisetum telmateia* in Lithuania

An analysis of the available information revealed that *E. telmateia* currently occurs in seven localities in Lithuania, mainly in the southern part of the country. One formerly recorded population has become extinct. The species usually grows in river valleys or close to rivers and occupies alluvial forest habitats. According IUCN criteria, *E. telmateia* was categorized as a vulnerable species in Lithuania (Gudžinskas & Rasimavičius, *Botanica Lithuanica* 23 (1) (2017) 17–32).



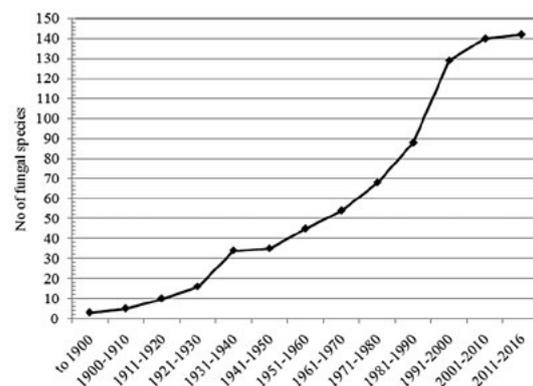
The Cultivation of Green Algae *Chlorella vulgaris* in Municipal Wastewater and Its Biomass Composition

The study showed that *C. vulgaris* is capable of a very efficient nutrient removal from municipal wastewater (up to 86% of total nitrogen and 87% phosphorus was removed). There is strong positive correlation between the initial concentration of nitrogen, and in some cases phosphorus, in the media and content of proteins and carbohydrates in the biomass of *C. vulgaris* (Venckus et al. *J Environ Eng Landsc* 25 (1) (2017) 56–63).



Alien Fungi in Lithuania: A List of Species, the Current Status and Trophic Structure

An inventory of alien fungi recorded in Lithuania since the 19th century was performed in collaboration with the mycologists from the Nature Research Centre in Vilnius. The compiled list includes 142 fungal species, the major part of which are plant pathogens, while mycorrhizal and saprotrophic fungi are much less represented. The status of more than half of the listed species was categorized as unknown, as their records are too few (Motiejūnaitė et al., *Botanica Lithuanica* 23 (2) (2017) 139–152).



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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. Among natural factors, we focus on key-stone 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 behavior 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 [1]. The geomorphological and biocenotical impacts of the Eurasian beaver *Castor fiber* were studied using traditional field methods and remote sensing. The geographical distribution patterns of European Amphibia – an important functional group of an ecosystem – were explained using structural equation modeling [2]. Studies of energy and material flow between ecosystems using stable isotope analysis are being conducted in cooperation with the Center for Physical Sciences and Technology. A chemoreception of model indicator species *Plo-dia interpunctella* on substrates infected and not infected by *Micromycetes* allowed us to discover 3-methyl-1-butanol as the main biomarker [3]. 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 applied in effluent/wastewater and landfill leachate toxicity assessments, were reviewed [4].

SELECTED PUBLICATIONS

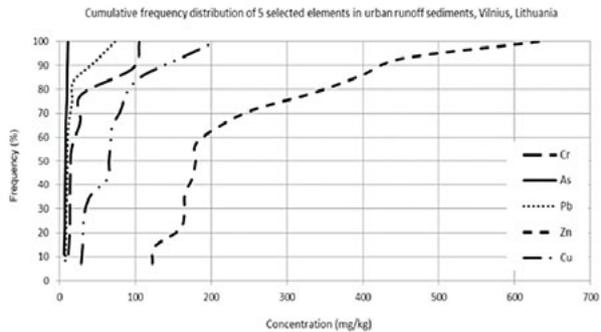


1. Ignatavičius G. et al. 2017. Est. J. Earth Sci. No. 66(01), p. 13-20.
2. Trakimas G. et al. 2016. Glob. Ecol. Biogeogr., 25(10):1228-1238.
3. Būda V. et al. 2016. J. Stored Prod. Res., 69: 152-158.
4. Četkauskaitė A. et al. 2016. J. Environ. Eng. Landsc. Manag. Vol. 24(2): 143- 156.



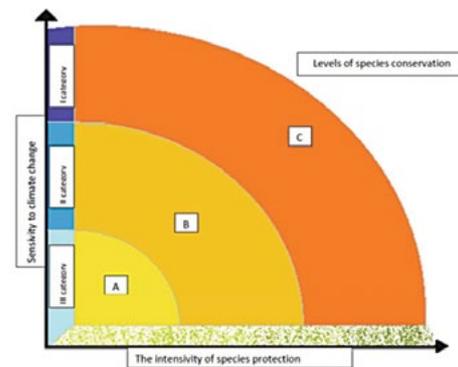
Heavy Metals Contamination in Surface Runoff Sediments

Sediments in untreated runoff from direct discharge stormwater systems significantly contribute to urban waterway pollution. The heavy metal contamination in surface runoff sediments was investigated. The geospatial analysis of the distribution of heavy metals shows that there are several active pollution sources supplying the dischargers with contaminated sediments. A PCA analysis and a *t*-test clearly depicted the significantly different chemical compositions of winter and autumn surface sediment samples. The results provide a useful tool for examining contamination in urban areas, distinguishing pollution sources and giving a better understanding of the importance of permeable surfaces and green areas (Ignatavičius et al. (2017) *Est. J. Earth Sci.* 66: 13-20).



The Optimization of the Conservation of Rare and Vulnerable Species in the Perspective of Climate Change in Lithuanian Reserves

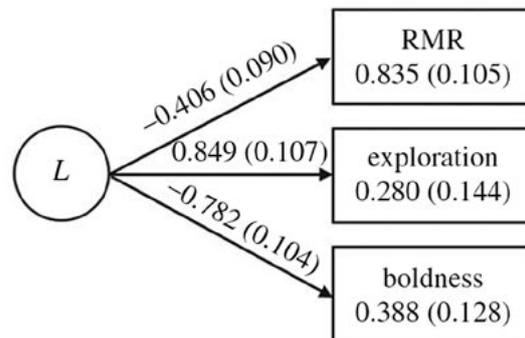
Nature reserves are one of the most important measures in saving biodiversity; however, during climate change, a real danger arises that these territories would not be able to fulfill the objectives. We evaluated the sensitivity of rare and vulnerable species to climate change in order to suggest measures for a better management of nature reserves. Different management measures are taken into account: (1) the mitigation of the direct effect of climate change, (2) an improvement of the existing level of rareness, (3) respecting the relation to the physical and biological environment, (4) a consideration of spread and geographical limits. Three management



intensity levels were suggested (Ignatavičius G., Toleikienė M. (2017) *Arch. Environ. Prot.* 43: 61-73).

The Metabolic Rate and Associated Behaviors in Insects

We measured the resting metabolic rate (RMR), boldness and exploration in a cricket, selected differentially for short and fast development over two generations. We applied structural equation models to an individual-level covariance matrix to examine whether the RMR generates any covariation between the measured behaviors. RMR and boldness were positively correlated, RMR and exploration were negatively correlated, and boldness and exploration were negatively correlated. However, the RMR was not a causal factor generating the covariation between boldness and exploration. The covariation between all three traits was explained by another, unmeasured mechanism (L) (Krams I. A., Niemela P. T., **Trakimas G.**, et al. 2017. *Proc. Royal Soc. B-Biol. Sci.*, 284: e20162481).



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 few aquatic species (fish and mussels). The effects of stressors will be assessed using an integrated assessment

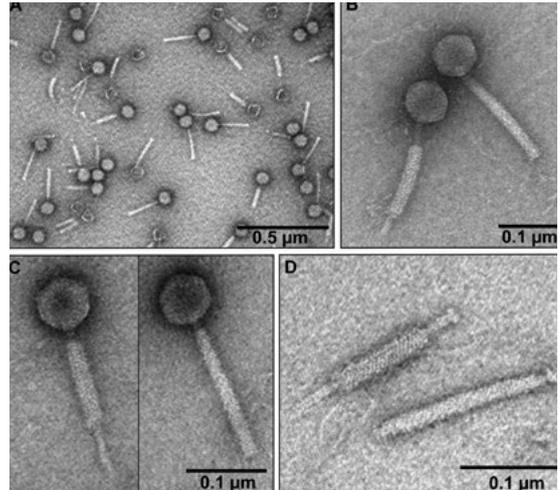
– from the subcellular to organism level. The biological response will be analyzed using cytogenetic, biochemical, cytological and physiological biomarkers. The integrated multibiomarker response will be estimated, which is useful in ecological risk assessment and can be applied in environment protection 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. And 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/fiber), are being developed.

The results obtained while studying a number of unique *Klebsiella*, *E. coli*, and *Arthrobacter* phages isolated by the scientists from our department show that the diversity of phages, in terms of virion structure, physiology and genetics, is enormous, and that we haven't even begun to properly harvest it. In fact, every single phage studied can either be used as a source of novel building blocks for the construction of multifunctional nanomaterials, or it can be exploited in both the detection and biocontrol of pathogenic bacteria.

The phage group of the department of Molecular Microbiology and Biotechnology has recently focused on the isolation and molecular characterization of novel phages with unique virion structure, host range or physiology. Over the last four years, five unique bacterial viruses – 2 *Arthrobacter* sp.- and 3 *Escherichia coli*-infecting viruses – have been isolated, characterized and published [1-4]. Also, based on the structural proteins encoded by these phages, the team has constructed a number of novel functionalized nanotubes that not only exhibit good physicochemical properties but can also be used as a platform for the construction of hybrid nanoparticles with the potential for use in a variety of applications. In 2015, the phage group, together with research groups from the Nature Research Center and the Institute of Biosciences, received a grant from the Research Council of Lithuania to investigate the impact of global warming on the diversity and co-evolutionary dynamics between microorganisms and the virus population in Lithuanian and Czech agroecosystems. More than 30 novel enterobacterial phages have been isolated during this project, and one of these phages, the only known psychrophilic *E. coli* siphophage vB_EcoS_NBD2, has been researched and published [Kaliniene et al. 2018].

SELECTED PUBLICATIONS

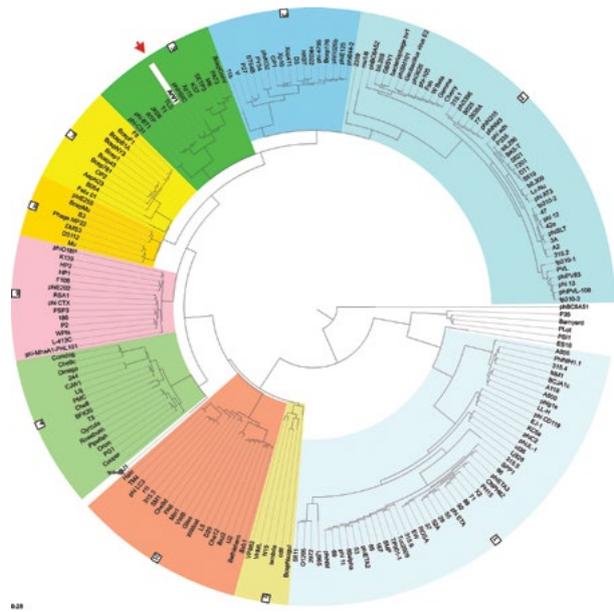


- 1: Kaliniene L, et al. Molecular Analysis of *Arthrobacter* Myovirus vB_ArtM-ArV1: We Blame It on the Tail. *J Virol*. 2017, 29:91(8).
- 2: Šimoliūnas E, et al. Incomplete LPS Core-Specific Felix01-Like Virus vB_EcoM_VpaE1. *Viruses*. 2015, 7(12):6163-81.
- 3: Kaliniene L, et al. Low-temperature bacterial viruses VR - a small but diverse group of *E. coli* phages. *Arch Virol*. 2015, 160(5):1367-70.
- 4: Šimoliūnas et al. Isolation and characterization of vB_ArS-ArV2 - first *Arthrobacter* sp. infecting bacteriophage with completely sequenced genome. *PLoS One*. 2014, 21:9(10):e111230.



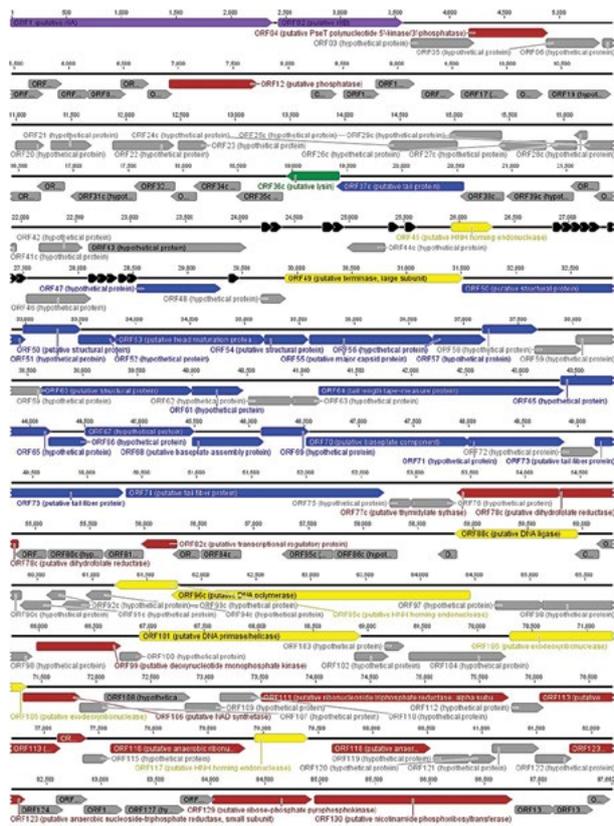
Molecular Analysis of *Arthrobacter Myovirus* vB_ArtM-ArV1: We Blame It on the Tail

Bacteriophages, which have likely originated in the early Precambrian Era, represent the most numerous population on the planet. Approximately 95% of the known phages are tailed viruses that comprise three families: *Podoviridae* (short tails), *Siphoviridae* (long noncontractile tails), and *Myoviridae* (contractile tails). Based on the current hypothesis, myophages, which may have evolved from siphophages, are thought to have first emerged among Gram-negative bacteria, whereas they emerged only later, among Gram-positive bacteria. The results of the molecular characterization of myophage vB_ArtM-ArV1 conform to the aforementioned hypothesis, since, at a glance, bacteriophage vB_ArtM-ArV1 appears to be a siphovirus that possesses a seemingly functional contractile tail. Our work demonstrates that such “chimeric” myophages are of cosmopolitan nature and are likely characteristic of the ecologically important soil bacterial genus *Arthrobacter* (Kaliniene et al. *J Virol.* 2017; 29;91(8).



Complete Genome Sequence of *Escherichia coli* Phage vB_EcoM_Alf5

With the emergence of multidrug-resistant strains, bacteriophages have been proposed as an alternative antimicrobial. A number of bacteriophages potentially suitable for the biocontrol of *E. coli* and *Salmonella* spp. have been isolated and sequenced, but only a few of them have been functionally characterized at the molecular level. The genome of the *E. coli*-specific Felix01virus Alf5 has a total of 133 ORFs and 19 tRNAs. The most obvious differences between the genomes of Alf5 and other Felix01viruses are observed in the ORFs, which encode hypothetical and tail fiber proteins, possibly accounting for their host range. Since Alf5 is capable of infecting *E. coli* K-12-derived laboratory strains including the KEIO collection mutants, this phage is a good model for both analysis of the differences in the host ranges and functional characterization of genetically related Felix01viruses (Alijošius et al. *Genome Announc.* 2017; 18;5(20).





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CRISPR-Cas Tools and Technologies

In the past few years, the RNA-guided Cas9 endonuclease from the type II CRISPR-Cas bacterial antiviral defense system has revolutionized the genome editing field and enabled a broad range of applications from basic biology to biotechnology and medicine. Simply by changing the RNA sequence, Cas9 can be reprogrammed to cleave, bind or nick a DNA target almost at any DNA sequence; however, the available sequence range is limited by the need of a short nucleotide sequence, termed a *protospacer adjacent motif* (PAM), that is absolutely required to initiate crRNA-mediated DNA binding [1, 2]. The PAM represents a nucleotide signature uniquely associated with each Cas9 protein and must be determined experimentally for each Cas9 variant. PAM sequence requirement may limit target site selection if genome-specific target sites are desired; therefore, Cas9 proteins with distinct PAM specificities may help expand the sequence space targeted by Cas9. The exploration of Cas9 orthologs could offer a diversity of PAM sequences and novel biochemical properties that may be beneficial for genome editing applications.

Together with Dr. Claudio Mussolino and Dr. Tony Cathomen (Freiburg University), we have shown that Cas9 orthologues requiring more stringent PAMs improve the overall specificity in the human genome by significantly lowering off-target cleavage activities to provide an alternative for safe human genome editing [3].

To expand the repertoire of Cas9s available for genome targeting, in collaboration with Dr. Joshua Young and Mark Cigan (DuPont Pioneer), we developed a new *in vitro* method for the simultaneous examination of guide RNA and protospacer adjacent motif (PAM) requirements [4]. The method relies on the *in vitro* cleavage of plasmid libraries containing a randomized PAM as a function of Cas9-guide RNA complex concentration. Using this method, we accurately reproduced the canonical PAM preferences for *Streptococcus pyogenes*, *Streptococcus thermophilus* CRISPR3 (Sth3) and CRISPR1 (Sth1). Additionally, the PAM and sgRNA solutions for a novel Cas9 protein from *Brevibacillus laterosporus* were provided by the assay and are demonstrated to support functional activity both *in vitro* and in plants.

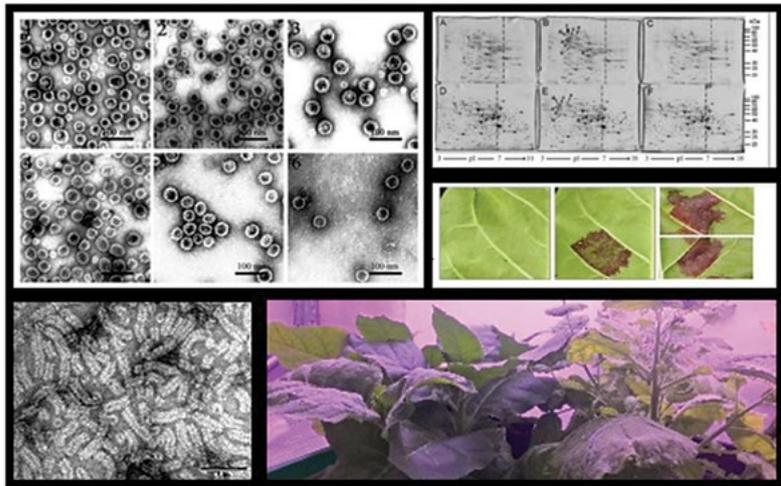
SELECTED PUBLICATIONS



1. Gasiunas et al. Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. *Proc Natl Acad Sci U S A*. 2012, 109: E2579-86
2. Šikšnys V, Gasiunas G, Karvelis T. RNA-directed DNA cleavage by the Cas9-crRNA complex. US patent. 9 637 739 2017.05.02
3. Muller et al. *Streptococcus thermophilus* CRISPR-Cas9 Systems Enable Specific Editing of the Human Genome. *Mol. Therapy* 2016, 24, 636-644
4. Karvelis et al. Rapid characterization of CRISPR-Cas9 protospacer adjacent motif sequence elements. *Genome Biology*. 2015, 16: 253



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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 harbor 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.

SELECTED PUBLICATIONS



1. Gedvilaite A. et al. Novel polyomaviruses in shrews (Soricidae) with close similarity to human polyomavirus 12. *J Gen Virol.* 2017. [Epub ahead of print]
2. Norkiene M. et al. Production of recombinant VP1-derived virus-like particles from novel human polyomaviruses in yeast. *BMC Biotechnol.* 2015, 15:68.
3. Paškevičius Š. et al. Plant-expressed pycnins for control of *Pseudomonas aeruginosa*. *PLoS One.* 2017 3;12(10).
4. Tamošiūnas, P.L. et al. Yeast-generated virus-like particles as antigens for detection of human bocavirus 1-4 specific antibodies in human serum. *Appl Microbiol Biotechnol.* 2016, 100(11):4935-46.

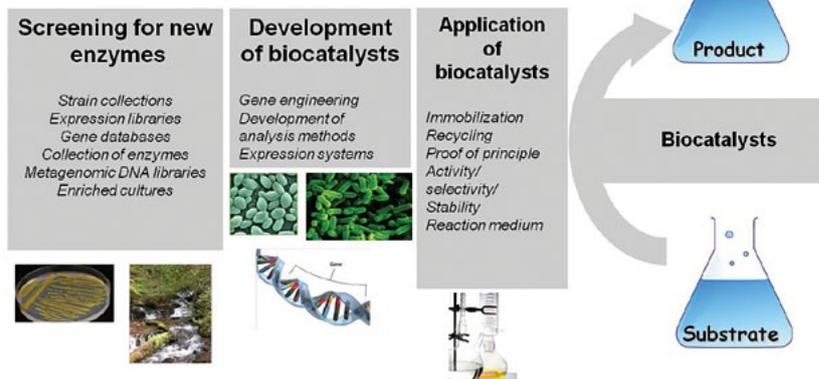
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Development and Application of Biocatalytic Systems

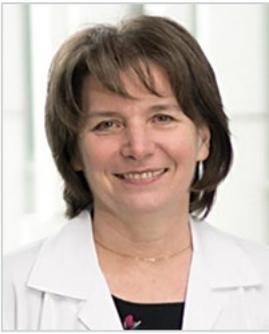
Biocatalysis, which applies natural biological substances (microorganisms, enzymes etc.) in various industrial processes, is one of the most popular alternatives to traditional technologies. The use of such biocatalysts fulfills the requirements that are needed for sustainable synthesis. They are very 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 to avoid 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 routes 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.

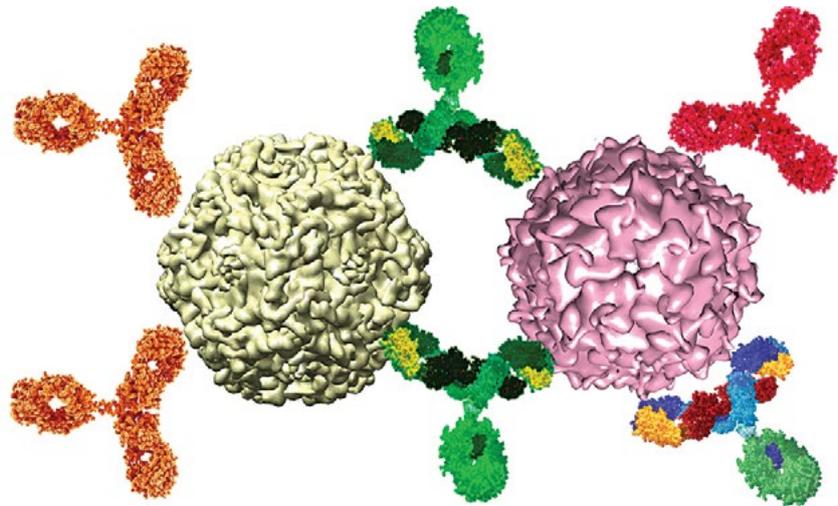
SELECTED PUBLICATIONS



1. A. Veteikytė, R. Šiekštelė, B. Tvaska, I. Matijošytė. Sequential application of waste whey as a medium component for *Kluyveromyces lactis* cultivation and a co-feeder for lipase immobilization by CLEA method. *Appl. Microbiol. Biotechnol.* 2017, 101:3617-3626.
2. R. Gruškienė, V. Kairys, I. Matijošytė. CLEA-based immobilization of methylotropic yeast alcohol oxidase: influence on storage stability and reaction efficiency. *Org. Process Res. Dev.*, 2015, 19 (12): 2025-2033.
3. R. Šiekštelė, A. Veteikytė, B. Tvaska, I. Matijošytė. Yeast *Kluyveromyces lactis* as host for expression of the bacterial lipase: cloning and adaptation of the new lipase gene from *Serratia* sp. *J. Indust. Microbiol. Biotechnol.*, 2015, 42: 1309-1317
4. M. Sulciene, A. Karalius, I. Matijošytė. Chemo-enzymatic route for the production of biopolyol from rapeseed oil. *Curr. Org. Chem.* 2014, 18: 3037-3043
5. E. Kleinaitė, V. Jaška, B. Tvaska, I. Matijošytė. A cleaner approach for biolubricant production using biodiesel as a starting material. *J. Clean Prod.*, 2014, 75:40-44



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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. So, 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. Also, 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).

SELECTED PUBLICATIONS



1. Simanavicius et al. Generation in yeast and antigenic characterization of hepatitis E virus capsid protein virus-like particles. *Appl Microbiol Biotechnol.*, 2018, 102 (1), 185-198.
2. Kailasan et al. Mapping antigenic epitopes on the human bocavirus capsid. *J Virol.*, 2016, 90 (9), 4670-4680
3. Zilnyte et al. The cytolytic activity of vaginolysin strictly depends on cholesterol and is potentiated by human CD59. *Toxins (Basel)*. 2015, 7(1) :110-128.
4. Pleckaityte et al. Construction of polyomavirus-derived pseudotype virus-like particles displaying a functionally active neutralizing antibody against hepatitis B virus surface antigen. *BMC Biotechnol.*, 2015, 15 (1): 85.

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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. The basic principle of droplet microfluidics is easy to appreciate: highly monodisperse, aqueous droplets are generated in an inert carrier oil in microfluidic channels on a chip, and each droplet functions as an independent microreactor. Hence, each droplet is the functional equivalent of a well (or tube), yet the volume of a droplet is roughly a thousand to a million times smaller. Such a massive reduction in reaction volume provides huge savings in reagent costs when performing large numbers of reactions in parallel. 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 sophisticated, yet highly controllable manner, therefore opening new opportunities for biology-related research.

Many useful microfluidic techniques have been developed to analyze single-cells or bio-molecules; however, there is an unmet demand for methods with improved analytical and high-throughput capabilities. Our multidisciplinary team is working at fulfilling this demand by developing a microfluidic droplet tool with higher throughput, reduced reagent cost, scalability and single-molecule resolution for a diverse set of quantitative experiments in cell biology and biomedicine. Over the last few years, we have developed (1) microfluidic methods for single-cell profiling, (2) an amplification of a single DNA molecule and its *in vitro* expression, (3) drug delivery systems, (4) an antibody screening and other novelties. Our current collaborators include Harvard University, Harvard Medical School, Caltech, Oxford, MSKCC and others.

SELECTED PUBLICATIONS



Zilionis R., Nainys J., Veres A., Savova V., Zemmour D., Klein MA., and Mazutis L. (2017) Single-cell barcoding and sequencing using droplet microfluidics, *Nature Protocols* 12, 44-73.

Kiseliovas V, Milosevic M, Kojic M, Mazutis L, Kai M, Liu YT, Yokoi K, Ferrari M, Ziemys A. Tumor progression effects on drug vector access to tumor-associated capillary bed, *J Control Release*. 2017 May 30. pii: S0168-3659(17)30626-0.

Galinis R, Stonyte G, Kiseliovas V, Zilionis R, Studer S, Hilvert D, Janulaitis A and Mazutis L. (2016) DNA nanoparticles for improved protein synthesis *in vitro*, *Angew Chem Int Ed Engl*. 2016 Feb 24;55(9):3120-3.

Mazutis L, Vasiliauskas R, Weitz D, (2015) Microfluidic Production of Alginate Hydrogel Particles for Antibody Encapsulation and Release, *Macromol. Biosci*. 2015, 15, 1641-1646.

Mazutis L., Gilbert J., Ung L., Weitz D., Griffiths A., Heyman J., (2013) Single-cell analysis and sorting using droplet-based microfluidics, *Nature Protocols*, 8(5): 870-891.



Single-Cell Barcoding and Sequencing Using Droplet Microfluidics

In collaboration with our colleagues from Harvard University, we have developed a droplet microfluidic technology, known as inDrops (for indexing droplets), for barcoding thousands of transcriptomes of single-cells. 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 next-generation sequencing. We are applying this technology to solve various biological questions, such as heterogeneity's role

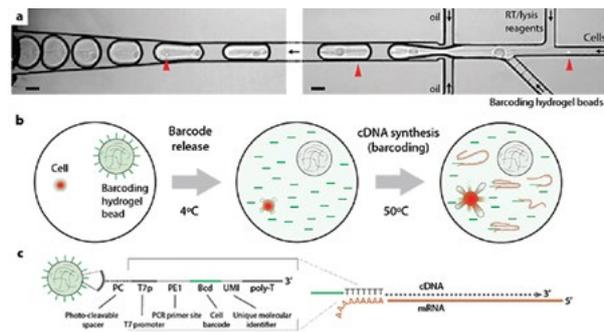


Figure 1. Single-cell transcriptome barcoding in drops.

in cancer, anti-tumor immune responses and the development of complex diseases.

2. The Effects of Tumor Progression on Drug Vector Access to A Tumor-Associated Capillary Bed

Capillaries associated with the tumor microenvironment are a crucial component of the enhanced permeability and retention effect. The imbalance of anti- and pro-angiogenic factors, such as the vascular endothelial growth factor (VEGF), leads to the formation of immature, highly permeable and abnormal vasculature that lacks smooth muscle cells and pericytes. In addition to the abnormal vasculature and the lack of a lymphatic drainage system, tumors exhibit high interstitial fluid pressure. Moreover, in contrast to normal blood vessels, which are in well-organized hierarchical structures, tumor vascular networks occur in a disorganized, chaotic architecture. We developed microfluidic devices mimicking tumor progression and examined the flow of drug particles in the presence of co-circulating red blood cells. Dysfunctional capillaries

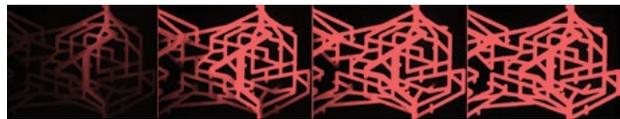


Figure 2. Still images of the flow of fluorescent dye in the tumor capillary model.

with no flow – a result of tumor progression – had limited access to all particles, while diffusion was shown to be the only prevailing transport mechanism. In view of drug vector distribution in tumors, independent of formulation and other pharmacokinetic aspects, our results suggest that the evolution of tumor vasculature during progression may influence drug delivery efficiency.

Single-Molecule Derived DNA Microparticle for *In Vitro* Protein Expression

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 μ m-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

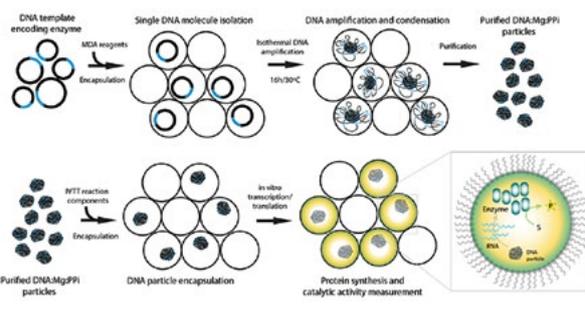


Figure 3. Single-molecule derived DNA microparticle generation.

droplets and in the 384-well format. The yield of protein obtained from the DNA particles was significantly higher than from the corresponding amount of free DNA templates, thus opening new possibilities for high throughput screening applications.



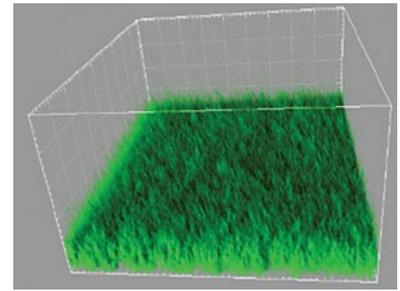
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Antibiotic Resistance and Pathogenesis

Infections caused by a 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 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 immunosuppressed patients (1). 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 to and colonize the host cells (1). 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 stabilization, regulation of cell growth, death under stress and mediation of bacterial persistence through the generation of cells tolerant to antibiotics (1). All the listed features are crucial in the life of pathogens and in understanding the role of TAs might bring novel insights into pathogenicity and the development of novel antibacterial strategies.

We focus our research toward understanding the molecular basis underlying the bacterial antibiotic resistance in the clinical setting and in the environment with an emphasis on novel resistance mechanisms and on the bacterial features contributing to pathogenesis. Toward this goal, we have recently identified and characterized the ubiquitous *A. baumannii* plasmid, which confers resistance to carbapenems – a broad spectrum of antibiotics used to treat *A. baumannii* infections (1). The current trend shows the spread of *A. baumannii* isolates harboring this plasmid or its derivatives in different countries. Moreover, we have characterized *A. baumannii* TA systems, including a pair of TAs carried by this plasmid, and showed their involvement in the *A. baumannii* stress response and plasmid stabilization functions (1, 2). By using our developed specific genetic engineering tools, we seek to identify novel factors that are important for the virulence of this opportunistic pathogen. We are also looking for novel antibiotic resistance mechanisms that have evolved in other gram-negative bacteria present in the clinical environment as well as in non-clinical habitats, such as soil and water (3). In parallel, we are developing sensitive molecular target-based methods for the detection of bacterial pathogens (4).

SELECTED PUBLICATIONS



1. Sužiedėlienė et al. 2016. Identification and characterisation of type II toxin-antitoxin systems in the opportunistic pathogen *Acinetobacter baumannii*. In: de Bruijn, F.J., (ed.) Stress and environmental control of gene expression in bacteria. Wiley-Blackwell.
2. Armalytė et al. The higBA Toxin-Antitoxin Module from the Opportunistic Pathogen *Acinetobacter baumannii* – Regulation, Activity and Evolution. *Frontiers in Microbiology* 2018, in Press.
3. Krasauskas et al. Purification and characterization of a new beta-lactamase OXA-205 from *Pseudomonas aeruginosa*. *Ann Clin Microbiol Antimicrob* 2015, 14:52.
4. Skerniškytė et al. Detection of *Salmonella* spp., *Y. enterocolitica*, *L. monocytogenes* and *Campylobacter* spp. by real-time PCR using amplicon DNA melting analysis and probe-based assay. *Int J Food Science and Technology* 2016, 51, 519-529.

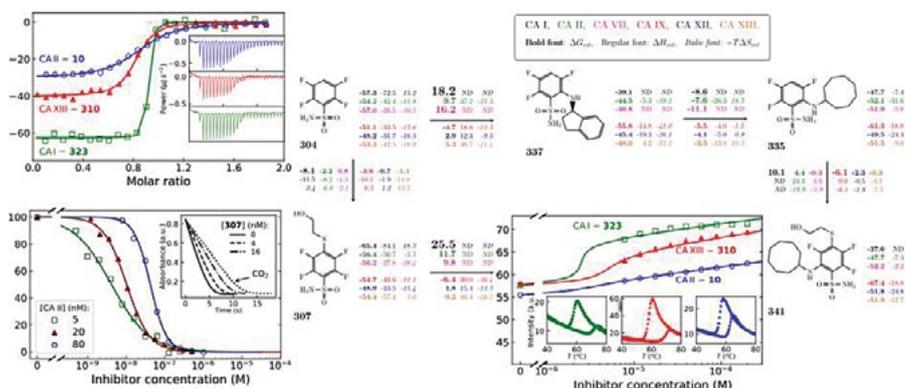
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Drug Design via Thermodynamics

Rational drug design should be able to make chemical compounds that bind to disease-causing target proteins with high affinity and specificity over all remaining proteins to avoid toxicity. Unfortunately, such a design is possible only in theory 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 efficacy of the designed compounds.

Our scientists come from various backgrounds including molecular biologists, biochemists, organic chemists, biophysicists, physicists, computer modelers, biologists and pharmacists. There are 6 teams in the department: 1) The team for organic synthesis performs the organic synthesis of novel compounds; 2) The team for molecular and cellular biology performs 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); 3) The team for biothermodynamics determines the energetics of binding between the synthesized compounds and the target proteins; 4) The team for *in silico* modeling and crystallography determines the X-ray crystallographic structures of protein-compound complexes, designs compounds, performs docking and searches for structure-energetics correlations; 5) The team for pharmaceutical development studies the effect of compounds in various biological systems including zebrafish and mice.

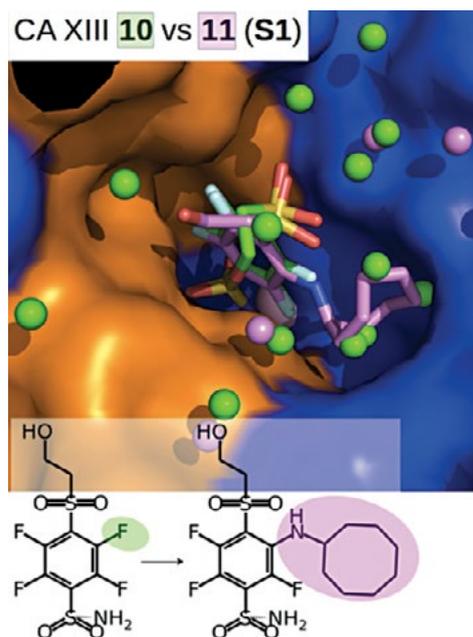
SELECTED PUBLICATIONS

- Linkuvienė, V., Talibov, V. O., Danielson, U. H. and Matulis, D. 2018. Introduction of Intrinsic Kinetics of Protein – Ligand Interactions and Their Implications for Drug Design. *J. Med. Chem.* 61. 2292-2302.
- Zubrienė, A., Smirnov, A., Dudutienė, V., Timm, D. D., Matulienė, J., Michailovienė, V., Zakšauskas, A., Manakova, E., Gražulis, S., Matulis, D. 2017. Intrinsic Thermodynamics and Structures of 2,4- and 3,4-Substituted Fluorinated Benzenesulfonamides Binding to Carbonic Anhydrases. *ChemMedChem.* 12. 161-176.
- Smirnov, A., Zubrienė, A., Manakova, E., Gražulis, S., Matulis, D. 2018. Crystal structure correlations with the intrinsic thermodynamics of human carbonic anhydrase inhibitor binding. *PeerJ.* doi: 10.7717/peerj.4412.



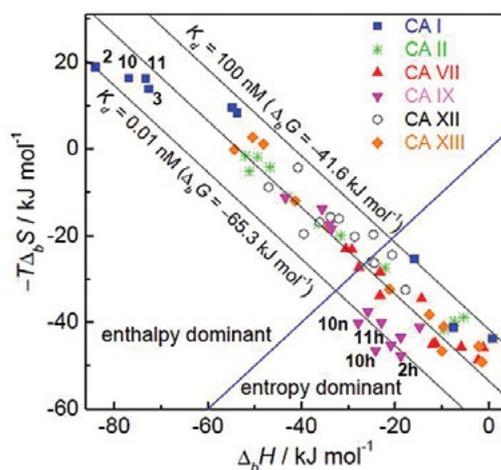
Crystal Structure – Thermodynamics Correlations of CA Inhibitors

The structure-thermodynamics correlation analysis was performed for a series of fluorine- and chlorine-substituted benzenesulfonamide inhibitor binding to several human carbonic anhydrase (CA) isoforms. The total of 24 crystal structures of 16 inhibitors bound to isoforms CA I, CA II, CA XII, and CA XIII provided the structural information of selective recognition between a compound and CA isoform. The binding thermodynamics of all structures were determined by the analysis of binding-linked protonation events, yielding the intrinsic parameters, i.e., the enthalpy, entropy and Gibbs energy of binding. Inhibitor binding was compared within the structurally similar pairs that differ by para- or metasubstituents enabling to obtain the contributing energies of ligand fragments. A deeper understanding of the energies contributing to the protein-ligand recognition should lead toward the eventual goal of rational drug design, where the chemical structures of ligands could be designed based on the target protein structure (Smirnov et al. PeerJ.4412).



The Intrinsic Thermodynamics of the Fluorinated Benzenesulfonamide Inhibitor Binding to CAs

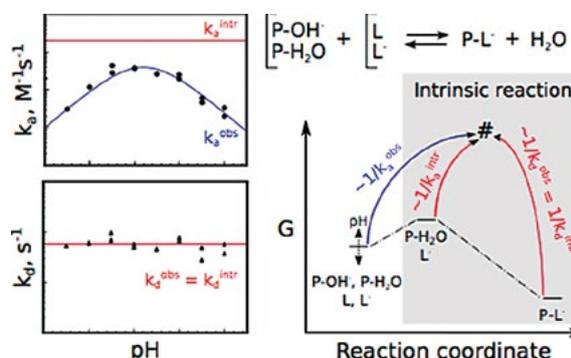
The goal of rational drug design is to understand the structure-thermodynamics correlations to predict the chemical structure of the drug that would exhibit an excellent affinity and selectivity to a target protein. Here we explore the contribution of the added functionalities of inhibitors to the intrinsic binding affinity, enthalpy and entropy. 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 intrinsic parameters of binding were determined by subtracting the linked protonation reactions. The development of meta- or ortho-substituted, fluorinated benzenesulfonamides toward a highly potent compound exhibiting the observed $K_{d,obs}$ of 43 pM and intrinsic K_d of 1.1 pM toward CA IX, an anticancer target, is described by applying the



FTSA, ITC, and X-ray crystallography (Zubriene et al. ChemMedChem 2017, 12, 161-176).

The Intrinsic Kinetics of Protein-Ligand Interactions

Structure-kinetic relationship analyses and the identification of dominating interactions for the optimization of lead compounds should ideally be based on intrinsic rate constants instead of the more easily accessible observed kinetic constants. The intrinsic rate constants were determined by a surface plasmon resonance (SPR). The observed association rates were pH-dependent and correlated with the fraction of a deprotonated inhibitor and a protonated zinc-bound water molecule. The intrinsic association rate constants were pH independent. By contrast, the observed and intrinsic dissociation rate constants were identical and pH-independent, demonstrating that the observed association and dissociation mechanisms are inherently different. A model accounting for the



differences between intrinsic and observed rate constants was developed (Linkuviene et al. J.Med.Chem. 2018, 61, 2292-2302).



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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 tumors has encouraged the development of molecular biomarker systems 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 clinical 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, our group aims at the (epi)genetic characterization of various human tumors (prostate, kidney, breast, lung and others) and the development of molecular biomarker systems for cancer detection, prognosis and disease monitoring during treatment (resistance development). Primarily focusing on altered DNA methylation and miRNA expression patterns, we have recently proposed biomarker panels for prostate cancer detection using liquid biopsy samples [1-4]. Epigenome-wide profiling let us identify novel biomarkers showing both diagnostic and prognostic potential [1-3]. The methylation of the RAS association domain family member 1 gene (RASSF1) was the first ever reported prognostic prostate cancer biomarker in urine [4]. Our miRNA analyses not only revealed the diagnostic value of a set of two miRNAs in a noninvasive assay [2] but also led to an identification of clinical importance of a particular miRNA host gene promoter methylation in prostate tumors [1]. In collaboration with the National Cancer Institute of Lithuania, we investigate the molecular profile of various tumors and apply modern single-cell, genome-wide approaches to explore tumor complexity and mechanisms of treatment resistance.

SELECTED PUBLICATIONS

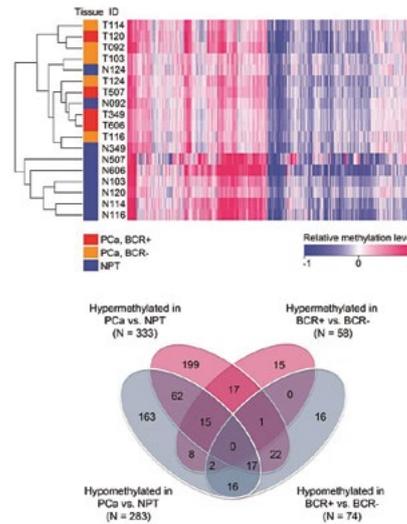


1. Daniunaite K, Dubikaityte M, Gibas P, Bakavicius A, Lazutka JR, Ulys A, Jankevicius F, Jarmalaite S. *Hum Mol Genet.* 2017; 26(13):2451-61.
2. Stuopelyte K, Daniunaite K, Bakavicius A, Lazutka JR, Jankevicius F, Jarmalaite S. *Br J Cancer.* 2016; 115(6):707-15.
3. Demidenko R, Razanauskas D, Daniunaite K, Lazutka JR, Jankevicius F, Jarmalaite S. *BMC Cancer.* 2015; 15:683.
4. Daniunaite K, Jarmalaite S, Kalinauskaite N, Petroska D, Laurinavicius A, Lazutka JR, Jankevicius F. *J Urol.* 2014; 192(6):1849-55.



The Clinical Significance of the miRNA Host Gene Promoter Methylation in Prostate Cancer

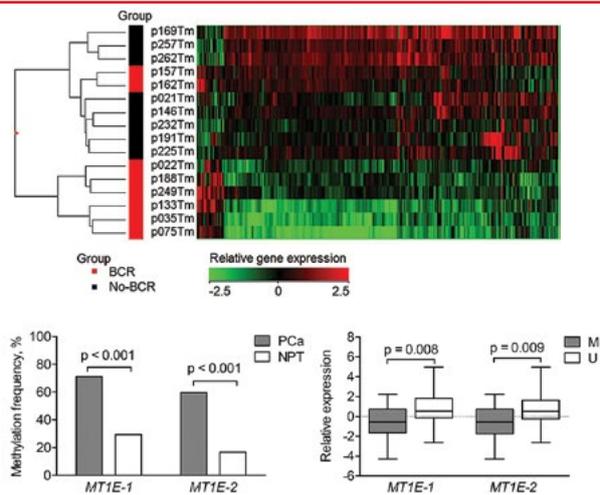
Methylome screening using microarray-based technology led us to an identification of multiple miRNA host genes with differentially methylated regulatory regions in prostate tumors. Host genes of miR-155-5p, miR-152-3p, miR-137, miR-31-5p and miR-642a, -b were analyzed for promoter methylation in prostate tumors and control samples and compared to the expression of mature miRNAs and their selected targets (*DNMT1*, *KDM1A* and *KDM5B*). The methylation of miR-155, miR-152 and miR-137 host genes was PCa-specific, and the downregulation of miR-155-5p significantly correlated with promoter methylation. A higher *KDM5B* expression was observed in samples with methylated miR-155 or miR-137 promoters, whereas the upregulation of *KDM1A* and *DNMT1* was associated with miR-155 and miR-152 methylation status, respectively. The promoter methylation of miR-155, miR-152 and miR-31 was predictive of biochemical disease recurrence (BCR)-free survival in various Cox models and increased the prognostic value of clinicopathologic factors. This study revealed



the potential of methylated miR-155, miR-152, miR-137 and miR-31 host genes as promising diagnostic and/or prognostic biomarkers of prostate cancer.

A Decreased Expression of *MT1E* Indicates Prostate Cancer Progression

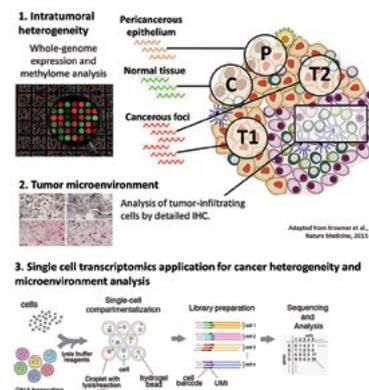
The differentiation of indolent and aggressive prostate tumors at the time of diagnosis is currently one of the major challenges in the field. This study aimed at the identification of prognostic biomarkers to aid in predicting the BCR of the disease. Microarray-based gene expression profiling in tissues of 8 BCR and 8 No-BCR cases revealed expression differences of 455 genes, most of which were down-regulated in BCR cases. Eleven genes were selected for validation in two independent cohorts. The down-regulation of the *MT1E* and *GPR52* expression and up-regulated levels of *EZH2* were the specific biomarkers of BCR in at least one of the two PCa cohorts, but only a *MT1E* expression retained the independent prognostic value in a multivariate analysis. A DNA methylation analysis showed a frequent *MT1E* methylation in PCa and was associated with the down-regulated expression in one PCa



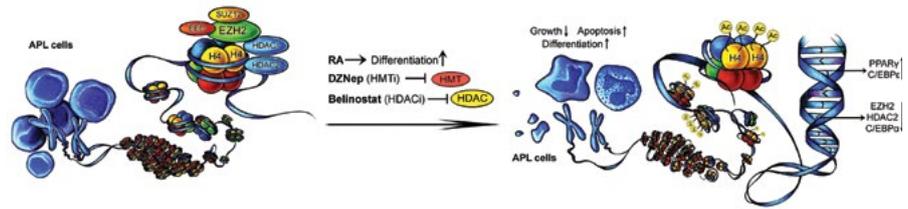
cohort. The results of this study suggested *MT1E* down-regulation as a potential feature of aggressive PCa.

Modern Technologies for Resolving the Complex Structure of a Tumor

Each tumor evolves through a complex accumulation process of mutations and epimutations and, finally, is composed of a complex mixture of cell subpopulations that contains molecularly variant subclones of malignant cells and cells infiltrating the tumor from its microenvironment, including fibroblasts, immune cells and precursors of vasculature. Due to the complex cellular and genetic architecture of each tumor, therapeutic and clinical outcomes vary in tumors that are characterized by the same category, stage and grade. The main objective of this ongoing study is to investigate the intrinsic and extrinsic complexity of renal and pulmonary tumors for an improved understanding of molecular mechanisms of cancer development and progression. The genomic and epigenomic profile of cell subclones (cancerous, pericancerous and normal-like epithelium) from the same tumor will be analyzed in order to resolve the intratumoral and extratumoral heterogeneity of cancer.



Single-cell technology will enable a thorough examination of cell subpopulations infiltrating the tumor. The intrinsic and extrinsic heterogeneity of tumors will be associated with clinical parameters for a better understanding of the clinical consequences of the tumor's cellular and genetic complexity.



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Leukemia: New Strategies in Prognosis and Treatment

Acute myeloid leukemia (AML) is an aggressive, heterogeneous group of malignancies with different clinical behaviors and different responses to therapy. AML karyotypes are most commonly classified into 3 prognostic categories with differing median survivals as: favorable risk, 7.6 years; intermediate risk, 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 few screening tests on the market for an early detection of certain cancers in people without any symptoms. But at this time, there are no special tests recommended to find acute myeloid leukemia (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 leukemia differentiation and epithelial cancer growth inhibition/death molecular mechanisms. Our group members manage on daily bases such techniques as culturing different tumor cell lines, total protein isolation and proteomic analysis: SDS/PAGE and two dimensional electrophoresis (2DE), protein native PAGE, mass spectrometry, methylation analysis and other cell molecular techniques. Our team members have extensive experience in project management. Prof. Dr. Rūta Navakauskienė has successfully led and implemented projects from the National Programs "Chronic Noninfectious Diseases" (2 projects), "Healthy Ageing" (ongoing project), led the Action of the project under the National Integrated Program of Biotechnology & Biopharmaceutics, European Social Fund project (ESF).

Today, our research is focused on the following:

- On the pharmacological manipulation of chromatin remodeling that might develop into a potent and specific strategy for the treatment of leukemia;
- On funding novel, potentially prognostic biomarkers useful for the diagnostics of leukemia or disease outcome predictions.

SELECTED PUBLICATIONS



1. G.Valiuliene, J.Stirblyte, M.Jazdauskaite, V.Borutinskaite, R.Navakauskiene. Anti-leukemic effects of HDACi Belinostat and HMTi 3-Deazaneplanocin A on human acute promyelocytic leukemia cells. *European Journal of Pharmacology*, 2017. 799:143-153.
2. V. Borutinskaite, J. Bauraitė-Akatova, R. Navakauskiene. Anti-leukemic activity of DNA methyltransferase inhibitor procaine targeted on human leukaemia cells. *Open Life Sciences*. 2016. 11: 322-330.
3. V. Borutinskaitė, R. Navakauskienė. The histone deacetylase inhibitor BML-210 influences gene and protein expression in human promyelocytic leukemia NB4 cells via epigenetic reprogramming. *International Journal of Molecular Sciences*. 2015. 16: 18252-18269.
4. R. Navakauskiene, V.V. Borutinskaite, G. Treigyte, J. Savickiene, D. Matuzevicius, D. Navakauskas, K.-E. Magnusson. Epigenetic changes during hematopoietic cell granulocytic differentiation--comparative analysis of primary CD34+ cells, KG1 myeloid cells and mature neutrophils. *BMC Cell Biology*. 2014, 15: 4.



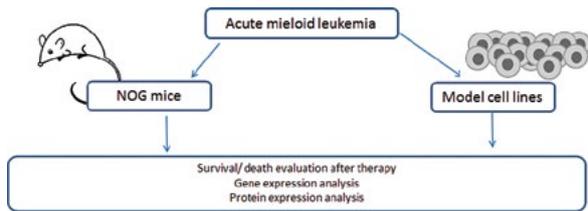
The Effect of Combined Epigenetic Therapy: Studies In Vitro and In Vivo

The development of acute myeloid leukemia is usually sustained by a deregulated epigenome. Alterations in DNA methylation and histone modifications are common manifestations of the disease. Acute promyelocytic leukemia (APL) is not an exception. Therefore, drugs that target epigenetic processes suggest an appealing strategy for APL treatment.

In this study, we tested the anti-leukemic activity of the histone deacetylase inhibitor (HDACi) Belinostat (PXD101, (2E)-N-Hydroxy-3-[3-(phenylsulfamoyl)phenyl]prop-2-enamide) and the histone methyltransferase inhibitor (HMTi) 3-Deazaneplanocin A (DZNep, 5R-(4- mino-1H-imidazo[4,5-c]pyridin-1- yl)-3-(hydroxymethyl)-3-cyclopentene-1S,2R-diol) combined with a retinoic acid (RA) in APL cells and in Xenograft models.

We demonstrated that:

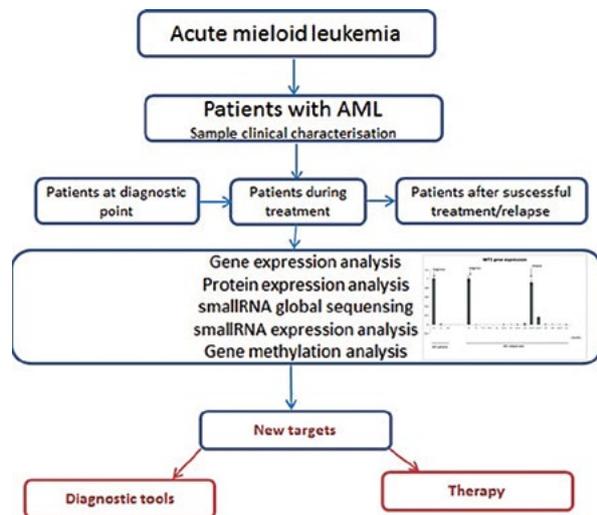
1. A combined treatment with RA, Belinostat and 3-Deazaneplanocin A caused a depletion of leukemia cell growth and viability, initiated an apoptosis and exaggerated the RA-induced granulocytic differentiation;
2. Epigenetic therapy protects APL xenograft NOG mice from tumor formation and prolongs their lifespan.



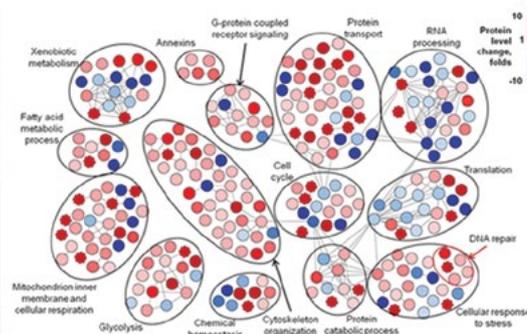
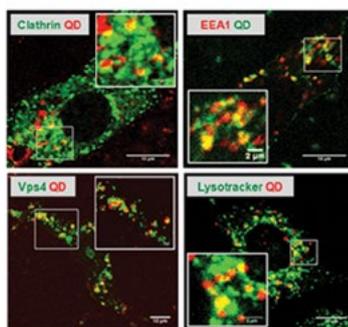
G.Valiuliene, J.Stirblyte, M.Jazdauskaite, V.Borutinskaite, R.Navakauskiene. Anti-leukemic effects of HDACi Belinostat and HMTi 3-Deazaneplanocin A on human acute promyelocytic leukemia cells. *European Journal of Pharmacology*, 2017. 799:143-153.
Valiulienė G, Treigyte G, Savickienė J, Matuzevičius D, Alksnė M, Jarašienė-Burinskaja R, Bukelskienė V, Navakauskas D, Navakauskienė R. Histone modifications patterns in tissues and tumours from acute promyelocytic leukemia xenograft model in response to combined epigenetic therapy. *Biomedicine & Pharmacotherapy*, 2016. 79:62-70.

Biomarkers as Novel Predictors of an APL Relapse

Acute promyelocytic leukemia (APL), the M3 subtype of acute myeloid leukemia (AML), accounts for 10% of all AML cases. The t(15;17) translocation that generates the PML-RAR α fusion mRNA is detected in as many as 90% of APL patients and has become the definitive marker of the disease. The APL relapse cases are very rare, but early diagnosis and the possibility of prediction of APL relapse is important, because patients can develop bone marrow failure and life-threatening coagulopathies. In our study, in collaboration with hematologists (at the Vilnius University Hospital Santaros Klinikos), we performed a gene expression analysis at diagnosis, during treatment and at a complete remission of APL patients and also of patients with a relapse history. We choose genes that are involved in cell cycle, apoptosis and proliferation processes like PPAR γ , C/EBP (crucial for the granulocytic differentiation process), p21, p53, ATM (regulates the cell cycle), polcomb complex proteins SUZ12, EZH2, EED, HDAC, DNMT (crucial for epigenetic regulation) and others. Our results have revealed for the first time that a detailed analysis of bone marrow samples for the c-Myc gene expression of APL patients can predict the success of treatment. The WT1 gene expression can predict relapse cases earlier than changes in peripheral blood parameters or bone marrow blast count.



This study was funded by the National Science program project "The Role of Molecular Modulators in the Hematological System during Cell Senescence, Differentiation and Regeneration" (No. SEN-12/2015, PI: Prof. Rūta Navakauskienė).

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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 tumors 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 tumor therapy. However, chemotherapy often fails due to the ability of the tumor 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 signaling *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.

To this end, we have shown that the surface properties of quantum dots play a crucial role in defining their cellular routes and biological activity. This study 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, 2). 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. Our data show that 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 (3).

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. 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 (4).

SELECTED PUBLICATIONS

1. Karabanovas V, Zitkus Z, Kuciauskas D, Rotomskis R, Valius M. Surface properties of quantum dots define their cellular endocytic routes, mitogenic stimulation and suppression of cell migration. *J Biomed Nanotechnol.* 2014; 10(5):775-86.
2. Damalakiene L, Karabanovas V, Bagdonas S, Pupelis L, Valius M, Rotomskis R. Suppression of a Specific Intracellular Uptake Pathway by a Saturating Accumulation of Quantum Dots. *J Biomed Nanotechnol.* 2015; 11(5):841-53.
3. Nissinen T, Näkki S, Laakso H, Kuciauskas D, Kaupinis A, Kettunen MI, Liimatainen T, Hyvönen M, Valius M, Gröhn O, Lehto VP. Tailored Dual PEGylation of Inorganic Porous Nanocarriers for Extremely Long Blood Circulation *In Vivo*. *ACS Appl Mater Interfaces.* 2016; 8(48):32723-32731.
4. Ger M, Kaupinis A, Nemeikaite-Ceniene A, Sarlauskas J, Cicenias J, Cenas N, Valius M. Quantitative proteomic analysis of anticancer drug RH1 resistance in liver carcinoma. *Biochim Biophys Acta.* 2016; 1864(2):219-32.



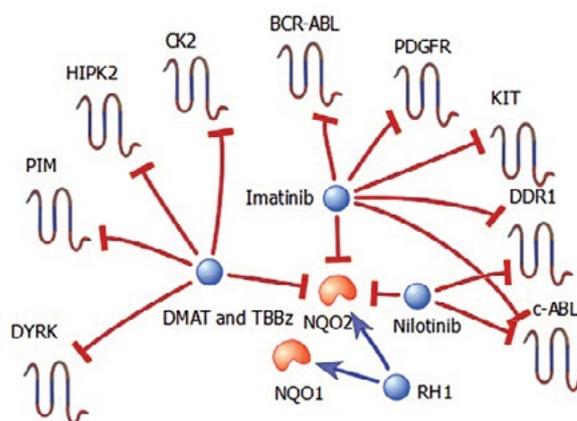
Molecular Modeling and a Structure-Based Drug Discovery Approach Reveals Protein Kinases as Off-Targets for a Novel Anticancer Drug RH1.

Potential drug target identification and the mechanism of action is an important step in the drug discovery process, which can be achieved by biochemical methods, genetic interactions or computational conjectures. Sometimes, more than one approach is implemented to mine out the potential drug target and characterize the on-target or off-target effects. A novel anticancer agent RH1 is designed as a pro-drug to be activated by NQO1, an enzyme overexpressed in many types of tumors. However, increasing data show that RH1 can affect cells in NQO1-independent fashion.

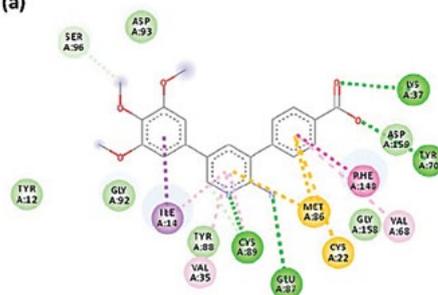
Here, we have implemented the bioinformatics approach of modeling and molecular docking for the search of RH1 targets among protein kinase species. We have examined 129 protein kinases in total, where 96 protein kinases are in complexes with their inhibitor, 11 kinases were in the unbound state with any ligand and, for 22 protein kinases, a 3D structure was modeled. A comparison of the calculated free energy of the binding of RH1 with indigenous kinase inhibitors, the binding efficiency as well as the alignment of their pharmacophoric maps allowed us to predict and rank protein kinases such as KIT, CDK2, CDK6, MAPK1, NEK2 and others as the most prominent off-targets of RH1.

The ranked kinases were also subjected to bioinformatics analysis in order to show which signaling pathways and biological processes these kinases are involved in and whether they could be participating in the cancers of other diseases. A functional annotation showed that the kinases are involved in processes, and the pathways, which are known to be important for carcinogenesis and medical annotation, confirmed that these kinases are known to participate in several cancer types. In conclusion, our results suggest that RH1 can potentially inhibit the function of some kinases and therefore could be used as an anticancer drug to treat carcinoma, kidney and lung cancer, pancreatic carcinoma, colon carcinoma and even arthritis.

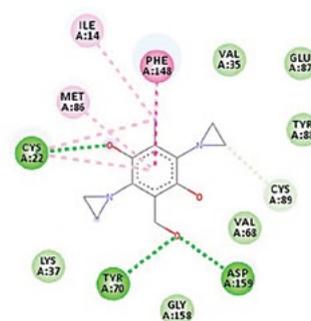
Gupta PP, Bastikar VA, Kuciauskas D, Kothari SL, Cicenas J, Valius M. *Med Oncol.* 2017 Sep 6; 34(10):176.



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Molecular Virology: Mechanisms, Evolution, Antivirals

The *Totiviridae* family dsRNA viruses from the yeast *Saccharomyces cerevisiae* 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 and proteomic 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, for instance) 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], constituent genes of a virus genome were re-introduced into model hosts to manipulate the phenotype conferred by the virus. We were able to achieve either 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.

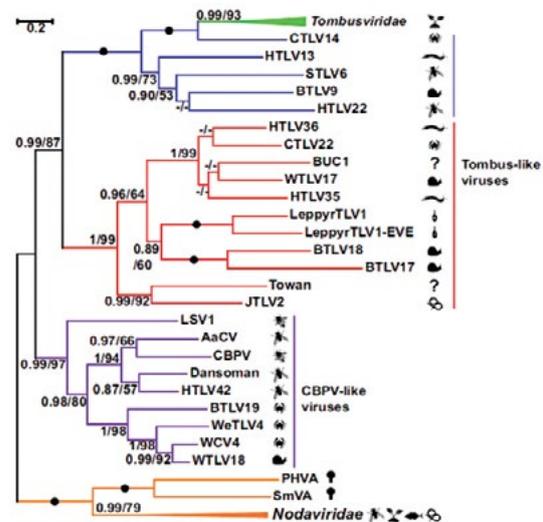
SELECTED PUBLICATIONS

1. A. Mikalkėnas et al. (2018) Conjugation of phosphonoacetic acid to nucleobase promotes a mechanism-based inhibition, *Journal of Enzyme Inhibition and Medicinal Chemistry*, 33:1, 384-389.
2. Grybchuk D. et al. Viral discovery and diversity in trypanosomatid protozoa with a focus on relatives of the human parasite *Leishmania*. *Proc Natl Acad Sci U S A*. 2018 Jan 16; 115(3):E506-E515.
3. Konovalovas A et al. 2016. Genome sequence of *Saccharomyces cerevisiae* double-stranded RNA virus L-A-28. *Genome Announc.* 4(3):e00549-16.
4. Serva S. et al. A. Direct conjugation of peptides and 5-hydroxymethylcytosine in DNA. *Bioconjugate Chem.* 2015, 26, 1008-1012.



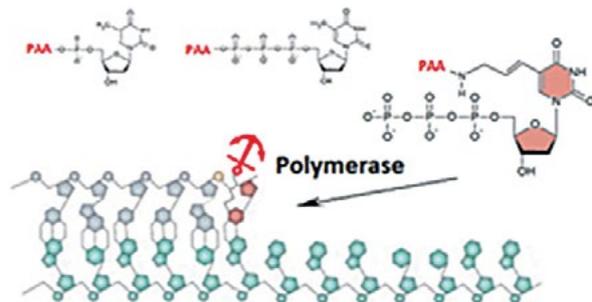
Multihost dsRNA Viruses

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 D. et al. (2018) Proc Natl Acad Sci U S A. 2018 Jan 16; 115(3):E506-E515).



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 (A. Mikalkenas et al., (2018) Journal of Enzyme Inhibition and Medicinal Chemistry, 33:1, 384-389).





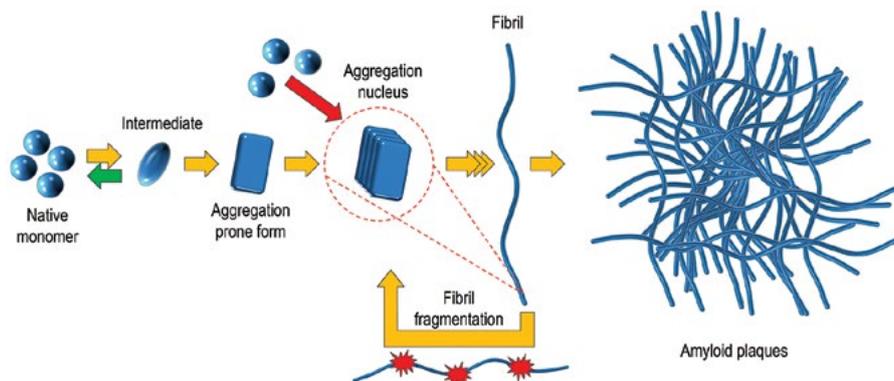
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Protein Misfolding and Aggregation

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.

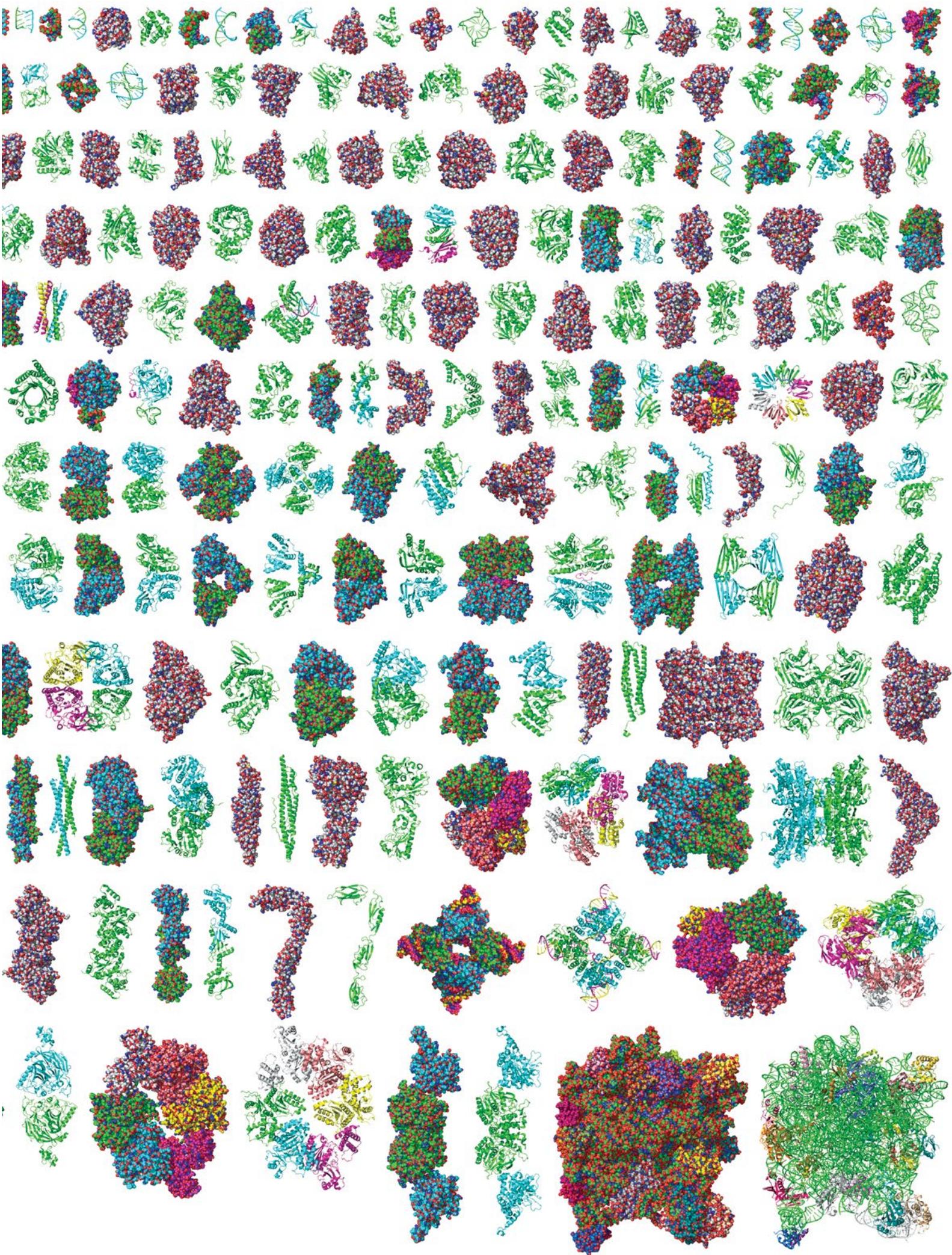
We are interested in comparing the aggregation profiles of different proteins and testing possibilities of their co-aggregation. The group has experience in the expression and purification of recombinant amyloid beta, alpha-synuclein, different isoforms of full-length Tau proteins, a variety of mammalian 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 2017 is the study of amyloid formation of the pro-inflammatory S100A9 protein, performed in collaboration with Umea University (Prof. L.Morozova-Roche). The application of the generic Finke-Watzky two-step nucleation-autocatalytic growth model to the kinetics of S100A9 aggregation demonstrated that a single molecule may become an aggregation nucleus at a higher rate than the subsequent fibrillar growth.

SELECTED PUBLICATIONS



1. Iashchishyn, I. A., Sulskis, D., Nguyen Ngoc, M., Smirnovas, V. & Morozova-Roche, L. A. Finke-Watzky Two-Step Nucleation-Autocatalysis Model of S100A9 Amyloid Formation: Protein Misfolding as "Nucleation" Event. *ACS Chemical Neuroscience* 8, 2152-2158 (2017).
2. Sneideris, T., Milto, K. & Smirnovas, V. Polymorphism of amyloid-like fibrils can be defined by the concentration of seeds. *PeerJ* 3, e1207 (2015).
3. Sneideris, T. et al. pH-Driven Polymorphism of Insulin Amyloid-Like Fibrils. *PloS one* 10, e0136602 (2015).
4. Šneideris, T. et al. Looking for a generic inhibitor of amyloid-like fibril formation among flavone derivatives. *PeerJ* 3, e1271 (2015).



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Molecular Signaling Pathways for Synaptic Pruning

The development of the mammalian nervous system is associated with a 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.

For microglia to discriminate between subsets of synapses that need to be removed or maintained, there must be (a) molecular signal(s) exposed on the surface of the synapse to trigger or inhibit microglial recognition and engulfment. Therefore, our focus is 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. Furthermore, we collaborate with the artificial intelligence company Oxipit (Lithuania) aiming to develop automated 3D image analysis tools based on machine learning. 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.

SELECTED PUBLICATIONS

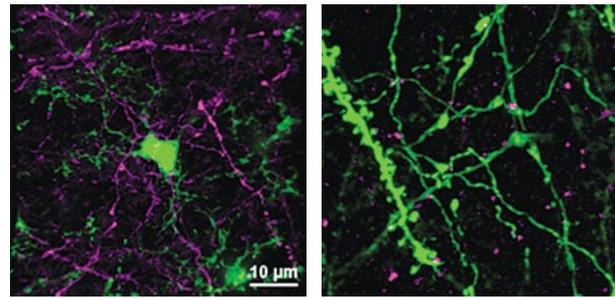


1. L. Weinhard et al., Microglia remodel synapses by presynaptic trogocytosis and spine head filopodia induction. *Nat Commun*, 10.1038/s41467-018-03566-5 (2018).
2. L. Weinhard et. al., Sexual dimorphism of microglia and synapses during mouse postnatal development. *Dev Neurobiol*, 10.1002/dneu.22568 (2017).
3. U. Neniškyte and C. T. Gross, Errant gardeners: glial-cell-dependent synaptic pruning and neurodevelopmental disorders. *Nat Rev Neurosci*, 10.1038/nrn.2017.110 (2017).



Lipid Scrambling as a Signal for Synaptic Pruning

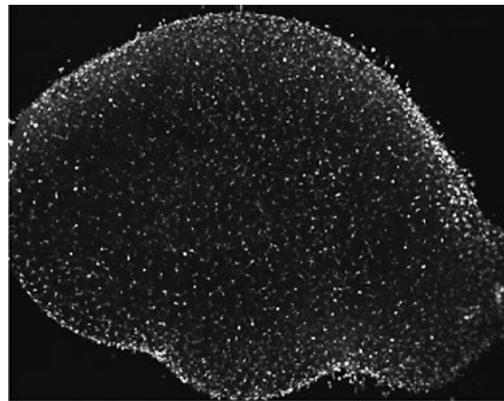
The risks underlying developmental circuit disorders and the reason why synaptic pruning is required to achieve the final connectome has been recently associated with the function of glial cells (Neniskyte and Gross, *Nat Rev Neurosci*, 2018). Using *ex vivo* tissue cultures and genetically modified mouse lines, we have identified that synapses that are targeted for phagocytic removal expose the lipid phosphatidylserine (PtdSer), which is then recognized by the microglia and leads to the pruning of unnecessary synapses. Mice, whose excitatory neurons lack phosphatidylserine transporters, have aberrations of neural circuit development. Our current investigations into the role of PtdSer during the postnatal pruning of synapses is supported by the European Union's Horizon 2020 research and innovation programme under the Marie Skłodowska-Curie grant agreement No. 705452 (2018-2021), the International



Brain Research Organization Return Home Fellowship (2016-2018) and the L'ORÉAL Baltic "For Women In Science" fellowship with the support of the Lithuanian National Commission for UNESCO and the Lithuanian Academy of Sciences (2017).

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

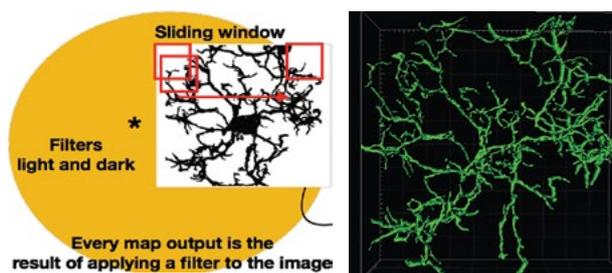
Capturing interactions between microglia and synapses during synaptic pruning requires advanced imaging techniques. Recently, together with our collaborators, we have employed light sheet fluorescent microscopy and correlative light and electron microscopy to visualize, for the first time, how the microglia "nibble" synapses and promote their plasticity through the growth of spine filopodia (Weinhard, Neniskyte *et al.*, *Nat Commun*, 2018). To define molecular interactions in microglia-synapse contacts, we use highly specific and sensitive click chemistry tools and develop molecular labeling techniques to define enzymatic cascades that control the balance of "eat-me" and "spare-me" signals. To visualize individual lipids in the synapse, we collaborate with the developers of nonlinear microscopy techniques (e.g., coherent Raman imaging).



We can both image and modulate neuronal activity, thus correlating synapse elimination signals with neuronal function.

Advanced 3D Image Analysis and Machine Learning for 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 (Weinhard, Neniskyte *et al.*, *Dev Neurobiol*, 2017). To avoid the commonly used, highly subjective classification of microglial cells by visual investigation, we are developing an artificial intelligence-based platform for the automatic classification of microglial cells based on their



morphology. Machine-classified microglia cells then are subjected to a comprehensive 3D morphological and functional analysis to define the microglial developmental profiles in relation with synaptic pruning.

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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 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 in the brain along with a tight collaboration with scientists from the US, Poland, Switzerland, Chile, the Czech Republic and New Zealand emerged into several successful international projects and an introduction of certain developed approaches into clinical settings both in Lithuania and abroad. A collaboration with neuroscientists, mathematicians and physicists 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) and signaling 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 behavioral, electrophysiological and neurovascular levels.

SELECTED PUBLICATIONS

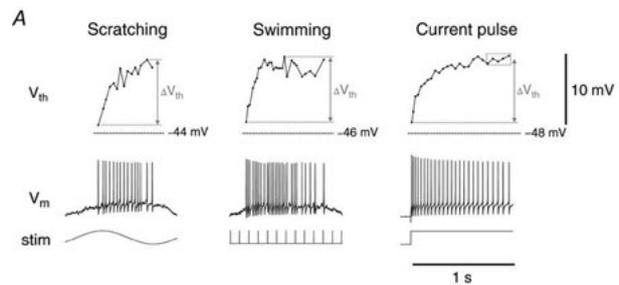


1. Pipinis E, Melynyte S, Koenig T, Jarutyte L, Linkenkaer-Hansen K, Ruksenas O, Griskova-Bulanova I. (2017) Association between resting-state microstates and ratings on the Amsterdam Resting-State Questionnaire. *Brain Topography* 30(2):245-248.
2. Binder, M., Gorska, U., Griskova-Bulanova, I. (2017) 40 Hz auditory steady-state responses in patients with disorders of consciousness: Correlation between phase-locking index and Coma Recovery Scale-Revised score. *Clinical neurophysiology*. Volume: 128 Issue: 5 Pages: 799-806.
3. Guzulaitis R., Hounsgaard J., Alaburda A. Irregular firing and high-conductance states in spinal motoneurons during scratching and swimming", *J Neurosci*. 2016 May 25;36(21):5799-807
4. Griškova-Bulanova, Inga; Hubl, Daniela; Van Swam, Claudia; Dierks, Thomas; Koenig, Thomas. Early- and late-latency gamma auditory steady-state response in schizophrenia during closed eyes: Does hallucination status matter? // *Clinical neurophysiology* 2016, Vol. 127, Iss. 5, p. 2214-2221.



Spike Threshold Dynamics in Spinal Motoneurons during Scratching and Swimming

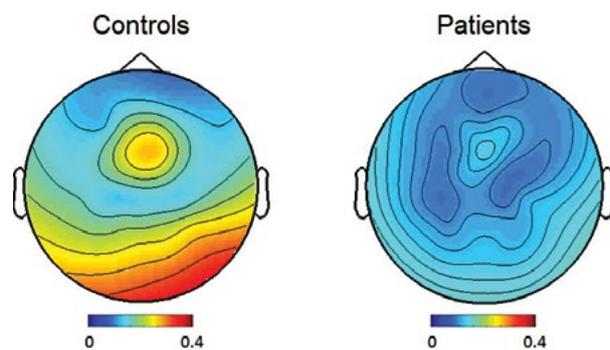
Intracellular recordings from spinal motoneurons in an *ex vivo* carapace-spinal cord preparation from adult turtles were performed during two distinct types of motor behavior – fictive scratching and fictive swimming. We found that the threshold of the first spike in episodes of scratching and swimming was the lowest. The threshold potential depolarizes by about 10 mV within each burst of spikes generated during scratch and swim network activity and recovers between bursts to a slightly depolarized level. The depolarization of the threshold potential results in a decreased excitability of motoneurons. The slow synaptic integration that results in a wave



of membrane potential depolarization rather than fast synaptic events preceding each spike is the factor influencing the threshold potential within firing bursts during motor behaviors (J Physiol. 2017 Sep 1;595(17):5843-5855).

40Hz Auditory Steady-State Responses in Patients with Disorders of Consciousness: A Correlation between the Phase-Locking Index and the Coma Recovery Scale-Revised Score

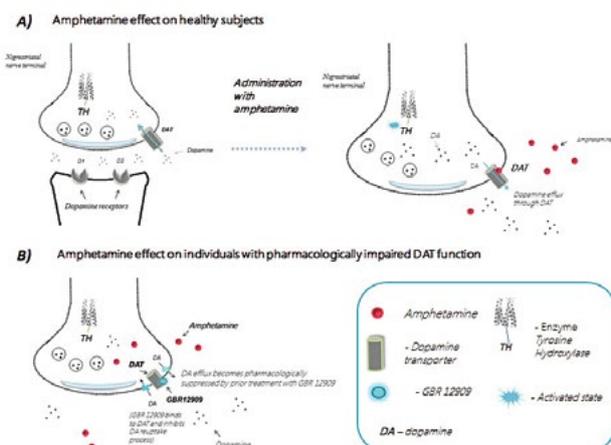
We aimed to elucidate whether a 40Hz auditory steady-state response (ASSR) could be sensitive to the state of patients with disorders of consciousness (DOC) as estimated with the Coma Recovery Scale-Revised (CRS-R) diagnostic tool. Fifteen DOC patients and 24 healthy controls took part in the study. The 40Hz click trains were used to evoke ASSRs. The mean evoked amplitude (EA) and phase-locking index (PLI) within a 38-42Hz window were calculated for 100ms bins, starting from 200 to 700ms and relative to the onset of stimulus. The PLI values from the patient group in the period of 200-500ms after the stimulus onset positively correlated with the CRS-R total score and with the scores of the Auditory and Visual subscales. The phase-locking index of 40Hz auditory steady-state responses can be an indicator of the level of dysfunction of the



central nervous system in DOCs (Binder, M., Gorska, U., Griskova-Bulanova, I. 40 Hz auditory steady-state responses in patients with disorders of consciousness: Correlation between phase-locking index and Coma Recovery Scale-Revised score. Clinical neurophysiology. Volume: 128 Issue: 5 Pages: 799-806).

The Potential Role of Tyrosine Hydroxylase in the Loss of the Psychostimulant Effect of Amphetamine under Conditions of Impaired Dopamine Transporter Activity

Amphetamine induces a paradoxical calming effect in humans with attention-deficit hyperactivity disorders. The underlying mechanism of this paradoxical calming effect of amphetamine in ADHD patients is poorly understood. Besides an inhibition of the dopamine transporter (DAT), amphetamine has also been demonstrated to regulate the activity of tyrosine hydroxylase (TH). Hence, the aim of this study was to determine the effect of amphetamine on TH activity in hyperactive rats. Our results indicate that amphetamine treatment alone increased locomotor activity in rats, whereas a pretreatment of rats with GBR12909 counteracted this effect. However, the phosphorylation levels of TH were not affected by the treatment with amphetamine, GBR12909 or the combination of both. Therefore, other mechanisms than phosphorylation-regulated TH activity changes are responsible



for the paradoxical calming effect of amphetamine (Janěnáitě, Eglé; Vengeliienė, Valentina; Běspalov, Anton; Behl, Berthold. // Behavioural brain research. 2017, Vol. 334, p. 105-108).





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Crystallography Open Database

Preview

$a=7.850 \text{ \AA}$
 $b=9.833 \text{ \AA}$
 $c=14.853 \text{ \AA}$
 $\alpha=90.122^\circ$
 $\beta=92.405^\circ$
 $\gamma=93.374^\circ$

Display in Jmol

Coordinates [7120377.cif](#)

Structure parameters

Chemical name	4,7-dimethyl-5-[3-[4-(2-fluorophenyl)piperazin-1-yl]propoxy]coumarin
Formula	C ₂₄ H ₂₇ F N ₂ O ₃
Calculated formula	C ₂₄ H ₂₇ F N ₂ O ₃
SMILES	<chem>O1C(=O)C=C(c2c(ccc12)C)OCCCN1CCN(C1)C1=CC=CC=C1F</chem>
Title of publication	Development of selective agents targeting serotonin 5HT _{1A} receptors with subnanomolar activities based on a coumarin core
Authors of publication	Ostrowska, Kinga; Grzeszczak, David; Gluch-Lutwin, Monika; Grybos, Anna; Sivek, Agata; Dobrzycki, Lukasz; Trzaskowski, Bartosz
Journal of publication	Med. Chem. Commun.
Year of publication	2017
a	$7.7595 \pm 0.0006 \text{ \AA}$
b	$9.8117 \pm 0.0006 \text{ \AA}$

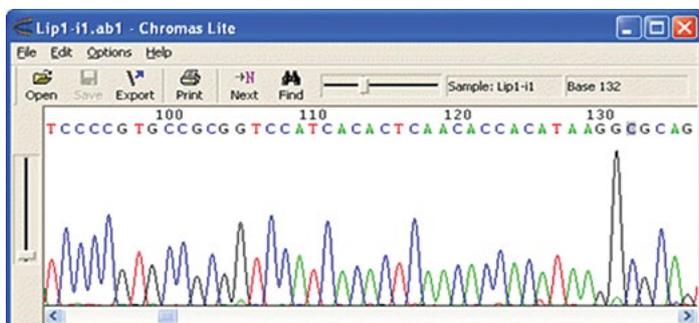
The Crystallography Open Database (COD, <http://www.crystallography.net/cod/>) is the largest to date open access collection of small molecule crystal structures, including organic non-polymer, inorganic and metal-organic compounds and minerals. All data are available in standard Crystallographic Interchange 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 formula 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.

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DNA Sequencing Center



The DNA Sequencing Center (SC), part of the Institute of Biotechnology (IBT) at the Life Sciences Center of Vilnius University, has been successfully running since March 27, 2003. The SC was founded to help researchers, both at IBT as well as other institutions in Lithuania, to process DNA samples in an efficient and economical manner. The Center is equipped with the Applied Biosystems 3130xl Genetic Analyzer 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 turn-around 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).

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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 Center 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 designed 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), hematology analyzer (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 Center and all external users. Regulatory and customized training courses on animal experimentation are regularly organized. The Laboratory Animal Science training program is certificated by the Lithuanian State Food and Veterinary Service.

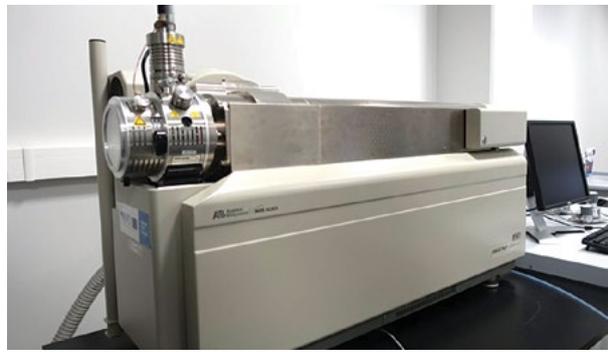
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Proteomics and Confocal Microscopy



The Proteomics Center is designated to perform high throughput, differential, quantitative proteome analyses and analyze protein localization and functions in fixed or live cells. The center is equipped with the Waters Synapt G2 higher definition mass spectrometer and the Sciex Qtrap4000 linear trap mass spectrometer, both directly coupled to nano-liquid chromatography systems and indirectly connected with a capillary range Dionex chromatography system. This allows us to offer the following services to our users: 1) Protein identification and quantitation in low and highly complex protein mixtures; 2) The implementation of a de novo sequencing of proteins from organisms with unknown or incomplete genomes; 3) 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 chemotherapeutic resistance of colon cancer chemotherapy (in collaboration with A. Laurinavicius, the National Center 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, US and A. Skebrdis, Lithuanian University of Health Sciences, Kaunas, Lithuania).

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

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Chemical Synthesis of Organic Compounds for Industry and Academy

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 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 or S-heterocyclic compounds, thiols, thioethers and thioamides, stereoisomeric disubstituted cyclohexane derivatives, aromatic carboxylic acids, amino acid derivatives, mono- and disubstituted cyclic polyethers, monodisperse derivatives of polyethylene glycols. These high-quality fine chemicals for scientific and commercial purposes are produced 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 execute 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 Biotecha (Lithuania), UAB Ekorama (Lithuania), UAB Vilniaus Ventos puslaidininkiai (Lithuania).

CONTACT INFORMATION:

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X-Ray Diffractometry and Crystal Growth Equipment



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 three-dimensional structures by means of single crystal X-ray crystallography techniques. The current diffractometer (Fig. 1b) comprises the Rigaku MM-007HF rotating anode microfocus generator with a Cu anode, VariMax focusing mirrors and two detectors: the Raxis-IV++ Image Plate detector (for protein crystallography) and the Pilatus 200k direct-conversion detector with a kappa stage (suitable for both small molecule and protein crystals). The Cu K_{α} radiation used in experiments is suitable for most organic crystals with light

elements, and it allows 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.

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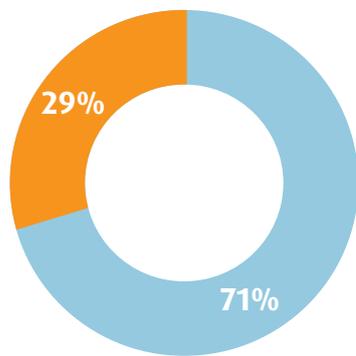
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Staff and Students

Staff

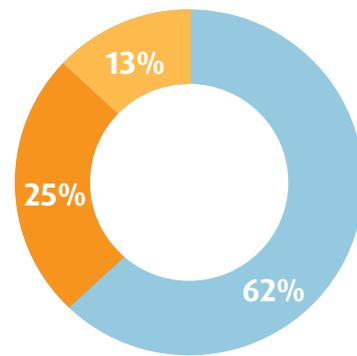
Academic staff	244
Non-academic staff	99
Total staff	343



■ Academic staff ■ Non-academic staff

Students

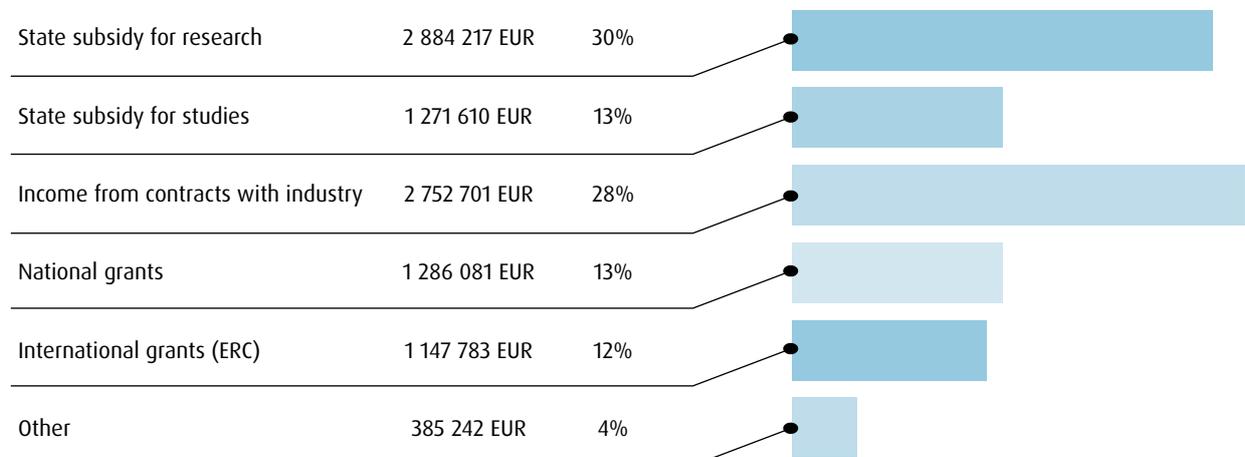
	2017/18
Bachelor's students	559
Master's students	226
PhD students	116
Total students:	901



■ Bachelor's students ■ Master's students ■ PhD Students

Financing Sources 2017

Total – 9,7 M EUR



An European Research Council (ERC) Advanced Grant

Prof. Saulius Klimašauskas, Director and Chief Scientist of the Institute of Biotechnology at the Life Sciences Center of Vilnius University, has been awarded an advanced ERC grant worth of 2.5 million euros for his 5-year project “Single-Cell Temporal Tracking of Epigenetic DNA Marks (EpiTrack).” Prof. Saulius Klimašauskas will be the first scientist to carry out an ERC-funded research at a Lithuanian institution.

This project is concerned with the mechanisms of epigenetic regulation of gene function in mammalian organisms. Over the past decade, epigenetics, the study of heritable changes of gene expression that are brought about without alterations of the genetic code itself, has taken central stage in our understanding of cellular differentiation, development and human disease. The process of adding a methyl group to a DNA molecule (DNA methylation) is one of the most prevalent epigenetic modifications in mammals. Prof. Klimašauskas’s team aims to deepen our understanding of how the genomic methylation patterns are established and how they govern cell variability and the ability to differentiate during development. Although DNA methylation has been extensively investigated, the key mechanisms of these fascinating events remain obscure.

The most dramatic epigenomic reprogramming in mammalian development occurs after fertilization, whereby a global loss of DNA methylation is followed by a massive reinstatement of new methylation patterns, different for each cell type. The goal of this project is to determine, with high precision, where and when the methylation marks are deposited by each of the three known DNA methylation enzymes (DNA methyltransferases) and how these methylation marks affect gene expression. To achieve this ambitious goal, the professor’s team will metabolically engineer mouse cells to permit cofactor, analog-based chemical pulse-tagging of their methylation sites *in vivo*. The scientists will advance the profiling of DNA modifi-



cations to the single cell level with an innovative integration of microdroplet-based barcoding, precise genomic mapping and super-resolution imaging. Using this unique experimental system, the researchers will determine, with unprecedented detail and throughput, the dynamics and variability of DNA methylation and gene expression patterns during the differentiation of mouse embryonic cells to neural lineages. The project will provide a comprehensive view of the roles that the three known DNA methylation enzymes play in mammalian development, thereby advancing our understanding of human development and disease.

International Grants

Horizon 2020

<i>Title</i>	<i>Head of the project</i>	<i>Duration</i>
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
<i>Title</i>	<i>Lead scientist</i>	<i>Duration</i>
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

International Brain Research Organization (IBRO) Return Home Fellow

<i>Title</i>	<i>Head of the project</i>	<i>Duration</i>
Investigation of molecular mechanisms that guide synaptic pruning in developing brain during brain circuit maturation. IBRO 2017	U. Neniškytė	2017

Lithuanian-Latvian-Taiwan Cooperation Program

<i>Title</i>	<i>Head of the project</i>	<i>Duration</i>
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

Lithuanian-Japan research program

<i>Title</i>	<i>Lead scientists</i>	<i>Duration</i>
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

COST

<i>Title</i>	<i>Lead scientist</i>	<i>Duration</i>
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
Chemical Approaches to Targeting Drug Resistance in Cancer Stem Cells. No. CM1106	R. Navakauskienė V. Borutinskaitė	2011-2016
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
Systems Biocatalysis. No. CM1303.	S. Klimašauskas I. Matijošytė	2013-2017

Doctoral Theses

<i>Name</i>	<i>Title</i>	<i>Supervisor</i>
J. Butkuvienė	<i>Batrachium</i> in Lithuanian Rivers: Morphological and Genetic Diversity and Related Factors	D. Žvingila
K. Daniūnaitė	The Diagnostic and Prognostic DNA Methylation Biomarkers of Prostate Cancer	S. Jarmalaitė
V. Grajevskaja	Studying the Mechanisms of Cardiovascular Development and Regeneration in <i>Danio Rerio</i> (Hamilton 1822)	R. Rakauskas
G. Kostiuk	Single Molecule Studies of the Monomeric Restriction Endonuclease BcnI-DNA Interactions	V. Šikšnys
V. Linkuvienė	Observed and Intrinsic Thermodynamic and Kinetic Parameters of Sulfonamide Derivative Binding to Carbonic Anhydrases	D. Matulis
K. Olechnovič	Methods for the Analysis and Assessment of the Three-Dimensional Structures of Proteins and Nucleic Acids: Development and Applications	Č. Venclovas
R. Pranckutė	An Evaluation of Lactic Acid and Thermophilic Bacteria Antibacterial Activity and Compatibility with Prebiotic Oligosaccharides for the Development of New Synbiotics	N. Kuisienė D. Čitavičius
T. Ragaliauskas	The Immobilization of Lipid Membranes on the Planar Surfaces. A Surface Plasmon Resonance Study	G. Valinčius
V. Raškevičius	The Computational Studies of Protein and Ligand Interactions	V. Kairys
R. Rimgailė-Voicik	The Organization and Functioning Patterns of <i>Lycopodium L.</i> and <i>Diphasiastrum Holub</i> Populations with an Emphasis on Gametophytes and Juvenile Sporophytes in Dry Pine Forests	J. Naujalis
V. Simanavičienė	The Molecular Diagnostics of Human Papillomavirus (HPV) and Studies on HPV Prevalence	A. Žvirblienė
I. Šakinytė	Reagentless Enzymatic Systems Consisting of Carbonaceous Structures	J. Razumienė
E. Šimoliūnas	The Construction of Self-Assembling Nanostructures Based on the Structural Proteins of Bacteriophages	R. Meškys
P. L. Tamošiūnas	Yeast-Generated, Parvoviral Virus-Like Particles and Their Use in Diagnostics	K. Sasnauskas
V. Valskys	The influence of the Conditions of Sapropel Formation on Its Chemical Composition and Contamination	G. Ignatavičius

International Study Programs

The VU Life Sciences Center (LSC) offers 12 different bachelor's and master's study programs. For international students interested in studying life sciences, the LSC offers five 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.

Please refer to the full description for more detailed information regarding the program:
https://www.vu.lt/site_files/TPRS/2018_degree_programmes/2018_BIOCHEMISTRY_K.pdf

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.

Please refer to the full description for more detailed information regarding the program:
https://www.vu.lt/site_files/TPRS/2018_degree_programmes/2018_Biophysics_K.pdf

Academic contact: Prof. Aidas Alaburda.

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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.

Please refer to the full description for more detailed information regarding the program:

https://www.vu.lt/site_files/TPRS/2018_degree_programmes/2018_GENETICS_K.pdf

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.

Please refer to the full description for more detailed information regarding the program:

https://www.vu.lt/site_files/TPRS/2018_degree_programmes/2018_MOLECULAR_BIOLOGY_K.pdf

Academic contact: Prof. Edita Sužiedėlienė.

Email: edita.suziedeliene@gf.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, behavior 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 and integrate knowledge from different scientific fields.

Please refer to the full description for more detailed information regarding the program:

https://www.vu.lt/site_files/TPRS/2018_degree_programmes/2018_NEUROBIOLOGY_K.pdf

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Admission contact: admissions@cr.vu.lt

Awards

INTERNATIONAL AWARDS



The Novozymes Prize

The 2017 Novozymes Prize was awarded to Prof. Dr. Virginijus Šikšnys, Head of the Department of Protein-DNA Interactions at the VU Life Sciences Center, and Dr. Emmanuelle Charpentier, Director and Scientific Member at the Max Planck Institute for Infection Biology in Germany, for their contributions in the development of the CRISPR-Cas9 gene editing tool – one of the most important scientific breakthroughs that opened new opportunities for researchers to edit and modify genes of various organisms.

The Novozymes Prize is awarded to recognize outstanding European research or technology contributions that benefit the development of biotechnological science. The Novozymes Prize consists of a funding amount for the awardees' research (DKK 2.5 million) and a personal award (DKK 0.5 million). An additional part of the Prize is an international symposium within the awardees' field of research.



The Lithuanian-American Innovation Award

Four VU LSC researchers – Dr. L. Mažutis, Prof. Dr. V. Šikšnys, Dr. G. Gasiūnas and Dr. T. Karvelis – were awarded the Lithuanian-American Innovation Award. Dr. L. Mažutis received the award for developing a technique for the efficient isolation and sequencing of single cells in collaboration with scientists from Harvard University. Prof. Dr. V. Šikšnys, Dr. G. Gasiūnas and Dr. T. Karvelis were awarded for the development of the CRISPR-Cas9 technology, which enables the editing of genomes. Their technology patent has been licensed to DuPont Pioneer, the world's

leading developer and supplier of advanced plant genetics. Vilnius University and DuPont have entered into a multiyear research collaboration to advance the development of the technology.

The Lithuanian-American Innovation Award was established by the American-Lithuanian Business Council (ALBC), the Baltic American Freedom Foundation (BAFF) and the US Embassy in Vilnius as a reward to a Lithuanian individual, organization, university or firm that have developed cutting-edge technologies or solutions in collaboration with US partners.

A New Member of EMBO

On June 16 EMBO announced that Prof. S. Klimašauskas has been elected a member of EMBO and joined the group of more than 1 700 of the best researchers in the world. “EMBO Members are leading scientists working across all of the life sciences. They also strengthen the research community in Europe and beyond through their international collaborations and connections,” says EMBO Director Maria Leptin.

EMBO Members are actively involved in the execution of the organization's initiatives by evaluating applications for EMBO funding and by serving on EMBO Council, Committees and Editorial Boards. Prof. V. Šikšnys has been elected an associate EMBO member in 2016, making it two representatives of Lithuania at this prestigious organization.





L'Oréal-UNESCO Baltic "For Women in Science" Fellowship

Dr. Urtė Neniškytė was awarded the prestigious L'Oréal-UNESCO Baltic "For Women in Science" fellowship on 26 May 2017 with the support of the Lithuanian National Commission for UNESCO and the Lithuanian Academy of Sciences. It is a reward for women scientists who have achieved outstanding contributions to the advancement of scientific knowledge and to the benefit

of society. Dr. Neniškytė is the first scientist in Lithuania ever to have received this award. She obtained her PhD at the University of Cambridge (UK) in molecular developmental neuroscience and is currently investigating molecular mechanisms that guide synaptic pruning in the developing brain during brain circuit maturation.



The Grand Prize in the iGEM Competition



The Vilnius-Lithuania iGEM team won the Grand Prize and received a gold medal as well as three special awards – Best New Basic Part, Best New Composite Part and Best Part Collection – in the largest and most prestigious Synthetic Biology competition iGEM (International Genetically Engineered Machine), where more than 300 students' teams from leading world universities worked all year long to solve real-world challenges by

building genetically engineered biological systems with standard, interchangeable parts.

In 2017, our team competed with their project SynORI – a framework designed to make working with single and multi-plasmid systems precise, easy and more functional. This system will help with everyday lab work, and it can also be used for biological computing and the assembly of large protein complexes or metabolic engineering.

NATIONAL AWARDS

The Lithuanian Science Award



In 2017, VU LSC scientists Prof. Valdemaras Razumas and Dr. Gintaras Valinčius from the Institute of Biochemistry have been awarded “The Lithuanian Science Prize” for a series of works “2D and 3D self-assembled systems: synthesis, properties and applications. The Lithuanian Science Award is granted every year by the Government of Lithuania for outstanding contribution to science and technological developments of national importance.



The Global Lithuanian Leaders Award



For bringing scientific innovations to Lithuania, Dr. Linas Mažutis 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.

The Order of the Lithuanian Grand Duke Gediminas



In 2017, Prof. Dr. Virginijus Šikšnys received the Cross of the Order of the Lithuanian Grand Duke Gediminas. It is the Lithuanian Presidential Award that honors the citizens of Lithuania for outstanding performance in civil and public offices.



VU Rectors Award

The VU Rectors Award for Excellence in Teaching was granted to Dr. Edita Sužiedelienė. The VU Rectors Award for Excellence in Science was granted to Dr. Gintautas Tamulaitis and Dr. Linas Mažutis.

Selected Events

The COINS

The COINS is the 13th international conference of life sciences that has been organized by the Vilnius University Students Representation. The event gathers not only students and scholars but various scientists working in the life science field as well.

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's students and doctorates of the Life Sciences Center to present their research results and benefit from the experience of internationally-known researchers.



Mobile Bioclass

The Mobile Bioclass is a mobile laboratory in Lithuania. It aims to promote biosciences among school children and inspire them to pursue careers in sciences. During the Bioclass, pupils get a chance to become scientists, to 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 class rooms.

The Mobile Bioclass is a joint project of the company Thermo Fisher Scientific Baltics and Vilnius University, which has been carried out since 2011. Until end of 2017, the Mobile Bioclass has visited over 100 schools in 70 cities of Lithuania.



The First Meeting of the Lithuanian Microbiologists' Society

The first meeting of the Lithuanian Microbiologists' Society was held on June 16, 2017. Microbiologists from the whole country discussed the possibility of the society becoming a member of the

Federation of European Microbiological Societies (FEMS), reviewed achievements from all sections of microbiology in Lithuania, talked about the need of common terminology of microbiology as well as the creation of a national collection of microorganisms.

Night of the Researchers

LSC researchers actively take part in the European research festival "European Night of the Researchers" – an annual scientific event held in all Europe that aims 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 place for visitors of all ages.



National Science Festival “Spaceship Earth”

The national science festival “Spaceship Earth” took place during September 11-20, 2017 at VU LSC. It is an annual festival organized in two biggest Lithuanian cities – Vilnius and Kaunas. Since 2004, this annual festival became 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, which now annually exceed 300), each September, more than 30 000 participants visit all the main universities, laboratories of the biggest technological companies, other innovative companies and museums. Children of all ages, school pupils and families are taking part at the concomitant open-air events (science fairs) in Vilnius and Kaunas.



Science Day

The annual event “Science Day,” organized by LSC’s industrial partner Thermo Fisher Scientific, was held on October 12, 2017. Visitors had the opportunity to see a broad selection of products used in life sciences, participate in seminars discussing new technologies and join guided tours through the LSC research laboratories.



Exhibition „For Women in Science“

VU LSC hosted photo exhibition „For Women in Science“ that was dedicated to five laureates of L’Oréal-UNESCO Baltic „For Women in Science“ fellowship: Jekaterina Ivanova, Marina Sokolova, dr. Renate Ranka (Latvia), dr. Els Heinsalu (Estonia) and dr. Urtė Neniškytė (Lithuania).

Urtė Neniškytė works at VU LSC. She is the first scientist in Lithuania to ever receive this award. Her research related to medicine biotechnology, to reveal the molecular basis of certain neurodevelopmental and neuropsychiatric diseases such as autism or schizophrenia.

The opening of this photo exhibition was held on 24 of August, 2017. The minister of science and education of Lithuania Jurgita Petrauskienė, vice-rector of Vilnius University Rimantas Jankauskas and Lithuanian National Commission for UNESCO representative Miglė Mašanauskienė delivered their speeches during the opening ceremony.



Visits

In 2017, Lithuania became the fifth associate member of CERN, joining a group of twenty-two member states and four associate members. Lithuania's membership agreement with CERN was signed at the Presidential Palace on June 27. On that occasion, CERN's Director-General, Fabiola Gianotti, visited the VU Life Sciences Center. Fabiola Gianotti was accompanied by CERN's Director for International Relations Charlotte Lindberg Warakaulle and PhD Christoph Schäfer, Advisor of CERN for Non-Member States and International Relations. The honorable guests met with the leading researchers and visited research laboratories.



Jean-David Malo, Director for *Open Innovation and Open Science*, Directorate-General for Research and Innovation at the European Commission, visited VU LSC on November 20, 2017. The commissioner met with Prof. Virginijus Šikšnys, who has significantly contributed to the development of the CRISPR/Cas9 system, and PhD Linas Mažutis, whose multidisciplinary team is developing droplet microfluidic techniques as a tool to analyze and profile single-cells, sequence individual cells in clinical samples, screen antibody producing B-cells, perform digital DNA quantification and many more biological and biomedical applications.



Craig L. Tucker, Vice President of Policy & Public Affairs for Life Sciences Pennsylvania, visited VU LSC on December 11, 2017. Inga Matijošytė, PhD, a researcher at the VU Life Science Center, who is also the President of the Lithuanian Biotechnology Association, discussed further trends of collaboration in the field

of life sciences. At the beginning of the December Association, President Inga Matijošytė and Christopher P. Molineaux, President & CEO of Life Sciences Pennsylvania, signed the memorandum of understanding, which opened new ways of collaboration between scientists and businessmen in both the US and Lithuania.

Invited Speakers

A Nobel Prize laureate, Director of the Max Planck Institute of Biochemistry, Professor of the Technical University of Munich and Doctor Honoris Causa of Vilnius University, Robert Huber gave a lecture "New Ways of Vision: Protein Structures in Translational Medicine and Business Development – My Experience" on July 5 at VU LSC.



A Nobel Prize laureate in Economic Sciences and Professor of Yale University, Robert J. Shiller visited VU LSC and delivered a lecture "Narrative Economics and Neuroeconomics," which attracted a large audience from the whole Vilnius University. R. J. Shiller's talk involved some topics that are extremely relevant in the scope of Physics of Risk, including the "epidemic" approach to economics and the importance of narration to humans as species.



Publications in 2017

- Abdelwahid, E.; Stulpinas, A.; Kalvelytė, A. V. Effective agents targeting the mitochondria and apoptosis to protect the heart. *Current Pharmaceutical Design*. 2017, 23(8): 1153-1168.
- Bagdžiūnas, G.; Stončius, S.; Butkus, E. Synthesis of star-shaped aromatic derivatives with chirality predetermined by bicyclo[3.3.1] nonane framework. *Synlett*. 2017, 28(20): 2790-2794.
- Balčiūnaitė, G.; Dam, S.; Pesliakas, H.; Žvirblis, G.; Mištinienė, E.; Ragažinskienė, O.; Pamparienė, I.; Žymantienė, J.; Baniulis, D.; Savickienė, N. Investigation of *Echinacea purpurea* root proteins with hemagglutinating activity. *Natural Product Communications*. 2017, 12(6): 947-950.
- Baltruškevičienė, E.; Schweigert, D.; Stankevičius, V.; Mickys, U.; Žvirblis, T.; Bublevič, J.; Sužiedėlis, K.; Aleknavičius, E. Down-regulation of miRNA-148a and miRNA-625-3p in colorectal cancer is associated with tumor budding *BMC Cancer*. 2017, 17, Art. No. 607.
- Baranauskas, M.; Grabauskaitė, A.; Griškova-Bulanova, I. Brain responses and self-reported indices of interoception: Heartbeat evoked potentials are inversely associated with worrying about body sensations. *Physiology & Behavior*. 2017, 180: 1-7.
- Baranauskienė, L.; Matulis, D. Herbicide oryzalin inhibits human carbonic anhydrases *in vitro*. *Journal of Biochemical and Molecular Toxicology*. 2017, 31(6), Art. No. e21894: 5.
- Barševičiūtė, R.; Skipitytė, R.; Pukienė, R.; Lapeikaitė, I.; Kakaras, I.; Remeikis, V. Climatic sensitivity of $\delta^{13}C$ in tree rings of *Quercus robur L.*, *Populus tremula L.* and *Pinus sylvestris L.* in Vilnius region (eastern Lithuania). *Dendrobiology*. 2017, 78: 1-9.
- Bikulčius, G.; Valius, M.; Ručinskienė, A.; Jankauskas, S.; Asadauskas, S. Measurement of lubricant layer thickness in anodised alumina using confocal fluorescence microscopy. *Transactions of the IMF*. 2017, 95(4): 226-232.
- Binder, M.; Gorska, U.; Griškova-Bulanova, I. 40Hz auditory steady-state responses in patients with disorders of consciousness: Correlation between phase-locking index and Coma Recovery Scale-Revised score. *Clinical Neurophysiology*. 2017, 128(5): 799-806.
- Borutinskaitė, V. V.; Treigytytė, G.; Matuzevičius, D.; Zaikova, I.; Čeksterytė, V.; Navakauskas, D.; Kurtinaitienė, B.; Navakauskienė, R. Proteomic analysis of pollen and blossom honey from rape seed *Brassica napus L.* *Journal of Apicultural Science*. 2017, 61(1): 73-92.
- Buchovec, I.; Lukševičiūtė, V.; Kokštaitė, R.; Labeikytė, D.; Kaziukonytė, L.; Lukšienė, Ž. Inactivation of Gram (-) bacteria *Salmonella enterica* by chlorophyllinbased photosensitization: Mechanism of action and new strategies to enhance the inactivation efficiency. *Journal of Photochemistry and Photobiology B*. 2017, 172: 1-10.
- Bulavaitė, A.; Lasickienė, R.; Tamošiūnas, P. L.; Šimanavičius, M.; Sasnauskas, K.; Žvirblienė, A. Synthesis of human parainfluenza virus 4 nucleocapsid-like particles in yeast and their use for detection of virus-specific antibodies in human serum. *Applied Microbiology and Biotechnology*. 2017, 101(7): 2991-3004.
- Bulkescher, R.; Starkuvienė, V.; Erfle, H. Solid-phase reverse transfection for intracellular delivery of functionally active proteins. *Genome Research*. 2017, 27(10): 1752-1758.
- Butkuvienė, J.; Sinkevičienė, Z.; Naužemys, D.; Patamsytė, J.; Žvingila, D. Genetic diversity of *Batrachium (Ranunculaceae)* species reveals the necessity of their protection in Lithuanian rivers. *Aquatic Botany*. 2017, 142: 61-70.
- Čėsniėnė, T.; Kleizaitė, V.; Bondzinskaitė, S.; Taraškevičius, R.; Žvingila, D.; Šiukšta, R.; Rančelis, V. P. Metal bioaccumulation and mutagenesis in a *Tradescantia* clone following long-term exposure to soils from urban industrial areas and closed landfills. *Mutation Research / Genetic Toxicology and Environmental Mutagenesis*. 2017, 823: 65-72.
- Chmieliauskas, S.; Banionis, D.; Laima, S.; Andriuskevičiūtė, G.; Mažeikienė, S.; Stasiūnienė, J.; Jasulaitis, A.; Jarmalaitė, S. Autopsy relevance determining hemochromatosis: case report *Medicine*. 2017, 96(49), Art. No. e8788: 1-4.
- Cicėnas, J.; Tamošaitis, L.; Kvederavičiūtė, K.; Tarvydas, R.; Staniūtė, G.; Kalyan, K.; Meškinytė-Kaušilienė, E.; Stankevičius, V.; Valius, M. KRAS, NRAS and BRAF mutations in colorectal cancer and melanoma. *Medical Oncology*. 2017, 34(2), Art. No. 26.
- Costa, F. A.; Scullin, C.; Al-Taani, G.; Hawwa, A. F.; Anderson, C.; Bezverhni, Z.; Binakaj, Z.; Cordina, M.; Foulon, V.; de Bikuña, B. G.; de Gier, H.; Granas, A. G.; Grinstova, O.; Griese-Mammen, N.; Grincevičius, J.; Grincevičienė, Š.; Kaae, S.; Kubilienė, L.; Marino, E. L.; Martins, S.; Modamio, P.; Nadin, G.; Nørgaard, L. S.; Obarcanin, E.; Tadic, I.; McElnay, J. C.; Hersberger, K. E.; Westerlund, T. Provision of pharmaceutical care by community pharmacists across Europe: is it developing and spreading? *Journal of Evaluation in Clinical Practice*. 2017, 23(6): 1336-1347.
- Dagys, M.; Laurynėnas, A.; Ratautas, D.; Kulys, J.; Vidžiūnaitė, R.; Talaiškis, M.; Niaura, G.; Marcinkevičienė, L.; Meškys, R.; Shleev, S. Oxygen electroreduction catalysed by laccase wired to gold nanoparticles via trinuclear copper cluster. *Energy & Environmental Science*. 2017, 10(2): 498-502.
- Daniūnaitė, K.; Dubikaitytė, M.; Gibas, P.; Bakavičius, A.; Lazutka, J. R.; Ulys, A.; Jankevičius, F.; Jarmalaitė, S. Clinical significance of miRNA host gene promoter methylation in prostate cancer. *Human Molecular Genetics*. 2017, 26(13): 2451-2461.
- Dankinas, D.; Mėlynyntė, S.; Šiurkutė, A.; Dapšys, K. Pathological imitative behavior and response preparation in schizophrenia. *Archives of Clinical Neuropsychology*. 2017, 32(5): 533-540.
- Dapkūnas, J.; Timinskas, A.; Olechnovič, K.; Margelevičius, M.; Dičiūnas, R.; Venclovas, Č. The PPI3D web server for searching, analyzing and modeling protein-protein interactions in the context of 3D structures. *Bioinformatics*. 2017, 33(6): 935-937.
- Dapkūnas, Ž.; Baranauskas, A.; Mickienė, G.; Plečkaitytė, M.; Žvirblis, G. Generation of dimeric single-chain antibodies neutralizing the cytolytic activity of vaginolysin. *Electronic Journal of Biotechnology*. 2017, 28: 52-57.
- Dapkutė, D.; Steponkienė, S.; Bulotienė, D.; Saulite, L.; Riekstina, U.; Rotomskis, R. Skin-derived mesenchymal stem cells as quantum dot vehicles to tumors. *International Journal of Nanomedicine*. 2017, 12: 8129-8142.
- Demidenko, R.; Daniūnaitė, K.; Bakavičius, A.; Sabaliauskaitė, R.; Skeberdytė, A.; Petroška, D.; Laurinavičius, A.; Jankevičius, F.; Lazutka, J. R.; Jarmalaitė, S. Decreased expression of MTIE is a potential biomarker of prostate cancer progression. *Oncotarget*. 2017, 8(37): 61709-61718.
- Dittrich, A.; Wehrden, H.; Abson, D. J.; Bartkowski, B.; Cord, A. F.; Fust, P.; Hoyer, C.; Kambach, S.; Meyer, M. A.; Radzevičiūtė, R.; Nieto-Romero, M.; Seppelt, R.; Beckmann, M. Mapping and analysing historical indicators of ecosystem services in Germany. *Ecological Indicators*. 2017, 75: 101-110.
- Donders, G.G.G.; Bellen, G.; Grincevičienė, Š.; Ruban, K.; Vieira-Baptista, P. *Aerobic vaginitis*: no longer a stranger. *Research in Microbiology*. 2017, 168(9-10): 845-858.
- Dulinskaskas, R.; Buišas, R.; Vengeliėnė, V.; Rukšėnas, O. Effect of ethanol on the visual-evoked potential in rat: dynamics of ON and OFF responses. *Acta Neurobiologiae Experimentalis*. 2017, 77(2): 190-197.
- Engblom, C.; Pfirsche, C.; Žilionis, R. Osteoblasts remotely supply lung tumors with cancer-promoting SiglecF(high) neutrophils. *Science*. 2017, 358(6367), Art. No. eaal5081: 15.
- Gaidukevič, J.; Razumienė, J.; Šakinytė, I.; Rebelo, S. L.H.; Barkauskas, J. Study on the structure and electrocatalytic activity of graphene-

- based nanocomposite materials containing (SCN)n. Carbon. 2017, 118: 156-167.
31. Gedvilaitė, A.; Tryland, M.; Ulrich, R. G.; Schneider, J.; Kurmauskaitė, V.; Moens, U.; Preugschas, H.; Calvignac-Spencer, S.; Ehlers, B. Novel polyomaviruses in shrews (*Soricidae*) with close similarity to human polyomavirus 12. *Journal of General Virology*. 2017, 98(12): 3060-3067.
 32. Ghosh, S.; Kuisienė, N.; Cheeptham, N. The cave microbiome as a source for drug discovery: reality or pipe dream? *Biochemical Pharmacology*. 2017, 134: 18-34.
 33. Grabauskaitė, A.; Baranauskas, M.; Griškova-Bulanova, I. Interoception and gender: What aspects should we pay attention to? *Consciousness and Cognition*. 2017, 48: 129-137.
 34. Griffin, Sh.; Preta, G.; Sheldon, I. M. Inhibiting mevalonate pathway enzymes increases stromal cell resilience to a cholesterol-dependent cytotoxicity. *Scientific Reports*. 2017, 7, Art. No. 17050: 1-13.
 35. Grigonis, R.; Alaburda, A. Spike threshold dynamics in spinal motoneurons during scratching and swimming. *Journal of Physiology*. 2017, 595(17): 5843-5855.
 36. Grincevičienė, Š.; Bellen, G.; Ruban, K.; Donders, G.G.G. Non-response to fluconazole maintenance treatment (ReCiDiF regimen) for recurrent vulvovaginal candidosis is not related to impaired glucose metabolism. *Mycoses*. 2017, 60(8): 546-551.
 37. Grincevičienė, Š.; Donders, G.G.G. Comment on treatment for recurrent vulvovaginal candidiasis. *American Journal of Obstetrics & Gynecology*. 2017, 216(4): 426-427.
 38. Gudiukaitė, R.; Gricajeva, A. Microbial lipolytic fusion enzymes: current state and future perspectives. *World Journal of Microbiology and Biotechnology*. 2017, 33(12): 1-8.
 39. Gudiukaitė, R.; Sadauskas, M.; Gegeckas, A.; Malūnavičius, V.; Čitavičius, D. J. Construction of a novel lipolytic fusion biocatalyst GDEst-lip for industrial application. *Journal of Industrial Microbiology and Biotechnology*. 2017, 44: 799-815.
 40. Gupta, P. P.; Bastikar, V. A.; Kučiauskas, D.; Lal Kothari, Sh.; Cičenas, J.; Valius, M. Molecular modeling and structure-based drug discovery approach reveals protein kinases as off-targets for novel anticancer drug RH1. *Medical Oncology*. 2017, 34(10), Art. No. 176.
 41. Gyvytė, U.; Juzėnas, S.; Šaltenienė, V.; Kupčinskas, J.; Poškienė, L.; Kučiauskas, L.; Jarmalaitė, S.; Stuoopelytė, K.; Steponaitienė, R.; Hemmrich-Stanisak, G.; Hübenthal, M.; Link, A.; Franke, S.; Franke, A.; Pangonytė, D.; Lesauskaitė, V.; Kupčinskas, L.; Skiecevičienė, J. MiRNA profiling of gastrointestinal stromal tumors by next-generation sequencing. *Oncotarget*. 2017, 8(23): 37225-37238.
 42. Havelka, J.; Rakauskas, R.; Turčinavičienė, J. Exotic aphid species *Brachycaudus divaricatae* in Central Europe: Distribution, host specificity and molecular diversity. *Biologia*. 2017, 72(3): 342-349.
 43. Hutchinson, J. M.C.; Reise, H.; Skujienė, G. Life cycles and adult sizes of five co-occurring species of *Arion* slugs. *Journal of Molluscan Studies*. 2017, 83(1): 88-105.
 44. Iashchishyn, I. A.; Šulskis, D.; Ngoc, M. N.; Smirnovas, V.; Morozova-Roche, L. A. Fink-Watzky two-step nucleation-autocatalysis model of S100A9 amyloid formation: protein misfolding as “nucleation” event. *ACS Chemical Neuroscience*. 2017, 8(10): 2152-2158.
 45. Ignatavičius, G.; Valskys, V.; Bulskaya, I.; Paliulis, D.; Zigmontienė, A.; Satkūnas, J. Heavy metal contamination in surface runoff sediments of the urban area of Vilnius, Lithuania. *Estonian Journal of Earth Sciences*. 2017, 66(1): 13-20.
 46. Ivanauskas, F.; Laurinavičius, V. S.; Sapagovas, M.; Nečiporenko, A. Reaction-diffusion equation with nonlocal boundary condition subject to PID-controlled bioreactor. *Nonlinear Analysis: modelling and control*. 2017, 22(2): 261-272.
 47. Janėnaitė, E.; Venglienė, V.; Bepalov, A.; Behl, B. Potential role of tyrosine hydroxylase in the loss of psychostimulant effect of amphetamine under conditions of impaired dopamine transporter activity. *Behavioural Brain Research*. 2017, 334: 105-108.
 48. Janonienė, A.; Liu, Z.; Baranauskienė, L.; Mäkilä, E.; Ma, M.; Salonen, J.; Hirvonen, J.; Zhang, H.; Petrikaitė, V.; Santos, H. A. A versatile carbonic anhydrase ix targeting ligand functionalized porous silicon nanoplatfom for dual hypoxia cancer therapy and imaging. *ACS Applied Materials & Interfaces*. 2017, 9(16): 13976-13987.
 49. Janulaitienė, M.; Paliulytė, V.; Grincevičienė, Š.; Zakarevičienė, J.; Vladiškauskienė, A.; Marcinkutė, A.; Plečkaitytė, M. Prevalence and distribution of *Gardnerella vaginalis* subgroups in women with and without bacterial vaginosis. *BMC Infectious Diseases*. 2017, 17, Art. No. 394.
 50. Jarašienė-Burinskaja, R.; Alksnė, M.; Bartuškienė, V.; Voišnienė, V.; Burinskij, J.; Čėnas, N.; Bukelskienė, V. Study of the cytotoxic effects of 2,5-diaziridinyl-3,6-dimethyl-1,4-benzoquinone (MEDZQ) in mouse hepatoma cells. *EXCLI Journal*. 2017, 16: 151-159.
 51. Javali, A.; Misra, A.; Leonavičius, K.; Acharyya, D.; Vyas, B.; Sambasivan, R. Co-expression of Tbx6 and Sox2 identifies a novel transient neuromesoderm progenitor cell state. *Development*. 2017, 144(24): 4522-4529.
 52. Jurėnas, D.; Chatterjee, S.; Konijnenberg, A.; Sobott, F.; Droogmans, L.; Garcia-Pino, A.; Van Melderen, L. AtaT blocks translation initiation by N-acetylation of the initiator tRNA^{Met}. *Nature Chemical Biology*. 2017, 13(6): 640-646.
 53. Jurėnas, D.; Garcia-Pino, A.; Melderen, L. Van. Novel toxins from type II toxin-antitoxin systems with acetyltransferase activity. *Plasmid*. 2017, 93: 30-35.
 54. Kalinienė, L.; Šimoliūnas, E.; Truncaitė, L.; Zajančauskaitė, A.; Nainys, J.; Kaupinis, A.; Valius, M.; Meškys, R. Molecular analysis of *Arthrobacter myovirus* vB_ArtM-ArV1: we blame it on the tail. *Journal of Virology*. 2017, 91(8), Art. No. e00023.
 55. Kalvelytė, A. V.; Imbrasaitė, A.; Krestnikova, N.; Stulpinas, A. Adult stem cells and anticancer therapy // *Advances in molecular toxicology / Edited by James C. Fishbein, Jacqueline M. Heilman: Elsevier Academic Press*, 2017, 123-202.
 56. Kanopka, A. Cell survival: Interplay between hypoxia and pre-mRNA splicing. *Experimental Cell Research*. 2017, 356(2), spec. iss. SI: 187-191.
 57. Karvelis, T.; Gasiūnas, G.; Šikšnys, V. Harnessing the natural diversity and in vitro evolution of Cas9 to expand the genome editing toolbox. *Current Opinion in Microbiology*. 2017, 37: 88-94.
 58. Karvelis, T.; Gasiūnas, G.; Šikšnys, V. Methods for decoding Cas9 protospacer adjacent motif (PAM) sequences: A brief overview. *Methods*. 2017, 121: 3-8.
 59. Kaupinis, A.; Aitmanaitė, L.; Strepetkaitė, D.; Valius, M.; Lazutka, J. R.; Arbačiauskas, K. Proteomic and gene expression differences between post-diapause and subitaneous offspring phenotypes in the cyclic parthenogen *Daphnia pulex*. *Hydrobiologia*. 2017, 798(1): 87-103.
 60. Kazlauskas, D.; Dayaram, A.; Kraberger, S.; Goldstien, Sh.; Varsani, A.; Krupovic, M. Evolutionary history of ssDNA bacilladnaviruses features horizontal acquisition of the capsid gene from ssRNA nodaviruses. *Virology*. 2017, 504: 114-121.
 61. Kazlauskas, D.; Sezonov, G.; Charpin, N.; Venclovas, Č.; Forterre, P.; Krupovic, M. Novel families of archaeo-eukaryotic primases associated with mobile genetic elements of bacteria and archaea. *Journal of Molecular Biology*. 2017, First published online: 1-14.
 62. Kazlauskas, E.; Brukštus, A.; Petrikas, H.; Petrikaitė, V.; Čikotienė, I.; Matulis, D. Improving the Hsp90 Inhibitors Containing 4-(2,4-Dihydroxyphenyl)-1,2,3-thiadiazole Scaffold: Synthesis, Affinity and Effect on Cancer Cells. *Anti-cancer Agents in Medicinal Chemistry*. 2017, 17(11): 1593-1603.
 63. Kazlauskienė, M.; Kostiuk, G.; Venclovas, Č.; Tamulaitis, G.; Šikšnys, V. A cyclic oligonucleotide signaling pathway in type III CRISPR-Cas systems. *Science*. 2017, 357(6351): 605-609.
 64. Kazokaitė, J.; Aspatwar, A.; Parkkila, S.; Matulis, D.; Kairys, V. Fluorinated benzenesulfonamide anticancer inhibitors of carbonic anhydrase IX exhibit lower toxic effects on zebrafish embryonic development than ethoxzolamide. *Drug and Chemical Toxicology*. 2017, 40(3): 309-319.
 65. Kazokaitė J.; Aspatwar A.; Parkkila S.; Matulis D. An update on anticancer drug development and delivery targeting carbonic anhydrase IX. *PeerJ*. 2017, 23(5):e4068.
 66. Kettunen, E.; Hernandez-Vargas, H.; Cros, M.P.; Durand, G.; Le Calvez-Kelm, F.; Stuoopelytė, K.; Jarmalaitė, S.; Salmenkivi, K.; Anttila, S.; Wolff, H.; Herceg, Z.; Husgafvel-Pursiainen, K. Asbestos-associated genome-

- wide DNA methylation changes in lung cancer. *International Journal of Cancer*. 2017, 141(10): 2014-2029.
67. Kiseliovas, V.; Milosevic, M.; Kojic, M.; Mažutis, L.; Kai, M.; Liu, Y.T.; Yokoi, K.; Ferrari, M.; Žiemys, A. Tumor progression effects on drug vector access to tumor-associated capillary bed. *Journal of Controlled Release*. 2017, 261: 216-222.
 68. Kolosej, R.; Jonušienė, I.; Venskutonis, P.R.; Kazernavičiūtė, R.; Brazienė, Z.; Jakienė, E.; Kvederavičiūtė, K.; Kanopka, A.; Vilys, L.; Mickevičius, V. The influence of β -alanine derivative products on spring oilseed rape yield and oil quality. *Žemdirbystė-Agriculture*. 2017, 104(2): 139-146.
 69. Kostiuik, G.; Dikić, J.; Schwarz, F.W.; Sasnauskas, G.; Seidel, R.; Šikšnys, V. The dynamics of the monomeric restriction endonuclease BcnI during its interaction with DNA. *Nucleic Acids Research*. 2017, 45(10): 5968-5979.
 70. Krams, I. A.; Krama, T.; Trakimas, G.; Kaasik, A.; Rantala, M. J.; Skute, A. Reproduction is costly in an infected aquatic insect. *Ethology Ecology & Evolution*. 2017, 29(1): 74-84.
 71. Krams, I.A.; Kecko, S.; Jöers, P.; Trakimas, G.; Elferts, D.; Krams, R.; Luoto, S.; Rantala, M.J.; Inashkina, I.; Gudrā, D.; Fridmanis, D.; Contreras-Garduño, J.; Grantiņa-Ieviņa, L.; Krama, T. Microbiome symbionts and diet diversity incur costs on the immune system of insect larvae *Journal of Experimental Biology*. 2017, 220(22): 4204-4212.
 72. Krams, I.A.; Niemelae, P.T.; Trakimas, G.; Krams, R.; Burghardt, G.M.; Krama, T.; Kuusik, A.; Mand, M.; Rantala, M.J.; Mand, R.; Kekalainen, J.; Sirkka, I.; Luoto, S.; Kortet, R. Metabolic rate associates with, but does not generate covariation between, behaviours in western stutter-trilling crickets, *Gryllus integer*. *Proceedings of the Royal Society. B, Biological sciences*. 2017, 284(1851), Art. No 20162481: 1-10.
 73. Kukcinavičiūtė, E.; Sasnauskienė, A.; Dabkevičienė, D.; Kirvelienė, V.; Jonušienė, V. Effect of mTHPC-mediated photodynamic therapy on 5-fluorouracil resistant human colorectal cancer cells. *Photochemical and Photobiological Sciences*. 2017, 16(7): 1063-1070.
 74. Liu, Z.; Balasubramanian, V.; Bhat, Ch.; Vahermo, M.; Mäkilä, E.; Kemell, M.; Fontana, F.; Janonienė, A.; Petrikaitė, V.; Salonen, J.; Yli-Kauhaluoma, J.; Hirvonen, J.; Zhang, H.; Santos, H.A. Quercetin-based modified porous silicon nanoparticles for enhanced inhibition of doxorubicin-resistant cancer cells. *Advanced Healthcare Materials*. 2017, 6(3), Art. No 1601009: 1-11.
 75. Long, F.; Nicholls, R.A.; Emsley, P.; Gražulis, S.; Merkys, A.; Vaitkus, A.; Murshudov, G.N. AceDRG: a stereochemical description generator for ligands. *Acta Crystallographica. Section D: structural biology*. 2017, 73(2): 112-122.
 76. Long, F.; Nicholls, R.A.; Emsley, P.; Gražulis, S.; Merkys, A.; Vaitkus, A.; Murshudov, G.N. Validation and extraction of molecular-geometry information from small-molecule databases. *Acta Crystallographica. Section D: structural biology*. 2017, 73(2): 103-111.
 77. Lukša, J.; Ravoitytė, B.; Konovalovas, A.; Aitmanaitė, L.; Butenko, A.; Yurchenko, V.; Serva, S.; Servienė, E. Different metabolic pathways are involved in response of *Saccharomyces cerevisiae* to L-A and M viruses. *Toxins*. 2017, 9(8), Art. No. 233: 1-17.
 78. Mani, J.; Wang, L.; Hüchelhoven, A.G.; Schmitt, A.; Gedvilaitė, A.; Jin, N.; Kleist, Ch.; Ho, A.D.; Schmitt, M. Definition and characterization of novel HLA-A*02-restricted CD8+ T cell epitopes derived from JCV polyomavirus with clinical relevance. *Oncotarget*. 2017, 8(2): 2485-2500.
 79. Marčiulionienė, E. D.; Lukšienė, B.; Montvydienė, D.; Sakalauskas, V.; Sevirukova, O.; Druteikienė, R.; Jefanova, O.; Žukauskaitė, Z. Radiocesium phytotoxicity to single cell and higher plants // Impact of cesium on plants and the environment. Switzerland: Springer, 2017. 209-230.
 80. Mėlynytė, S.; Rukšėnas, O.; Griškova-Bulanova, I. Sex differences in equiprobable auditory Go/NoGo task: effects on N2 and P3. *Experimental Brain Research*. 2017, 235(5): 1565-1574.
 81. Mickevičiūtė, A.; Timm, D.D.; Gedgaudas, M.; Linkuvienė, V.; Chen, Zh.; Waheed, A.; Michailovienė, V.; Zubrienė, A.; Smirnov, A.; Čapkauskaitė, E.; Baranauskienė, L.; Jachno, J.; Revuckienė, J.; Manakova, E.; Gražulis, S.; Di Cera, E.; Sly, W.S.; Matulis, D. Intrinsic thermodynamics of high affinity inhibitor binding to recombinant human carbonic anhydrase IV. *European Biophysics Journal*. 2017, First on line: 1-20.
 82. Mickienė, G.; Dalgėdienė, I.; Dapkūnas, Ž.; Žvirblis, G.; Pesliakas, J.H.; Kaupinis, A.; Valius, M.; Mištiniene, E.; Plečkaitytė, M. Construction, purification, and characterization of a homodimeric granulocyte colony-stimulating factor. *Molecular Biotechnology*. 2017, 59(9-10): 374-384.
 83. Navakauskienė, R. Combination epigenetic therapy // *Handbook of epigenetics (second edition): the new molecular and medical genetics / Edited by: Trygve Tollefsbol. London: Academic Press - Elsevier science, 2017, 623-632.*
 84. Neniškytė, U.; Gross, C. Errant gardeners: glial-cell-dependent synaptic pruning and neurodevelopmental disorders. *Nature Reviews Neuroscience*. 2017, 18(11): 658-670.
 85. Novickij, V.; Girkontaitė, I.; Zinkevičienė, A.; Švedienė, J.; Lastauskienė, E.; Paškevičius, A.; Markovskaja, S.; Novickij, J. Reversible permeabilization of cancer cells by high sub-microsecond magnetic field. *IEEE Transactions on Magnetics*. 2017, 53(11): 1-4.
 86. Novickij, V.; Lastauskienė, E.; Švedienė, J.; Grainys, A.; Staigvila, G.; Paškevičius, A.; Girkontaitė, I.; Zinkevičienė, A.; Markovskaja, S.; Novickij, J. Membrane permeabilization of pathogenic yeast in alternating sub-microsecond electromagnetic fields in combination with conventional electroporation. *Journal of Membrane Biology*. 2017: 1-7.
 87. Olechnovič, K.; Venclovas, Č. VoroMQA: Assessment of protein structure quality using interatomic contact areas. *Proteins: Structure, Function, and Bioinformatics*. 2017, 85(6): 1131-1145.
 88. Osipenko, A.; Plotnikova, A.; Nainytė, M.; Masevičius, V.; Klimašauskas, S.; Vilkaitis, G. Oligonucleotide-addressed covalent 3'-terminal derivatization of small RNA strands for enrichment and visualization. *Angewandte Chemie International edition*. 2017, 56(23): 6507-6510.
 89. Paškevičius, Š.; Starkevič, U.; Misiūnas, A.; Vitkauskienė, A.; Gleba, Y.; Ražanskienė, A. Plant-expressed pycnins for control of *Pseudomonas aeruginosa*. *PLoS One*. 2017, 12(10): 1-17.
 90. Paškevičiūtė, M.; Petrikaitė, V. Differences of statin activity in 2D and 3D pancreatic cancer cell cultures. *Drug Design, Development and Therapy*. 2017, 11: 3273-3280.
 91. Pečiukaiytė-Alksnė, M.; Šarlauskas, J.; Misevičienė, L.; Marozienė, A.; Čėnas, N.; Krikštopaitis, K.; Staniulytė, Z.; Anusevičius, Ž. Flavoenzyme-mediated reduction reactions and antitumor activity of nitrogen-containing tetracyclic ortho-quinone compounds and their nitrated derivatives. *EXCLI Journal*. 2017, 16: 663-678.
 92. Petkauskaitė, R.; Blom, J.; Goesmann, A.; Kuisienė, N. Draft genome sequence of pectic polysaccharide-degrading moderate thermophilic bacterium *Geobacillus thermodenitrificans* DSM 101594. *Brazilian Journal of Microbiology / Sociedade Brasileira de Microbiologia*. 2017, 48(1): 7-8.
 93. Petrašiūnas, A.; Podėnas, S. New data on winter crane flies (*Diptera: Trichoceridae*) of Korea with description of a new species. *Zootaxa*. 2017, 4311(4): 561-575.
 94. Pipinis, E.; Mėlynytė, S.; Koenig, T.; Jarutytė, L.; Linkenkaer-Hansen, K.; Rukšėnas, O.; Griškova-Bulanova, I. Association between resting-state microstates and ratings on the Amsterdam resting-state questionnaire. *Brain Topography*. 2017, 30 (2): 245-248.
 95. Podėnienė, V.; Gelhaus, J.K. The first description of the larva and pupa of crane fly genus *Phyllolabis Osten Sacken, 1877 (Diptera, Limoniidae, Limnophilinae)*, with information for understanding the phylogenetic relationships and systematical position of the genus. *Zootaxa*. 2017, 4365(5): 559-570.
 96. Pudžiuvėlytė, L.; Stankevičius, Mantas, M.; Audrius S.; Petrikaitė, V.; Ragažinskienė, O.; Drakšienė, G.; Bernatoniene, J. Chemical composition and anticancer activity of *Elsholtzia ciliata* essential oils and extracts prepared by different methods. *Industrial Crops and Products*. 2017, 107: 90-96.
 97. Radzevičiūtė, R.; Theodorou, P.; Husemann, M.; Japoshvili, G.; Kirkitadze, G.; Zhusupbaeva, A.; Paxton, R.J. Replication of honey bee-associated RNA viruses across multiple bee species in apple orchards of Georgia, Germany and Kyrgyzstan. *Journal of Invertebrate*

- Pathology. 2017, 146: 14-23.
98. Radžiuvienė, G.; Rasmusson, A.; Augulis, R.; Leščiuotė-Krilavičienė, D.; Laurinavičienė, A.; Clim, E.; Laurinavičius, A. Automated image analysis of HER2 fluorescence in situ hybridization to refine definitions of genetic heterogeneity in breast cancer tissue. *BioMed Research International*. 2017, Art. No. 2321916: 11.
 99. Ragaliauskas, T.; Mickevičius, M.; Rakovska, B.; Penkauskas, T.; Vanderah, D.J.; Heinrich, F.; Valinčius, G. Fast formation of low-defect-density tethered bilayers by fusion of multilamellar vesicles. *Biochimica et Biophysica Acta: biomembranes*. 2017, 1859(5): 669-678.
 100. Raškevičius, V.; Kairys, V. Predicting isoform-specific binding selectivities of benzenesulfonamides using QSAR and 3D-QSAR. *Current Computer-aided Drug Design*. 2017, 13(1): 75-83.
 101. Ratautas, D.; Tetianec, L.; Marcinkevičienė, L.; Meškys, R.; Kulys, J. Bioanode with alcohol dehydrogenase undergoing a direct electron transfer on functionalized gold nanoparticles for an application in biofuel cells for glycerol conversion. *Biosensors and Bioelectronics*. 2017, 98: 215-221.
 102. Reil, D.; Imholt, C.; Rosenfeld, U.M.; Drewes, S.; Fischer, S.; Heuser, E.; Petraitytė-Burienkienė, R.; Ulrich, R.G.; Jacob, J. Validation of the *Puumala* virus rapid field test for bank voles in Germany. *Epidemiology & Infection*. 2017, 145(3): 434-439.
 103. Rutkauskas, K.; Zubrienė, A.; Tumosienė, I.; Kantminienė, K.; Mickevičius, V.; Matulis, D. Benzenesulfonamides bearing pyrrolidinone moiety as inhibitors of carbonic anhydrase IX: synthesis and binding studies. *Medicinal Chemistry Research*. 2017, 26(1): 235-246.
 104. Sadauskas, M.; Vaitekūnas, J.; Gasparavičiūtė, R.; Meškys, R. Indole biodegradation in *Acinetobacter sp* strain O153: genetic and biochemical characterization. *Applied and Environmental Microbiology*. 2017, 83(19), Art. No. UNSP e01453-17, Art. No. UNSP e01453-17: 1-10.
 105. Šarlauskas, J.; Tamulienė, J.; Čėnas, N. Aziridinyl-substituted benzo-1,4-quinones: A preliminary investigation on the theoretical and experimental studies of their structure and spectroscopic properties. *Spectrochimica Acta. Part A: Molecular and Biomolecular Spectroscopy*. 2017, 178: 136-141.
 106. Sasnauskas, G.; Tamulaitienė, G.; Tamulaitis, G.; Čalyšėva, J.; Laime, M.; Rimšėlienė, R.; Lubyš, A.; Šikšnyš, V. UbaLAI is a monomeric Type IIE restriction enzyme. *Nucleic Acids Research*. 2017, 45(16): 9583-9594.
 107. Saulite, L.; Dapkutė, D.; Pleiko, K.; Popena, I.; Steponkienė, S.; Rotomskis, R.; Rieksina, U. Nano-engineered skin mesenchymal stem cells: potential vehicles for tumour-targeted quantum-dot delivery. *Beilstein Journal of Nanotechnology*. 2017, 8: 1218-1230.
 108. Savickienė, J.; Matuzevičius, D.; Baronaitė, S.; Treigyte, G.; Krasovskaja, N.; Zaikova, I.; Navakauskas, D.; Utkus, A.; Navakauskienė, R. Histone modifications pattern associated with a state of mesenchymal stem cell cultures derived from amniotic fluid of normal and fetus-affected gestations. *Journal of Cellular Biochemistry*. 2017, 118(11): 3744-3755.
 109. Shemesh, K.; Sebesta, M.; Pacesa, M.; Sau, S.; Bronstein, A.; Parnas, O.; Liefshitz, B.; Venclovas, Č.; Krejci, L.; Kupiec, M. A structure-function analysis of the yeast Elg1 protein reveals the importance of PCNA unloading in genome stability maintenance. *Nucleic Acids Research*. 2017, 45(6): 3189-3203.
 110. Shubchynskyy, V.; Boniecka, J.; Schweighofer, A.; Šimulis, J.; Kvederavičiūtė, K.; Stumpe, M.; Mauch, F.; Balazadeh, S.; Mueller-Roeber, B.; Boutrot, F.; Zipfel, C.; Meškienė, I. Protein phosphatase AP2C1 negatively regulates basal resistance and defense responses to *Pseudomonas syringae*. *Journal of Experimental Botany*. 2017, 68(5): 1169-1183.
 111. Šimonis, P.; Keršulis, S.; Stankevič, V.; Kašėta, V.; Lastauskienė, E.; Stirkė, A. Caspase dependent apoptosis induced in yeast cells by nanosecond pulsed electric fields. *Bioelectrochemistry*. 2017, 115: 19-25.
 112. Smirnovienė, J.; Smirnovas, V.; Matulis, D. Picomolar inhibitors of carbonic anhydrase: Importance of inhibition and binding assays. *Analytical Biochemistry*. 2017, 522: 61-72.
 113. Smolskaitė, L.; Slapšytė, G.; Mierauskienė, J.; Dedonytė, V.; Venskutonis, P.R. Antioxidant and genotoxic properties of hispidin isolated from the velvet-top mushroom, *Phaeolus schweinitzii* (*Agaricomycetes*). *International Journal of Medicinal Mushrooms*. 2017, 19(11): 967-980.
 114. Smutok, O.; Karkovska, M.; Serkiz, R.; Vus, B.; Čėnas, N.; Gonchar, M. A novel mediatorless biosensor based on flavocytochrome b(2) immobilized onto gold nanoclusters for non-invasive L-lactate analysis of human liquids. *Sensors and Actuators B: Chemical*. 2017, 250: 469-475.
 115. Stankevičius, V.; Kunigėnas, L.; Stankūnas, E.; Kuodytė, K.; Strainienė, E.; Cicėnas, J.; Samalavičius, N.E.; Sužiedėlis, K. The expression of cancer stem cell markers in human colorectal carcinoma cells in a microenvironment dependent manner. *Biochemical and Biophysical Research Communications*. 2017, 484(4): 726-733.
 116. Stankevičius, V.; Kuodytė, K.; Schweigert, D.; Bulotienė, D.; Paulauskas, T.; Daniūnaitė, K.; Sužiedėlis, K. Gene and miRNA expression profiles of mouse Lewis lung carcinoma LLC1 cells following single or fractionated dose irradiation. *Oncology Letters*. 2017, 13(6): 4190-4200.
 117. Stankevičius, V.; Vasauskas, G.; Rynkevičienė, R.; Venius, J.; Pašukonienė, V.; Aleknavičius, E.; Sužiedėlis, K. Microenvironment and Dose-Delivery-Dependent Response after Exposure to Ionizing Radiation in Human Colorectal Cancer Cell Lines. *Radiation Research*. 2017, 188(3): 291-302.
 118. 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. *Molecular Cell*. 2017, 65(3): 554-564.
 119. Tamulaitienė, G.; Jovaišaitė, V.; Tamulaitis, G.; Songailienė, I.; Manakova, E.; Zaremba, M.; Gražulis, S.; Shuang-yong, X.; Šikšnyš, V. Restriction endonuclease Agel is a monomer which dimerizes to cleave DNA. *Nucleic Acids Research*. 2017, 45(6): 3547-3558.
 120. Tamulienė, J.; Šarlauskas, J.; Bekešienė, S. Modeling and investigation of new explosive materials based on N-(3,5-dimethyl-2,4,6-trinitrophenyl)-1H-1,2,4-triazol-3-amine. *Journal of Molecular Modeling*. 2017, 23(8), Art. No. 228.
 121. Tamulis, A.; Majauskaitė, K.; Talaikis, M.; Zborowski, K.; Kairys, V. Acetylcholine molecular arrays enable quantum information processing. *Chemical Physics Letters*. 2017, 684: 39-43.
 122. Tankovic, J.; Timinskas, A.; Janulaitienė, M.; Žilnytė, M.; Baudel, J.L.; Maury, E.; Žvirblienė, A.; Plečkaitytė, M. *Gardnerella vaginalis* bacteremia associated with severe acute encephalopathy in a young female patient. *Anaerobe*. 2017, 47: 132-134.
 123. Tauraitė, D.; Jakubovska, J.; Dabužinskaitė, J.; Bratčikov, M.; Meškys, R. Modified nucleotides as substrates of terminal deoxynucleotidyl transferase. *Molecules*. 2017, 22(4), Art. No. 672, Art. No. 672: 1-16.
 124. Tetianec, L.; Chaleckaja, A.; Kulys, J.; Jančienė, R.; Marcinkevičienė, L.; Meškienė, R.; Stankevičiūtė, J.; Meškys, R. Characterization of methylated azopyridine as a potential electron transfer mediator for electroenzymatic systems. *Process Biochemistry*. 2017, 54: 41-48.
 125. Theodorou, P.; Albig, K.; Radzevičiūtė, R.; Settle, J.; Schweiger, O.; Murray, T.E.; Paxton, R.J. The structure of flower visitor networks in relation to pollination across an agricultural to urban gradient. *Functional Ecology*. 2017, 31(4): 838-847.
 126. Toliušis, P.; Zaremba, M.; Šilanskas, A.; Szczelkun, M.D.; Šikšnyš, V. CgII cleaves DNA using a mechanism distinct from other ATP-dependent restriction endonucleases. *Nucleic Acids Research*. 2017, 45(14): 8435-8447.
 127. Tomkuvienė, M.; Ličytė, J.; Olendraitė, I.; Liutkevičiūtė, Z.; Clouet-D'Orval, B.; Klimašauskas, S. Archaeal fibrillarlin-Nop5 heterodimer 2'-O-methylates RNA independently of the C/D guide RNP particle. *RNA*. 2017, 23(9): 1329-1337.
 128. Tutkus, M.; Marčiulionis, T.; Sasnauskas, G.; Rutkauskas, D. DNA-endonuclease complex dynamics by simultaneous FRET and fluorophore intensity in evanescent field. *Biophysical Journal*. 2017, 112(5): 850-858.
 129. Tutkus, M.; Sasnauskas, G.; Rutkauskas, D. Probing the dynamics of restriction endonuclease NgoMIV-DNA interaction by single-molecule FRET. *Biopolymers*. 2017, 107(12), Art. No. 23075: 1-9.
 130. Urba, A.; Valiulis, D.; Šarlauskas, J.; Kvietkus, K.; Šakalys, J.; Selskis, A. A pilot study of different materials applied for active sampling of gaseous oxidized mercury in the atmospheric air. *Atmospheric Pollution Research*. 2017, 8(4): 791-799.

131. Valiauga, B.; Williams, E.M.; Ackerley, D.F.; Čėnas, N. Reduction of quinones and nitroaromatic compounds by *Escherichia coli* nitroreductase A (NfsA): characterization of kinetics and substrate specificity. *Archives of Biochemistry and Biophysics*. 2017, 614: 14-22.
132. Valiulienė, G.; Stirblytė, I.; Jasnauskaitė, M.; Borutinskaitė, V.V.; Navakauskienė, R. Anti-leukemic effects of HDACi Belinostat and HMTi 3-Deazaneplanocin A on human acute promyelocytic leukemia cells. *European Journal of Pharmacology*. 2017, 799: 143-153.
133. Van Melderen, L.; Jurėnas, D.; Garcia-Pino, A. Messing up translation from the start: how AtaT inhibits translation initiation in *E. coli*. *RNA Biology*. 2017, First on line: 1-5.
134. Vaškevičienė, I.; Paketurytė, V.; Zubrienė, A.; Kantminienė, K.; Mickevičius, V.; Matulis, D. N-Sulfamoylphenyl- and N-sulfamoylphenyl-N-thiazolyl-β-alanines and their derivatives as inhibitors of human carbonic anhydrases. *Bioorganic Chemistry*. 2017, 75: 16-29.
135. Veteikytė, A.; Šiekštelė, R.; Tvaska, B.; Matijošytė, I. Sequential application of waste whey as a medium component for *Kluyveromyces lactis* cultivation and a co-feeder for lipase immobilization by CLEA method. *Applied Microbiology and Biotechnology*. 2017, 101(9): 3617-3626.
136. Zhang, H.; Liu, D.; Wang, L.; Liu, Z.; Wu, R.; Janonienė, A.; Ma, M.; Pan, G.; Baranauskienė, L.; Zhang, L.; Cui, W.; Petrikaitė, V.; Matulis, D.; Zhao, H.; Pan, J.; Santos, H.A. Microfluidic encapsulation of prickly zinc-doped copper oxide nanoparticles with VD1142 modified spermine acetalated dextran for efficient cancer therapy. *Advanced Healthcare Materials*. 2017, 6(11): 1-13.
137. Žilionis, R.; Nainys, J.; Veres, A.; Savova, V.; Zemmour, D.; Klein, A.M.; Mažutis, L. Single-cell barcoding and sequencing using droplet microfluidics. *Nature Protocols*. 2017, 12(1): 44-73.
138. Zubaitė, G.; Šimutis, K.; Galinis, R.; Milkus, V.; Kiseliovas, V.; Mažutis, L. Droplet microfluidics approach for single-DNA molecule amplification and condensation into DNA-magnesium-pyrophosphate particles. *Micromachines*. 2017, 8(2), Art. No. 62: 1-13.
139. Zubrienė, A.; Smirnov, A.; Dudutienė, V.; Timm, D.D.; Matulienė, J.; Michailovienė, V.; Zakšauskas, A.; Manakova, E.; Gražulis, S.; Matulis, D. Intrinsic thermodynamics and structures of 2,4- and 3,4-substituted fluorinated benzenesulfonamides binding to carbonic anhydrases. *ChemMedChem*. 2017, 12(2): 161-176.

List of 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-dihydroxyphenyl)-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. Analysis of methylation sites (US9347093 B2)*
8. Fluorinated benzenesulfonamides as inhibitors of carbonic anhydrase (US9725467 B2)
9. RNA-directed DNA cleavage by the CAS9-CRRNA complex (US 9637739 B2)

PATENT APPLICATIONS

1. System and method for a biomimetic fluid processing (US20150336095A1; EP2941642A1; CA2896997A1; Nr. CN105308452A1)*
2. Fluorinated benzenesulfonamides as inhibitors of carbonic anhydrase (EP2914583A1)
3. Analysis of methylation sites (EP2594651A1)*
4. Production of selenoproteins (EP3019194A1)

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

GRANTED EU PATENTS

1. 5-Aryl-4(5-substituted 2,4-dihydroxyphenyl)-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 (M-tag-Primer) (EP2776575B1)

Key Performance Indicators	2017
Total number of patent applications	10
New patent applications filled in 2017	2
Total number of patents	15
US Patents Granted in 2017	2
EU Patents Granted in 2017	1
Licenses	11

Collaboration

RESEARCH INSTITUTIONS:

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

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