Institute of Biotechnology
Vilnius University

Institute of Biotechnology Vilnius University

Biennial Report 2015–2016

Institute of Biotechnology
Vilnius University

Report 2015–2016

CONTENTS

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Director's note

The 2015-2016 period was notable for several reasons.
In 2015 the Institute celebrated its 40th anniversary. Founded in 1975 as an independent research institution (All-union Research Institute of Applied Enzymology), from 2010 part of Vilnius University, it has become a recognized leader in life sciences.The institute strives to maintain the highest standards of scientific endeavour and technological advance in a broadly defined field of molecular biotechnology, including nucleic acid and protein technologies, bioinformatics, molecular and immune diagnostics, drug design, microfluidic, next generation genome editing and epigenomics. It also serves as an interface between advanced education, basic research and technological development for the economic and social benefit of Lithuania and the European Union. Surely, the most important landmark in the history of the Institute is creation of modern biotech industry in Lithuania. We take pride in our internationally recognized spin-offs Fermentas UAB (currently ThermoFisher Scientific Baltics), Sicor-Biotech UAB (Currently Teva), Biocentras UAB, Biok UAB, and the new spin-offs Profarma UAB (2007), Nomads UAB (2010), Baltymas UAB (2011), IMD Technologies UAB (2012), Thermofarma Baltic UAB (2014), Sekos MB (2015), and Droplet Genomics UAB (2016).
The highest level of research performed at the Institute in 2015-2016 was attested by scientific publications in top-tier journals (Cell, Molecular Cell, Nature Genetics, Nature Structural & Molecular Biology, Nature Protocols, PNAS, Nucleic Acid Research, Genome Biology, Blood, Angewandte Chemie International Edition, Trends in Microbiology), 4 European/US patents and 3 patent applications, some of which were successfully licensed. Our scientists have also won prestigious international prizes. In 2016, professor Virginijus Šikšnys was awarded the Warren Alpert Foundation Prize, established by Harvard University, and the Novozyme Prize, established by the Danish company Novo Nordisk, for the development of CRISPR/Cas9 technology, a revolutionary genome editing tool that opened new opportunities for medicine and agriculture. The team of bioinformaticians led by dr. Česlovas Venclovas was recognized as the best among 40 participants in the global protein interaction prediction competition CAPRI (Critical Assessment of Predicted Interactions). And the latest good news – a research proposal of professor Saulius Klimašauskas dedicated to developing an innovative experimental platform for in vivo tracking of epigenetic events in live mammalian cells has been selected for funding by the European Research Council. Prof. S. Klimašauskas group will be the first in Lithuania to be awarded this prestigious and highly recognized research grant.
In 2016 the Institute moved to the new building and has become part of the Life Sciences Centre of Vilnius University. This new centre brings together facilities and personnel from three separate entities of Vilnius University, namely, Faculty of Natural Sciences (currently Institute of Biosciences), Institute of Biochemistry, and Institute of Biotechnology. The Life Sciences Centre will serve as a platform for strengthening biomedical research and molecular biotechnology in Lithuania. We hope that integrating scientific research and studies under the same roof will be beneficial to both science and education.
In 2016 Lithuania has become an associated member of EMBC/EMBO and EMBL, and also joined the EMBO Installation Grants scheme. EMBL is an intergovernmental organization conducting research in the field of life sciences. Participation in EMBL presents an opportunity to access international research infrastructure and cutting-edge technologies at various pan-European research centres. EMBO Installation Grants encourage talented young group leaders to establish laboratories in countries which might otherwise lose their top scientists abroad. Our professor Virginijus Šikšnys has become the first Lithuanian scientist to be elected to EMBO.
In retrospect, I am delighted with the overall progress made by the Institute over the last 10 years. As this is the last time that I am writing a preface to the Biennial Report, I would like to thank everyone who has contributed to the success of the Institute. I would also like to wish my colleagues the best of luck in all their future endeavours.

Prof. Kęstutis Sasnauskas

Institute of Biotechnology:

Just the Facts and Numbers

- The Institute of Biotechnology was established in 1990 after restructuring of the All Union Research Institute of Applied Enzymology. Since October 1, 2010 it has become an internal unit of Vilnius University. **In 2016 the Institute had moved to the new premises** and became part of the Life Sciences Centre of Vilnius University that united three separate entities of Vilnius University, namely, the Institute of Biosciences (former Faculty of Natural Sciences), the Institute of Biochemistry and the Institute of Biotechnology.
- Located at Saulėtekio al. 7, Vilnius.
- **Total staff number is 133; research staff number is 89, it includes 60 researchers (PhD).**
- The youngest Lithuanian research institute - average age – 38.
- Allocation of state budget (2015) comprises 32 % of income; other 68 % comes from outside sources (grants, programmes, contracts).
- 13 scientists of the Institute were awarded the Lithuanian Science Prize:
  prof. V. Butkus and prof. A. Janulaitis (1994),
  prof. S. Klimašauskas and prof. V. Šikšnys (2001),
  dr. A. Ražanskienė, dr. A. Gedvilaitė and
  prof. K. Sasnauskas (2003),
  dr. Č. Venclovas (2010),
  dr. D. Matulis (2012),
  dr. A. Žvirblienė (2013),
  dr. G. Sasnauskas, dr. G. Tamulaitis and dr. M. Zaremba (2014).
- In 2015-2016, 101 scientific papers were published in peer reviewed high impact journals; 7 international patent applications filled in and 2 foreign patents published.
- Successful participation in EC (FP5, FP6, FP7, H2020) and other competitive international programmes (HHMI, NIH).
- Selected as an EU Centre of Excellence in 2003 – EC FP5 tender – 600.000 Euros.
- A winner of the EC FW7 – Regional Research Potential: Coordination and Support action (FW7-REGPOT-2009—1) directed to the integration of European research entities into the European scientific research area - MoBiLi project – 1.600.000 Euros.
- Successful participation in projects of the European Social Fund under the Global Grant Measure – seven projects – 2.501.200 Euros for 2011-2015.
- **In 2016, prof. Virginijus Šikšnys was awarded the prestigious Warren Alpert Foundation Prize.**
- Since 2000 after long term abroad 30 researchers have returned to the Institute and were involved in the establishment of new laboratories.
- Involved in education of students at Vilnius University, Gediminas Technical University, Kaunas University of Technology. A lot of the Institute lecturers are members of Committees on preparing Study Programmes.
- 40—45 students accomplish Bachelor or Master theses at the Institute each year.
- 46 students are currently involved in Biochemistry or Chemical Engineering PhD studies at the Institute; in 2015-2016, 5 PhD theses were defended.
- Major Lithuanian biotech spin-off companies emerged from the Institute (UAB Fermentas (presently Thermo Fisher Scientific Baltics) - 1995, UAB Sicor - Biotech (presently TEVA) - 1995, UAB Biocentras - 1991, UAB Biok – 1991, UAB Profarma – 2007, UAB Nomads – 2010, UAB Baltymas – 2011, UAB IMD technologies – 2012, UAB ThermoPharma Baltic – 2014, MB Sekos – 2015, UAB Droplet Genomics – 2016).
- Industrial Biotechnology Programme was initiated by the Institute 2007-2009; 2011-2013.
- National Integrated Programme of Biotechnology & Biopharmacy was initiated by the Institute in 2012-2015.

Doctoral theses

	Name	*Title*	*Supervisor*
2015	T. Šinkūnas	In vitro reconstitution of DNA interference in a type I CRISPR-Cas system	prof., dr. V. Šikšnys
2016	E. Kazlauskas	Thermodynamics of aryl-dihydroxyphenyl-thiadiazole binding to recombinant human HSP90p	prof., dr. D. Matulis
	S. Baranauskė	Structural-functional analysis of the plant small RNA methyltransferase HEN1	dr. G. Vilkaitis
	G. Alzbutas	Salt resistance mechanism of halotolerant / halophilic prokaryotic DNases and halotolerance induction for bovine DNase I	dr. A. Lagunavičius
	T. Karvelis	Type II CRISPR-Cas systems: from basic studies towards genome editing	prof. dr. V. Šikšnys

A new "born" doctor Tautvydas Karvelis with his supervisor Prof. Virginijus Šikšnys

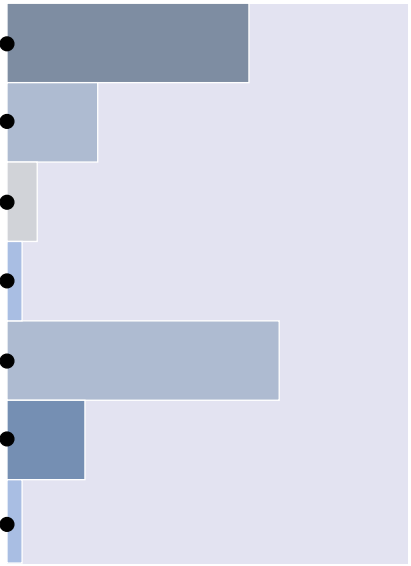
Just "released" Dr. Tomas Šinkūnas together with his supervisor Prof. Virginijus Šikšnys

Egidijus Kazlauskas enjoys a traditional gift from his colleagues

Financing Sources 2015 – 2016

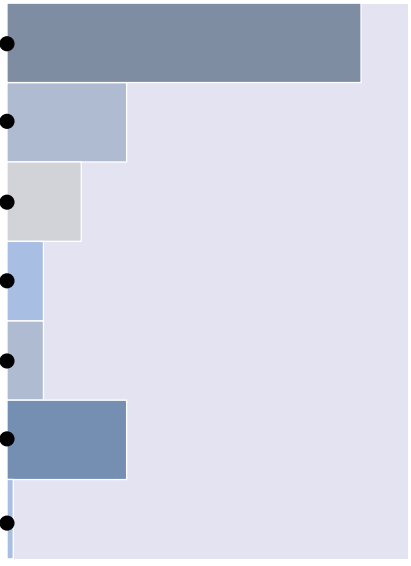
Funding 2015 4,2 MEuros

	K €	%
State Subsidy	1319.7	32 %
Research Council of Lithuania	491.0	12 %
Foreign Grants and Contracts	162.3	4 %
Agency for Science, Innovation and Technology	92.8	2 %
EU Structural Funds	1517.7	36 %
Industry	401.0	10 %
Other	165.3	4 %



Funding 2016 3,3 MEuros

	K €	%
State Subsidy	1565.2	47 %
Research Council of Lithuania	539.5	16 %
Foreign Grants and Contracts	328.9	10 %
Agency for Science, Innovation and Technology	162.5	5 %
EU Structural Funds	170.6	5 %
Industry	516.0	16 %
Other	52.0	1 %



National and International Grants

EUROPEAN COMMUNITY GRANTS
Framework 7 and Horizon 2020 programmes

Title	*Head of the project*	*Financing EUR thousand*	*Duration*
Integrated microfluidic system for long term cell cultivation, monitoring and analysis (BioCellChip)	dr. L. Mažutis	239.0	2012–2015
Towards construction of a comprehensive map of amyloid-ligand interactions:(-)-epigallocatechin 3-Gallate and insulin amyloid (EGCG+INSULIN=)	dr. V. Smirnovas	100.0	2011–2015
Sonic drilling coupled with automated mineralogy and chemistry on-line-on-mine-real-time (SOLSA) (No. 689868)	dr. S. Gražulis	601.1	2016–2020
High throughput screening of single-cells using droplet microfluidics — cells-in-drops (No. 705791)	dr. L. Mažutis	130.8	2016–2018

OTHER INTERNATIONAL GRANTS
National Institutes of Health (USA)

Title	*Head of the project*	*Financing EUR thousand*	*Duration*
Direct single nucleotide mapping of genomic CpG marks	Prof. S. Klimašauskas	223.4	2013–2016

EU FUNDS
National Integrated Programe

Title	*Head of the project*	*Financing EUR thousand*	*Duration*
Biotechnology and biopharmacy: fundamental and applied research	prof. K. Sasnauskas	1437.7	2012–2015

JOINT RESEARCH PROGRAMME

Title	*Head of the project*	*Financing EUR thousand*	*Duration*
Development of new generation means for virus diagnostics and prophylaxis and application in veterinary medicine	dr. A. Gedvilaitė	435.9	2013–2015

EUROPEAN SOCIAL FUND
Under the Global Grant Measure

Title	*Head of the project*	*Financing EUR thousand*	*Duration*
Structure and molecular mechanisms of bacterial antivirus defence systems	prof. V. Šikšnys	458.4	2011–2015
Molecular tools for epigenomics and Rnomics	prof. S. Klimašauskas	456.4	2011–2015
The use of genome-wide analysis for engineering of new yeast strains with improved heterologous expression	dr. R. Slibinskas	379.2	2012–2015
Novel chimeric proteins with antiviral activity	dr. A. Žvirblienė	399.8	2012–2015
Design of selective carbonic anhydrase, Hsp90, and Hsp70 inhibitors and investigation of their anticancer properties	dr. D. Matulis	405.5	2012–2015
Exploring flavones as universal inhibitors of amyloid-like fibril formation	dr. V. Smirnovas	401.9	2012–2015
Making use of large-scale biological data for the development of a new method to assess protein models and for studying DNA replication and repair systems in bacteria and viruses	dr. Č. Venclovas	286.3	2013–2015

NATIONAL RESEARCH PROGRAMME
Healthy and Safe Food

Title	*Head of the project*	*Financing EUR thousand*	*Duration*
Expression analysis of anthocyanin biosynthesis genes in horticultural plants	dr. V. Kazanavičiūtė (partner of the Institute of Horticulture, Lithuanian Research Centre for Agriculture and Forestry)	197.1	2012–2015

NATIONAL RESEARCH PROGRAMME
Healthy Aging

Title	*Head of the project*	*Financing EUR thousand*	*Duration*
Investigation of synthesis regulation of proteins associated with Alzheimer disease development	prof. K. Sasnauskas	198.9	2015–2018
Investigation of genetic and epigenetic prognostic markers for prediction of clinical course of papillary thyroid cancer (PTC) in different age groups	dr. A. Žvirblienė	115.5	2015–2018
Investigation of human carbonic anhydrase IX as a cancer biomarker for application in cancer diagnostics, visualization and prognosis	dr. J. Matulienė	200.0	2015–2018
Age-related remodelling of aorta and dilatative pathology of ascending aorta: search for epigenetic biomarkers	dr. G. Vilkaitis	71.4	2016–2018

LITHUANIAN-SWISS COOPERATION PROGRAMME

Title	*Head of the project*	*Financing EUR thousand*	*Duration*
Directed evolution of computer engineered enzymes using droplet based microfluidics	prof. A. Janulaitis	703.9	2012-2016
Signaling control of pathogen induced plant immunity	dr. I. Meškienė	605.0	2012–2016

LITHUANIAN-LATVIAN-TAIWAN COOPERATION PROGRAMME

Title	*Head of the project*	*Financing EUR thousand*	*Duration*
Design of anticancer pharmaceutical compounds using structure and energetics of lead – target interaction	dr. D. Matulis	51.9	2016–2018

RESEARCH TEAM PROJECTS

Title	*Head of the project*	*Financing EUR thousand*	*Duration*
Expansion of the crystallography open database (COD) and statistical analysis of crystal Structures	dr. S. Gražulis	91.9	2013–2015
Investigation of RNA interference in bacteria	dr. G. Tamulaitis	101.1	2013–2015
Structural and functional studies of restriction enzyme family	dr. G. Tamulaitienė	100.9	2013–2015
The role of Cas1 ir Cas2 proteins in adaptation mechanism of CRISPR-Cas systems	dr. G. Gasiūnas	89.9	2014–2016
Single molecule and structural studines of a new type of restriction endonucleases	dr. M. Zaremba	100.0	2015–2017
Structure-function relationship of the B3 DNA binding domains	dr. G. Sasnauskas	100.0	2015–2018
Csm effector complex labelling for single molecule FRET experiments	dr. G. Tamulaitis	100.0	2016–2018
Genomic mapping of covalently tagged CpG sites	dr. E. Kriukienė	101.3	2013–2015
Identification and analysis of small non-coding RNAs of gram-positive lactic acids bacteria involved in resistance to antibacterial agents	dr. G. Vilkaitis	100.0	2015–2018
Signalling components in stem cells	dr. A. Schweighofer	86.9	2014–2016
Synthesis of Schmallenberg virus proteins and their application for diagnostic means	prof. K. Sasnauskas	99.3	2013–2015

Construction of antibody fragments with activity prolongation and development of sample (model) for medical device	dr. G. Žvirblis	115.1	2015–2016
Development of new diagnostic tools for hepatitis E virus (HEV) infection and studies on HEV prevalence in Lithuania	dr. I. Kučinskaitė-Kodzė	96.2	2015–2018
Protein ligand binding volume and its application in drug design	dr. V. Petrauskas.	84.0	2014–2016
Bayesian nonparametrics for detection of distant protein homology	dr. M. Margelevičius	99.6	2013–2015

Department of

Protein-Nucleic Acids Interactions

Chief Scientist and Head

Prof. Virginijus Šikšnys, PhD

phone: 370 5 223 4354 ; fax: 370 5 223 4367

e-mail: siksnys@ibt.lt; virginijus.siksnys@bti.vu.lt

http://www.ibt.lt/en/laboratories/department-of-protein---dna-interactions.html

Scientific staff

Saulius Gražulis, PhD
Giedrius Sasnauskas, PhD
Elena Manakova, PhD
Mindaugas Zaremba, PhD
Giedrė Tamulaitienė, PhD
Gintautas Tamulaitis, PhD
Arūnas Šilanskas, PhD
Tautvydas Karvelis, PhD
Tomas Šinkūnas, PhD
Giedrius Gasiūnas, PhD
Marija Mantvyda Grušytė

PhD students

Georgij Kostiuk, MSc
Paulius Toliušis, MSc
Evelina Stankė, MSc
Inga Songailienė, MSc
Andrius Merkys, MSc
Miglė Kazlauskienė, MSc
Algirdas Grybauskas, MSc
Antanas Vaitkus, MSc
Gediminas Drabavičius, MSc

Students

Augustė Ambrazaitė
Greta Bigelytė
Marius Jatautas
Vykintas Jauniškis
Marija Fiodorovaitė
Rokas Grigaitis
Kotryna Kauneckaitė
Marius Makaras
Rūta Masiulytė
Miglė Mikutėnaitė
Irmantas Mogila
Dalia Smalakytė
Edvinas Stankūnas
Donata Tuminauskaitė
Tomas Urbaitis

Technician

Ana Tunevič

Research overview

Phages the most abundant organisms in the biosphere and are major parasites of bacteria. They infect bacteria in order to replicate and usually kill bacteria when replication is completed. Therefore, phages represent a deadly threat to bacteria. Despite of this deadly threat bacteria thrive and multiply. This is because bacteria developed multiple defense barriers and tools to counter fight viral attacks. The overall research theme in our department is the structural and functional characterization of enzymes and enzyme assemblies that contribute to the bacteria defense systems that target invading nucleic acids. In particularly, we are interested in the molecular machinery involved in the CRISPR function and structural and molecular mechanisms of restriction enzymes. We are using X-ray crystallography, mutagenesis, and functional biochemical and biophysical assays to gain information on these systems.

Structure and molecular mechanisms of CRISPR-Cas systems

Recently, an adaptive microbial immune system CRISPR (clustered regularly interspaced short palindromic repeats) has been identified that provides acquired immunity against viruses and plasmids. CRISPR represents a family of DNA repeats present in most bacterial and archaeal genomes. CRISPR loci usually consist of short and highly conserved DNA repeats that are interspaced by variable sequences of constant and similar length, called spacers. CRISPR arrays are typically located in the direct vicinity of *cas* (CRISPR associated) genes (Figure 1). *Cas* genes constitute a large and heterogeneous gene family which encodes proteins that often carry functional nucleic-acid related domains such as nuclease, helicase, polymerase and nucleotide binding. In response to phage infection, some bacteria integrate new spacers that are derived from phage genomic sequences, which results in CRISPR-mediated phage resistance. Many mechanistic steps involved in invasive element recognition, novel repeat manufacturing, and spacer selection and integration into the CRISPR locus remain uncharacterized (see project overview for the details).

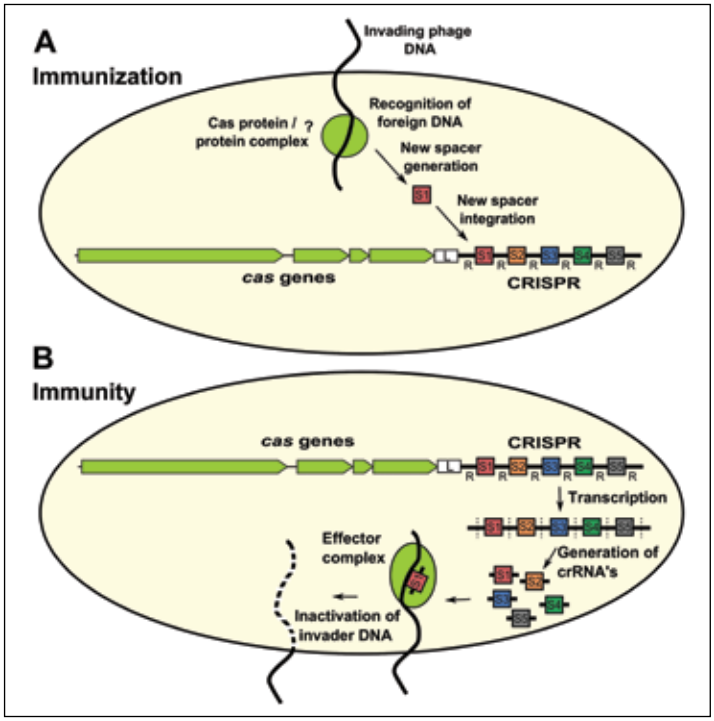


Figure 1. ***CRISPR-Cas system. CRISPR loci consist of short and highly conserved DNA repeats (R) interspaced by variable sequences of constant and similar length, called spacers (S). CRISPR repeat-spacer arrays are typically located in the direct vicinity of*** *cas* ***(CRISPR-associated) genes. In the immunisation step, Cas proteins incorporate foreign DNA fragments as new spacer sequences. New spacers are incorporated in a directional manner; therefore, the CRISPR array acts as a historical record of viral infections. In the immunity step, small crRNA molecules are produced form the precrRNA precursor transcribed from the CRISPR array. These crRNAs together with Cas proteins assemble into ribonucleoprotein complex that specifically targets invading nucleic acids for degradation.***

Structure and molecular mechanisms of CRISPR-Cas systems: projects overview

Streptococcus thermophilus DGCC7710 strain, for which biological activity of the CRISPR-Cas system has been directly demonstrated in a phage challenge assay, contains four distinct systems: CRISPR1, CRISPR2, CRISPR3 and CRISPR4, which belong to the three distinct Types. Direct spacer incorporation activity has been demonstrated for the CRISPR1 and CRISPR3 systems, with the former being more active. The CRISPR2 system seems to be disrupted and non-functional, whilst functional activity of CRISPR4 has not yet been demonstrated. We aim to establish molecular mechanisms of CRISPR-immunity provided the CRISPR1, CRISPR3 and CRISPR4 systems of S. thermophilus.

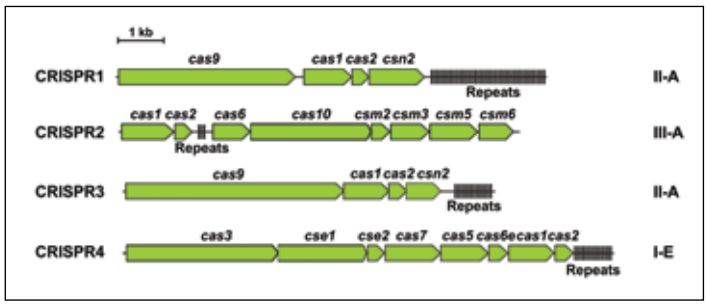


Figure 2. ***CRISPR/Cas systems of S. thermophilus DGCC7710. CRISPR1 and CRISPR3 systems belong to the Type II, CRISPR2 to the Type III whilst CRISPR4 belongs to the Type I (Ecoli subtype).***

Spatiotemporal Control of Type III-A CRISPR-Cas Immunity: Coupling DNA Degradation with the Target RNA Recognition

Streptococcus thermophilus (St) type III-A CRISPR-Cas system restricts MS2 RNA phage and cuts RNA *in vitro*. However, the CRISPR array spacers match DNA phages, raising the question: does the St CRISPR-Cas system provide immunity by erasing phage mRNA or/and by eliminating invading DNA? We show that it does both. We find that (1) base-pairing between crRNA and target RNA activates single-stranded DNA (ssDNA) degradation by StCsm; (2) ssDNase activity is confined to the HD-domain of Cas10; (3) target RNA cleavage by the Csm3 RNase suppresses Cas10 DNase activity, ensuring temporal control of DNA degradation; and (4) base-pairing between crRNA 5'-handle and target RNA 3'-flanking sequence inhibits Cas10 ssDNase to prevent self-targeting (Figure 3). We propose that upon phage infection, crRNA-guided StCsm binding to the emerging transcript recruits Cas10 DNase to the actively transcribed phage DNA, resulting in degradation of both the transcript and phage DNA, but not the host DNA.

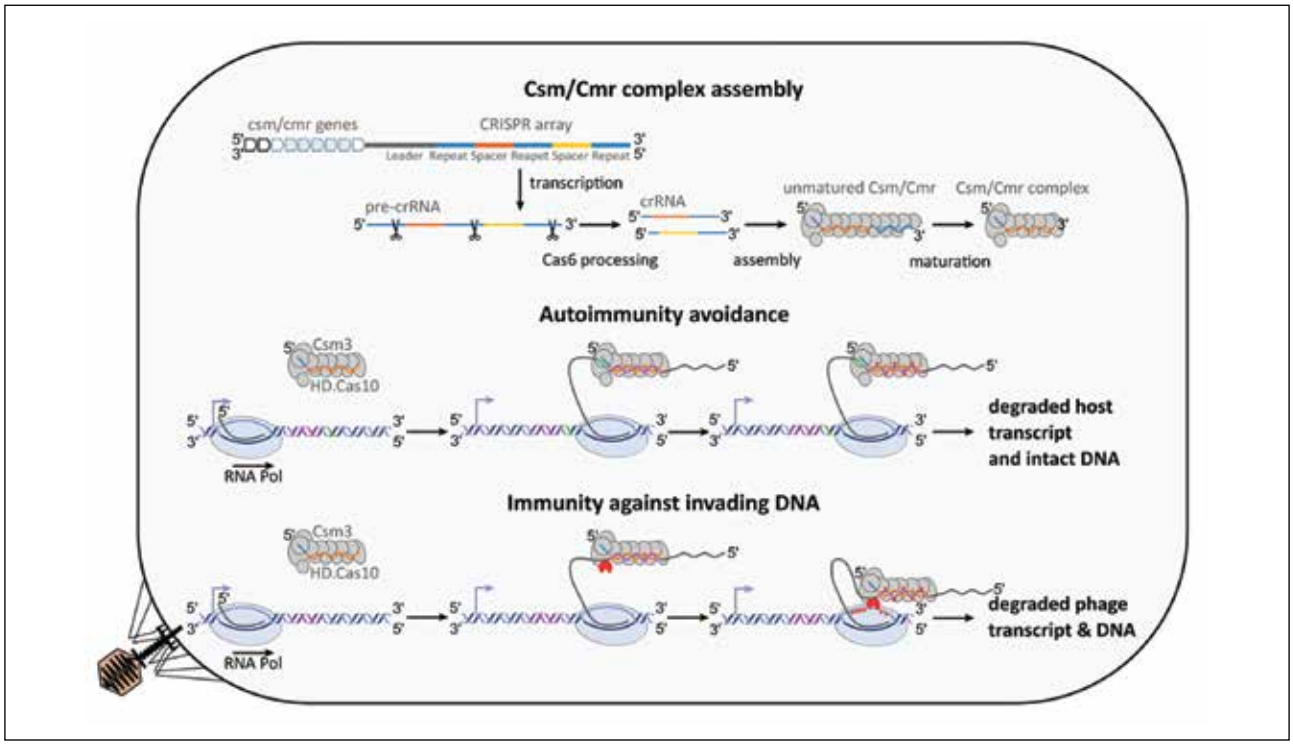


Figure 3. ***Mechanism for nucleic acid interference by the Csm (Type III-A) and Cmr (Type III-B) complexes. Csm/Cmr complex assembly: the assembly stage of effector complex involves transcription of the CRISPR array into a long precursor CRISPR RNA (pre-crRNA). The latter is cleaved at the repeats by the endoribonuclease Cas6, encoded in the vicinity of CRISPR array. The proccessed crRNA is bound by Csm or Cmr proteins to form a Csm/Cmr complex. The crRNA in the Csm/Cmr complex is further truncated by unknown nuclease. The mature crRNA guides the complex in the recognition of invader transcript at the interference stage. Autoimmunity avoidance: in the case of bidirectional transcription through the CRISPR array, anti-precrRNA (gray) transcribed from a putative promoter on the template strand will bind to the Csm/Cmr complex due to the complementarity to crRNA (orange). However, the complementary between the crRNA 5'-handle (blue) and the 3'- flanking sequence (green) will repress the ssDNase activity thus protecting the host DNA from cleavage. Immunity against invading DNA: in the case of phage infection, the RNA is transcribed and crRNA (orange) will basepair to the protospacer region (magenta) but not to the 5'-handle (blue) of crRNA. This will activate phage DNA degradation by the HD-domain (red) of Cas10.***

Rapid characterization of CRISPR-Cas9 protospacer adjacent motif sequence elements

To expand the repertoire of Cas9s available for genome targeting, we developed a new in vitro method for the simultaneous examination of guide RNA and protospacer adjacent motif (PAM) requirements. 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 reproduce the canonical PAM preferences for Streptococcus pyogenes, Streptococcus thermophilus CRISPR3 (Sth3), and CRISPR1 (Sth1). Additionally, PAM and sgRNA solutions for a novel Cas9 protein from Brevibacillus laterosporus are provided by the assay and are demonstrated to support functional activity *in vitro* and in plants.

Structure and function of restriction endonucleases

Restriction-modification (RM) systems

RM systems often function as the first antiviral defense line. RM system is comprised of two enzymes: DNA methyltransferase and restriction endonuclease. They serve as a defensive instrument in the cell protecting the cell from the invasion of the foreign, for example, viral DNA. In the cell the host DNA is protected from the cleavage by methylation. The accompanying methylase present in the cell modifies the same sequence recognized by the restriction enzyme and makes it resistant for cleavage by restriction enzyme. In this respect RM system can be treated as primitive innate immune system of bacteria that is able to differentiate between self and non-self DNA using a methyl tag in the specific sequence. RM systems are present in 90% of bacteria and archea and many bacteria contain multiple RM systems.

For a long time we tried to understand structure-function relationships in restriction enzymes using a combination of the X-ray crystallography and biochemical and biophysical methods. Specifically, we were interested to understand how restriction enzymes gain their unique specificity.

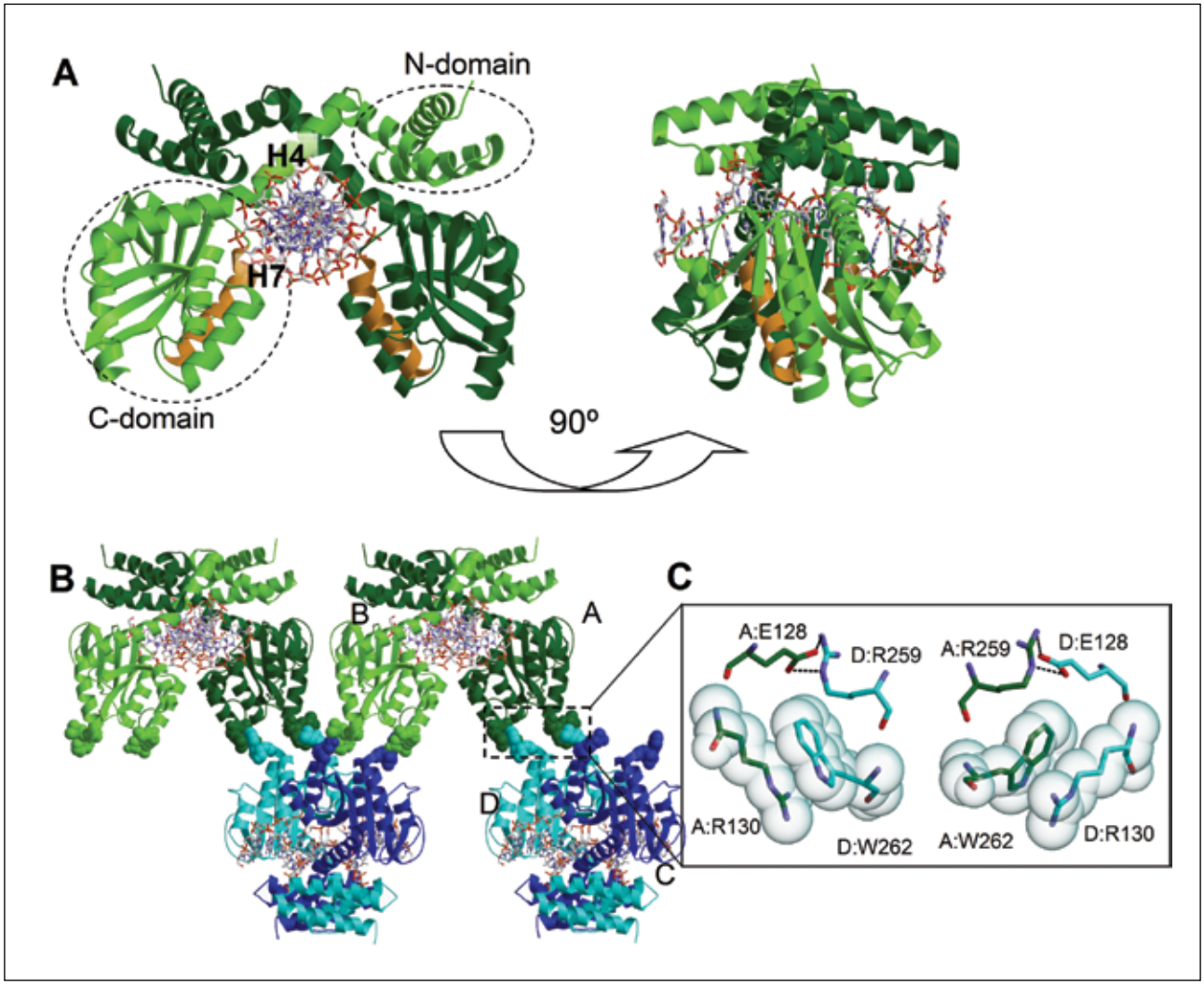
Structure and function of restriction endonucleases: projects overview

Restriction enzymes that recognize related sequences

We have focused on the family of restriction enzymes that recognizes a common CCGG (CCNGG) core that is flanked by various nucleotides. In total this group contains 12 restriction enzymes that show specificity for 10 nucleotide sequences. This group of restriction enzymes that recognize related nucleotide sequences includes 4, 6 and 8 bp palindrome cutters containing a conserved CCGG subsequence and a number of 5 and 7 bp cutters that interact with the pseudopalindromic recognition sites that contain an extra nucleotide interspaced between the conserved CCGG pattern. Crystal structures reveal that restriction enzymes belonging to this group share a conserved structural core and often conserved residues for the recognition of the conserved CCGG tetranucleotide. The conserved structural core is decorated by additional structural elements that provide specificity for the external base pairs.

Crystal structure of BsaWI restriction enzyme

Type II restriction endonuclease BsaWI recognizes a degenerated sequence 5'-W/CCGGW-3' (W stands for A or T, '/' denotes the cleavage site). It belongs to a large family of restriction enzymes that contain a conserved CCGG tetranucleotide in their target sites. These enzymes are arranged as dimers or tetramers, and require binding of one, two or three DNA targets for their optimal catalytic activity. Here, we present a crystal structure and biochemical characterization of the restriction endonuclease BsaWI (Figure 4). BsaWI is arranged as an 'open' configuration dimer and binds a single DNA copy through a minor groove contacts. In the crystal primary BsaWI dimers form an indefinite linear chain via the C-terminal domain contacts implying possible higher order aggregates. We show that in solution BsaWI protein exists in a dimer-tetramer-oligomer equilibrium, but in the presence of specific DNA forms a tetramer bound to two target sites. Site-directed mutagenesis and kinetic experiments show that



BsaWI is active as a tetramer and requires two target sites for optimal activity. We propose BsaWI mechanism that shares common features both with dimeric Ecl18kI/SgrAI and bona fide tetrameric NgoMIV/SfiI enzymes.

Figure 4. The crystal structure of the BsaWI-DNA complex. (A) BsaWI dimer. The BsaWI subunits are colored light green and dark green, respectively. Domains are shown by dotted circles. Helix H4 connects the C-domain to the N-domain. Helix H7 (orange) corresponds to the dimerization helices of Bse634I, Cfr10I, NgoMIV. (B) BsaWI oligomers. The BsaWI dimers (the primary dimers are colored green and blue) form a network in the crystal. The primary dimers AB and CD form a putative tetramer. The interacting residues are depicted in the space-fill mode. One of the putative tetramerization/oligomerization interfaces is boxed. (C) The putative tetramerization/oligomerization interface of BsaWI. In the close-up view the residues involved in the tetramerization contacts are shown and colored according to the subunit color.

Collaboration

Dr. Philippe Horvath, DuPont, France
Prof. dr. Mark Szczelkun, Bristol University, United Kingdom
Dr. Ralf Seidel, Technische Universitat Dresden, Germany
Dr. Mark Cigan, DuPont, USA
Dr. J. Young, DuPont, USA

Funding

European Social Fund under the Global Grant Measure
Research Council of Lithuania
Agency for Science, Innovation and Technology

Publications 2015-2016

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Cigan A.M., **Gasiunas G., Karvelis T., Siksnys V.**, Young J.K. Guide RNA/CAS endonuclease systems. WO/2016/186953, PCT/US2016/032073.

Siksnys V., Kazlauskiene M., Tamulaitis G. Programmable RNA Shredding by the Type III-A CRISPR-Cas System of Streptococcus thermophilus. PCT/IB2015/056756.

Department of

Biological DNA Modification

Chief Scientist and Head

Prof. Saulius Klimašauskas, PhD, FRSC

phone: +370 5 223 4350; fax: +370 5 223 4367

e-mail: saulius.klimasauskas@bti.vu.lt

http://www.ibt.lt/dmtl_en.html

Scientific staff

Giedrius Vilkaitis, PhD
Edita Kriukienė, PhD
Viktoras Masevičius, PhD
Rasa Rakauskaitė, PhD
Zita Liutkevičiūtė, PhD
Miglė Tomkuvienė, PhD
Juozas Gordevičius, PhD
Simona Baranauskė, PhD
Vaidotas Stankevičius, PhD
Zdislav Staševskij, M.Sc.
Giedrė Urbanavičiūtė, M.Sc.
Audronė Rukšėnaitė, M.Sc.

PhD Students

Povilas Gibas, M.Sc.
Aleksandr Osipenko, M.Sc.
Stasė Butkytė, M.Sc.
Milda Mickutė, M.Sc.
Janina Ličytė, M.Sc.
Milda Rudytė, M.Sc.

Students

Mantas Šarauskas
Indrė Kirilauskaitė
Monika Jazdauskaitė
Laurynas Čiupas
Ignas Maželis
Sigita Grigaitytė
Dominyka Grigaitė
Diana Ikasalaitė
Kotryna Skardžiūtė
Ieva Stirblytė
Eglė Mazgelytė

Technician

Daiva Gedgaudienė

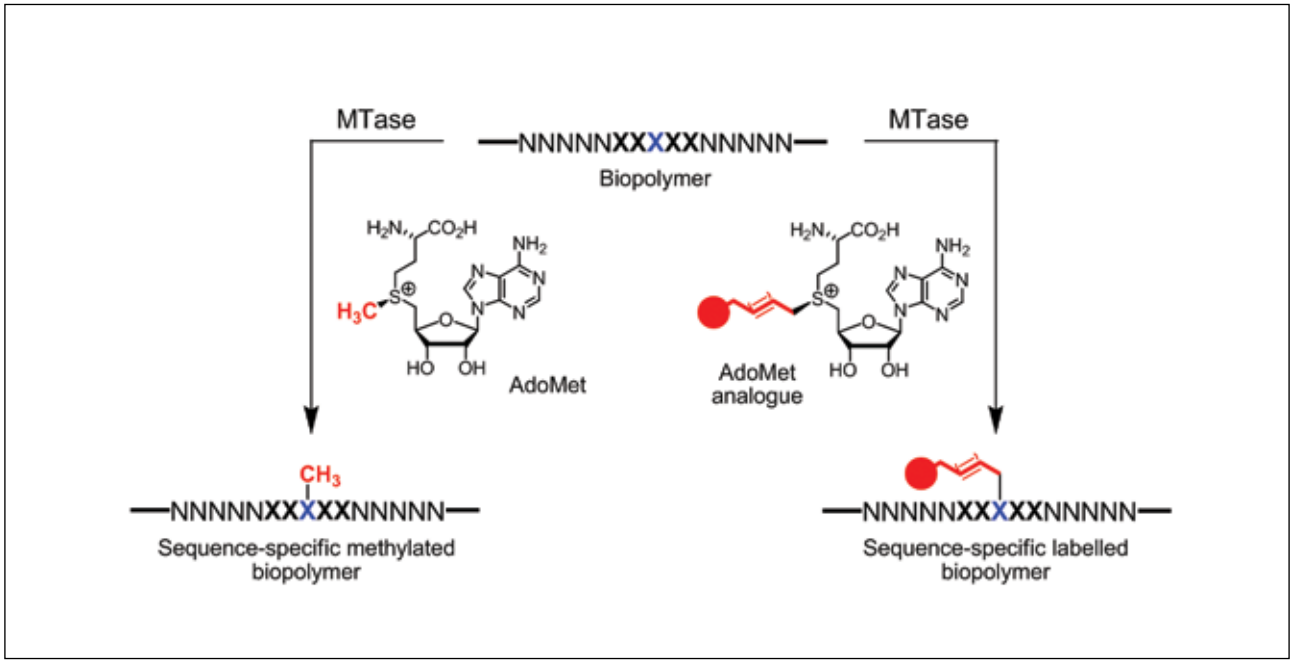


Figure 1. Enzymatic sequence-specific modification of biopolymers. Left, methyltransferase-directed biological AdoMet-dependent methylation. Right, methyltransferase-directed Transfer of Activated Groups (mTAG) from AdoMet analogs carrying a sulfonium-bound activating triple or double bond, a linker, and a functional or reporter group onto the target site. N, random nucleotide; XXXXX, recognition sequence of the directing MTase.

Engineering the catalytic reaction of methyltransferases for targeted covalent labeling of DNA and RNA

AdoMet-dependent methyltransferases (MTases), which represent more than 3% of the proteins in the cell, catalyze the transfer of the methyl group from S-adenosyl-L-methionine (SAM or AdoMet) to specific target sites in DNA, RNA, proteins or small biomolecules. Our long standing effort is aimed at **redesigning the methyltransferase reactions** for **targeted covalent deposition of desired functional or reporter groups** onto biopolymer molecules. We had synthesized a series of model AdoMet analogs with sulfonium-bound extended side chains replacing the methyl group and showed that propargylic side chains can be efficiently transferred by DNA MTases with high sequence- and base-specificity. Using DNA MTases along with their novel cofactors that carry useful functional or reporter groups, we demonstrated that our approach name mTAG (methyltransferase-directed Transfer of Activated Groups) can be used for sequence-specific functionalization and labeling of a wide variety of model and natural DNA substrates [6, 10].

Molecular tools for genome analysis: epigenome profiling by covalent capture of unmodified CpG sites

Modification of CpG dinucleotides in DNA is part of epigenetic regulation of gene function in vertebrates and is associated with complex human disease. Bisulfite sequencing permits high-resolution analysis of cytosine modification in mammalian genomes; however, its utility is often limited due to substantial cost. Here, we describe an new epigenome profiling approach, named Tethered Oligonucleotide-Primed sequencing, TOP-seq, which is based on covalent tagging of individual unmodified CpG sites followed by non-homologous priming of the DNA polymerase action at these sites to directly produce adjoining regions for their sequencing and precise genomic mapping. Pilot TOP-seq analyses of bacterial and human genomes showed a better agreement of TOP-seq with published bisulfite sequencing

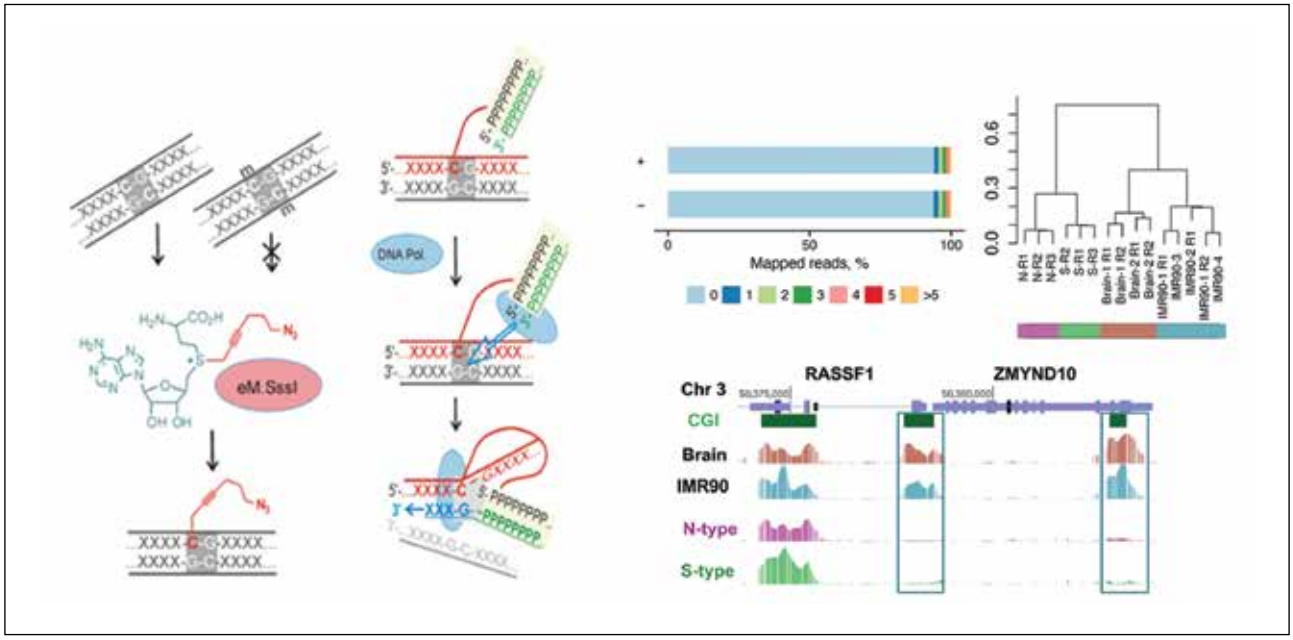


Figure 2. ***TOP-seq analysis of unmethylated CpG sites in the genome.. (A) Selective tagging of unmodified CpG sites with an azide group using an engineered variant of the SssI methyltransferase (eM.SssI) and a synthetic analog of the SAM cofactor (step 1 in C). (B) Tethered oligonucleotide-primed DNA polymerase activity at an internal covalently tagged CpG site. X and P denote generic nucleotides in genomic DNA and the tethered oligonucleotide, respectively.C. General outline of the TOP-seq profiling of unmodified CG sites in the genome (unmethylome).***

maps as compared to widely used MBD-seq and MRE-seq and permitted identification of long-range and gene-level differential methylation among human tissues and neuroblastoma cell types [9]. Altogether, we propose an affordable single CpG-resolution technique well suited for large-scale epigenome studies.

Splicing-dependent expression of mirtronic miRNAs in cancer cells

An abundant class of intronic miRNAs undergoes atypical Drosha-independent biogenesis in which the spliceosome governs the excision of hairpin miRNA precursors, called mirtrons (Figure 3). Although nearly 500 splicing-dependent miRNA candidates have been recently predicted via bioinformatic analysis of human RNA-Seq datasets, only a few of them have been experimentally validated. We experimentally examined whether biogenesis of certain miRNAs is under a splicing control by analyzing their expression levels in response to alterations in the 5'- and 3'-splice sites of a series of intron-containing minigenes carrying appropriate miRNAs. We found that biogenesis of the human hsa-miR-1227-3p, hsa-miR-1229-3p and hsa-miR-1236-3p is splicing-dependent, therefore these miRNAs can be assigned to the class of miRNAs processed by a non-canoni-

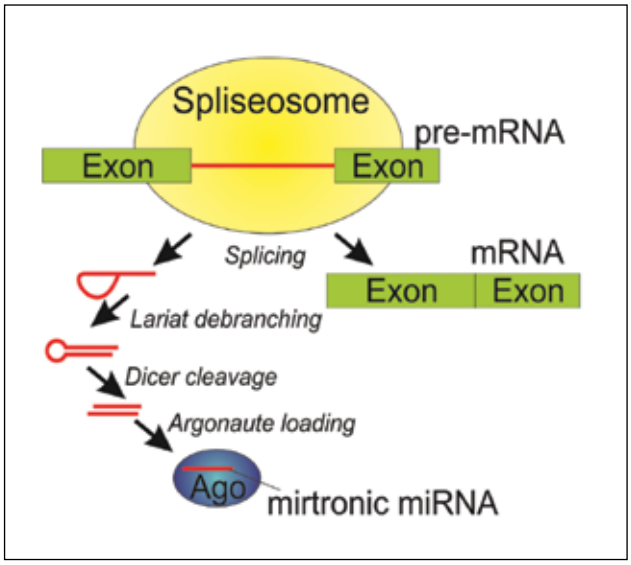


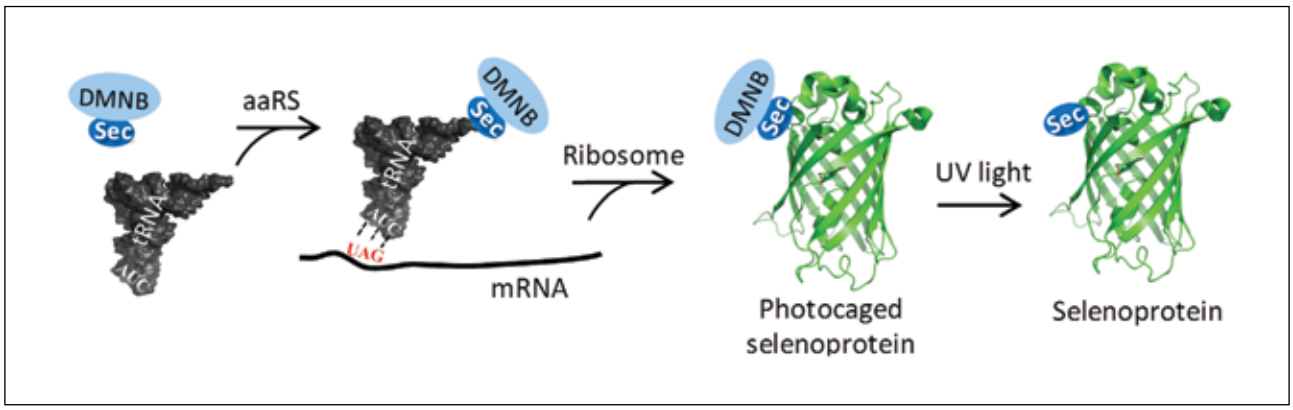
Figure 3. ***Splicing-dependent and Drosha-independent biogenesis of mirtronic miRNAs. The spliceosome, a multi-component ribonucleoprotein complex, cuts out introns from pre-mRNA transcripts and splices together the exons resulting in mature mRNA. Lariats of the introns are debranched by a lariat debranching enzyme. The produced structures fold into pre-miRNA hairpins that are exported to the cytoplasm. After cleavage by Dicer, the guide miRNA strand of mature miRNA/miRNA* duplex is loaded into a functional Ago complex.***

cal mirtron pathway. Different results were obtained with 5' and 3'-tailed mirtrons, where disruption of the splicing sites did not inhibit the accumulation of hsa-miR-3064-5p, hsa-miR-6515-5p, hsa-miR-3940-5p and hsa-miR-6850-5p. Also we determined the differential expression levels of the mirtronic miRNA in five digestive tract (pancreas PANC-1, SU.86.86, T3M4, stomach KATOIII, colon HCT116) and two excretory system (kidney CaKi-1, 786-O) carcinoma cell lines as well as in pancreatic, stomach and colorectal tumors. In particular, hsa-miR-1229-3p is selectively up-regulated in the pancreatic and stomach cancer cell lines derived from metastatic sites. Compared with the healthy controls, the expression of hsa-miR-1226-3p was significantly higher in stomach tumors, but extensively down-regulated in colorectal tumors. Furthermore, we provided evidence that over-expression of splicing factors SRSF1 or SRSF2 can up-regulate the processing of individual mirtronic miRNAs in HCT116 cells [7].

general approach for efficient biosynthesis of selenoproteins containing photocaged selenocysteine residues at genetically predetermined positions.

We explored a novel strategy based on a photolabile (4,5-dimethoxy-2-nitrobenzyl, DMNB) group to protect Sec in producing cells and during protein isolation using a yeast expression system originally designed to incorporate DMNB-Ser residues in proteins (Figure 4). We achieved efficient incorporation of DMNB-Sec in a model protein, EGFP, and also demonstrated an efficient photolytic removal of the protecting group from the Se atom, which has not been previously described for any protein or a synthetic peptide. Examples of light-con-

Figure 4. Strategy for in vivo incorporation of a photocaged L-selenocysteine (DMNB-Sec) into a genetically encoded position of a recombinant protein followed by its photochemical decaging. Shown are interactions between key components during incorporation of genetically encoded Sec in the form of a unnatural amino acid DMNB-Sec.



Biosynthesis of selenoproteins with genetically-encoded photocaged selenocysteines

Engineering and in-cell production of recombinant proteins with desired catalytic capacity is widely exploited for structural and functional studies and for practical applications in medicine and industry. L-Selenocysteine (Sec), the 21st amino acid, endows engineered proteins with new valuable properties due to its enhanced chemical reactivity (higher nucleophilicity, lower p*K*a, and a lower redox potential) as compared to cysteine. Despite its high technological potential, targeted incorporation of Sec into recombinant proteins is far from trivial. We developed the first

trolled dimerization and site-specific labeling of such recombinant proteins further illustrate robustness and practical utility of the new technique. The generality of this approach is attested by our recent successful production of a HpaII DNA cytosine-5 methyltransferase fusion protein, in which an essential catalytic Cys is replaced with Sec. This paves the way to direct comparison of S- and Se-nucleophiles in the natural and atypical reactions (see above) potentially leading to design of improved molecular tools for genome studies.

Collaborations

Prof. Arturas Petronis, CAMH, Toronto U., Canada

Dr. Andreas Finke, Max Planck Institute for Plant Breeding Research, Cologne, Germany

Dr. Marie-Pierre Chapot-Chartier ir Dr. Saulius Kulakauskas, INRA Institut Micalis, France

Dr. Nadine Martinet, Institut de Chimie, Universite de Nice-Sophia Antipolis, France

Prof. Emmanuel Mikros, School of Pharmacy, University of Athens, Greece

Dr. Andrey Kulbachinskiy, IMG, Moscow, Russia

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Contracts

Thermo Fisher Scientific Baltics

Selected publications 2015-2016

JOURNAL ARTICLES

1. **R. Rakauskaitė, G. Urbanavičiūtė, A. Rukšėnaitė, Z. Liutkevičiūtė, R. Juškėnas, V. Masevičius,** and **S. Klimašauskas**. Biosynthetic selenoproteins with genetically-encoded photocaged selenocysteines. *Chem. Commun.*, 2015, 51(39): 8245-8248.

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5. V. Myrianthopoulos, P. F. Cartron, **Z. Liutkevičiūtė, S. Klimašauskas**, D. Matulis, C. Bronner, N. Martinet, and E. Mikros. Tandem virtual screening targeting the SRA domain of UHRF1 identifies a novel chemical tool modulating DNA methylation. *Eur. J. Med. Chem.*, 2016, 114: 390–396.

6. **V. Masevičius, M. Nainytė,** and **S. Klimašauskas**. Synthesis of S-adenosyl-L-methionine analogs with extended transferable groups for methyltransferase-directed labeling of DNA and RNA. *Curr. Protoc. Nucleic Acid Chem.*, 2016, 64: 1.36.1-1.36.13.

7. **S. Butkytė, L. Čiupas, L. Vilys, P. Mocevicius, A. Kanopka, and G. Vilkaitis. S**plicing-dependent expression of microRNAs of mirtron origin in human digestive and excretory system cancer cells. *Clin. Epigenetics*, **2016, 8: 33.**

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

BOOK CHAPTERS

10. M. Tomkuvienė, E. Kriukienė, and **S. Klimašauskas**. DNA labeling using DNA methyltransferases. In *DNA Methyltransferases - Role and Function*. ed. A. Jeltsch and R.Z. Jurkowska (2016) Springer International Publishing, p. 511–535.

GRANTED PATENTS

Klimašauskas S., Liutkevičiūtė Z., Kriukienė E. Derivatization of biomolecules by covalent coupling of non-cofactor compounds using methyltransferases. EP2414528 (B1), US8822146 (B2).

Klimašauskas S., Liutkevičiūtė Z., Kriukienė E. Conversion of alpha-hydroxyalkylated residues in biomolecules using methyltransferases. EP2414527 (B1), US9505797 (B2)

Klimašauskas S., Staševskij Z. Nucleic acid production and sequence analysis. US9347093 (B2)

S. Klimašauskas, G. Vilkaitis, M. Mickutė. Analysis of single-stranded RNA. *LT6341 (B).*

S. Klimašauskas, Z. Liutkevičiūtė, V. Masevičius. Sequence analysis of 5-hydroxymethyl in DNA. *LT6340 (B).*

S. Klimašauskas, Z. Liutkevičiūtė, V. Masevičius. DNA strand scission analysis of 5-hydroxymethylated cytosines. *LT6339 (B).*

PATENT APPLICATIONS

Klimašauskas S., Rakauskaitė R., Masevičius V. Production of selenoproteins. EP3019194 (A1).

Klimašauskas S., Staševskij Z. Nucleic acid production and sequence analysis. US2017016055 (A1).

Klimašauskas S., Vilkaitis G., Mickutė M. Analysis of single-stranded RNA. WO2016148556 (A1).

Department of

Bioinformatics

Chief Scientist and Head

Dr. Česlovas Venclovas

phone: 370 5 223 4368; fax: 370 5 223 4367

e-mail: venclovas@ibt.lt; ceslovas.venclovas@bti.vu.lt

http://bioinformatics.lt

Scientific staff

Dr. Visvaldas Kairys

Dr. Mindaugas Margelevičius

Dr. Albertas Timinskas

Dr. Justas Dapkūnas

Dr. Darius Kazlauskas

Rytis Dičiūnas

PhD students

Kliment Olechnovič (until October 2016)

Kęstutis Timinskas (until October 2016)

Vytautas Raškevičius (until October 2016)

Aurimas Nausėdas

Students

Justina Jankauskaitė

Mantvydas Lopeta

Lukas Tutkus

Mindaugas Niunevas

Kristupas Šermokas

At present computational methods are playing an increasingly important role in biological research. Breakthroughs in technologies have resulted in a flood of various types of biological data such as genome sequences for different organisms, data on gene expression, protein-protein interactions, etc. Computational biology and bioinformatics are helping to make sense of all this vast biological data by providing tools for performing large-scale studies. In addition, computational biologists are utilizing experimental data to improve various analytical and predictive methods that could help address specific biological problems.

Research carried out in our department covers a broad range of topics that can be collectively described as *Computational Studies of Protein Structure, Function and Evolution*. There are two main research directions:

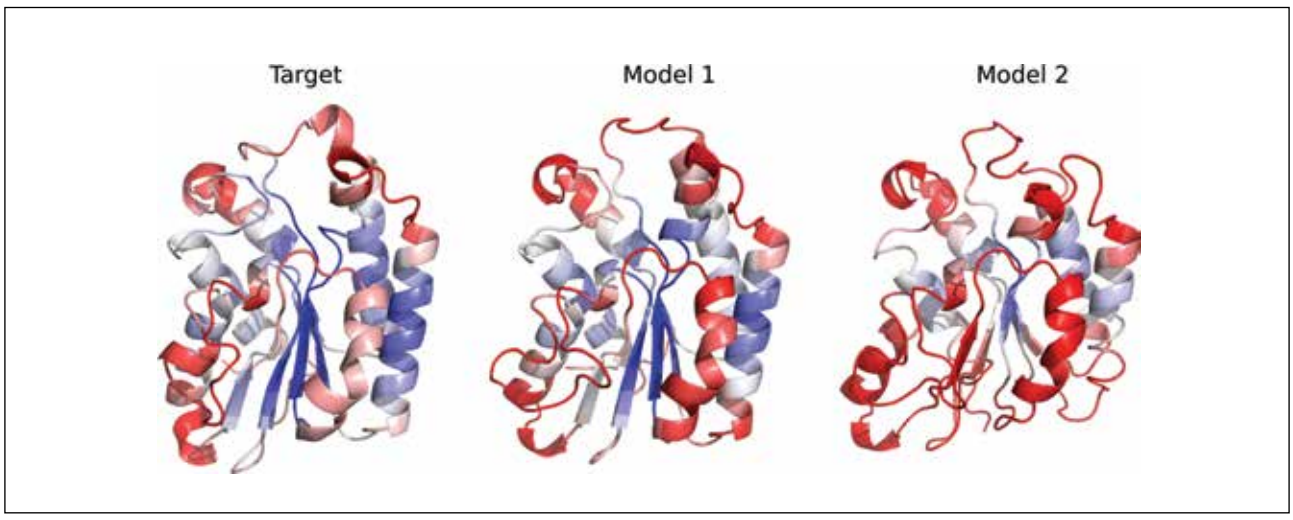
- Development of methods for detecting protein homology (common evolutionary origin) from sequence data, comparative protein structure modeling, analysis and evaluation of protein 3D structure, analysis of protein-protein interactions in 3D.
- Application of computational methods for discovering general patterns in biological data, structural/functional characterization of proteins and their complexes; design of novel proteins and mutants with desired properties. We address a variety of challenging biological problems, yet our main focus is on proteins and protein complexes that perform work on nucleic acids.

Development of computational methods

Our major methods development efforts were directed at evaluation and analysis of 3D structures of biological macromolecules and their complexes. We have also been actively working towards increasing the sensitivity of detecting distant homologies among protein families.

VoroMQA: assessment of protein structure quality using interatomic contact areas

In the absence of experimentally determined protein structure many biological questions can be addressed using computational structural models. However, the utility of protein structural models depends on their quality. Therefore, the estimation of the quality of predicted structures is an important problem. One of the approaches to this problem is the use of knowledge-based statistical potentials. Such methods typically rely on the statistics of distances and angles of residue-residue or atom-atom interactions collected from experimentally determined structures. We recently developed VoroMQA (Voronoi tessellation-based Model Quality Assessment), a new method for the estimation of protein structure quality. Our method combines the idea of statistical potentials with the use of interatomic contact areas instead of distances. Contact areas, derived using Voronoi tessellation of protein structure, are used to describe and seamlessly integrate both explicit interactions between protein atoms and implicit interactions of protein atoms with solvent. VoroMQA produces scores at atomic, residue and global levels, all in the fixed range from 0 to 1.



An example of VoroMQA local scores mapped onto the native structure (Target) and two models (Model 1 being more accurate). Blue and red colors indicate correspondingly favorable and unfavorable scores.

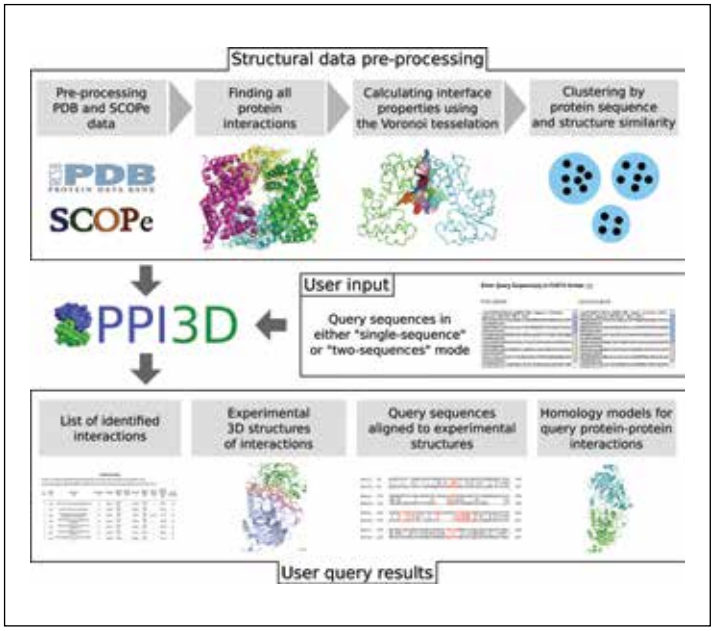
We tested the new method on publicly available model sets and compared it to several other single-model quality assessment methods. In summer of 2016, we tested VoroMQA even more thoroughly during the world-wide CASP12 experiment by participating in it as the human group "VoroMQA-select". We used VoroMQA to evaluate models available from various automated servers, and submitted the best five models as our predictions. According to the official CASP12 results (http://www.predictioncenter.org/casp12/zscores_final.cgi), VoroMQA-select showed top performance. In the overall ranking of 128 groups VoroMQA-select was 5th if the most confident model (model 1) was considered, but came up second if the best-of-five models were compared. These results clearly demonstrated the efficacy of interatomic contact areas in estimating protein structure quality.

The manuscript describing VoroMQA is submitted for publication and the corresponding software is freely available as both a standalone application and a web server at http://bioinformatics.lt/software/voromqa.

PPI3D: a web server for searching, analyzing and modeling protein-protein interactions in the context of 3D structures

Most of biological processes are driven by protein-protein interactions. Therefore, the comprehensive understanding of molecular mechanisms of various biological pathways is impossible without the detailed knowledge of protein-protein interactions. With current technologies it is relatively easy to find out whether proteins interact. However, without knowing how they interact, these data alone have limited value. The details regarding protein interactions can be obtained from the three-dimensional (3D) structure of corresponding protein complexes. A large number of experimentally determined structures of protein complexes are already available at the Protein Data Bank (PDB). However, although available 3D structures of protein complexes in PDB offer numerous possibilities, there are several important problems that have to be solved. One of them is the highly redundant nature of PDB. Both the analysis and modeling of protein-protein interactions in 3D necessitates having regularly updated non-redundant set of protein-protein interactions. A related problem is how to define a protein-protein interaction interface and how to assess the interface similarity in constructing a non-redundant data set.

To enable researchers, in particular experimentalists, to make effective use of PDB data for studies of protein-protein interactions, we have developed the PPI3D (**P**rotein-**P**rotein **I**nteractions in **3D**) web server. The web server provides a possibility to query experimentally determined 3D structures of protein complexes, to analyze the identified protein-protein interactions and to generate homology models of protein complexes. Structural data for experimentally determined protein-protein interactions are represented by PDB biological assemblies. All the protein-protein interactions accessible through PPI3D are clustered according to both sequence and interaction interface similarity. This removes the redundancy of structural data yet preserves alternative protein binding modes. The server enables users to explore interactions for individual proteins or interactions between a pair of proteins (protein groups). The PPI3D output enables users to interactively explore both the overall results and every detected interaction. The server is freely accessible at http://bioinformatics.lt/ppi3d/. Manuscript describing PPI3D has been published in *Bioinformatics*.



Schematic representation of data pre-processing, user input and results provided by the PPI3D server.

To test how useful PPI3D is in providing complete yet non-redundant set of protein-protein interactions we participated in the 2016 world-wide CAPRI experiment, designed to reveal the state-of-the-art in computational modeling of protein complexes. Combining selection of templates with PPI3D and model quality assessment with

VoroMQA, our group ("Venclovas") was able to get the best results (see the official CAPRI ranking at http://predictioncenter.org/casp12/doc/presentations/CASP12_CAPRI_Lensink.pdf; p.42).

Application of computational biology methods to specific biological problems

An important component of our research is the application of computational methods for addressing specific biological questions. Computational approach is often combined with experiments through collaboration with experimental labs. Biological questions we tackle are not restricted to a specific topic. Still, most research projects involve proteins participating in nucleic acids binding or metabolism. Some of the projects accomplished during the reported period or those still ongoing are listed below.

- Experimental-computational studies directed at understanding molecular mechanisms of CRISPR-Cas systems that provide protection to bacterial cells against invading foreign nucleic acids (collaboration with Prof. Virgis Siksnys at our Institute)
- Computational analysis of structural and functional properties of DNA polymerases and their distribution in bacterial genomes
- Computational analysis of the nature and distribution of DNA replication proteins in genomes of double-stranded DNA viruses (collaboration with Dr. Mart Krupovič, Institut Pasteur, Paris)
- Experimental-computational studies of the yeast alternative clamp loader Elg1RFC and its role in genome stability maintenance (collaboration with Prof. Martin Kupiec, Tel Aviv University)
- Experimental-computational studies of inhibitors of human carbonic anhydrase isoforms that are promising target candidates in various cancer types (collaboration with Prof. Daumantas Matulis at our Institute)

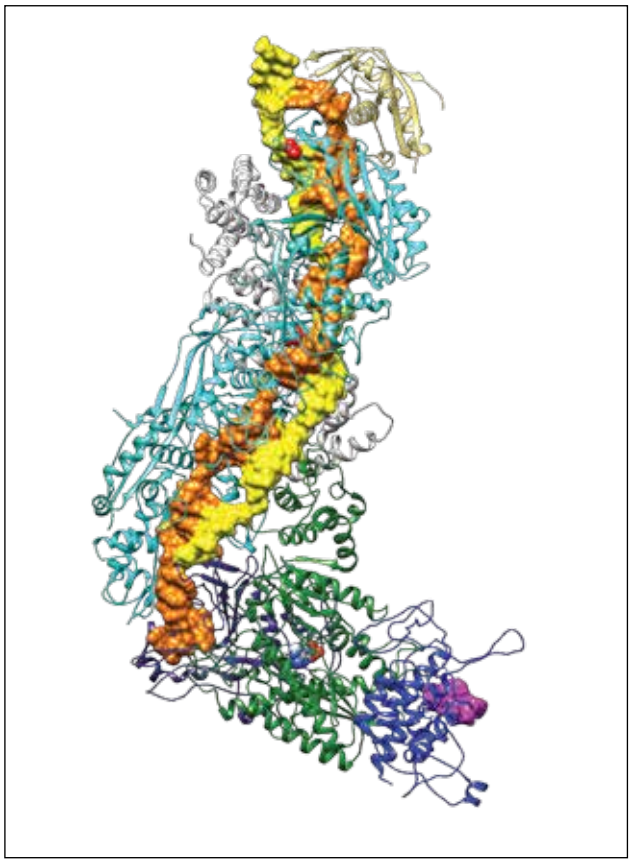
Two of these research projects are highlighted below.

Studies directed at understanding molecular mechanisms and the evolution of prokaryotic CRISPR-Cas immune systems

Approximately half of bacteria and the majority of archaea possess Clustered Regularly Interspaced Short Palindromic Repeat (CRISPR) arrays and CRISPR-associated (Cas) proteins. CRISPR-Cas systems function as adaptive immunity systems that protect their prokaryotic hosts from invading foreign mobile genetic elements. Currently, CRISPR-Cas systems are classified into two broad classes depending on the composition of the crRNA-effector complexes. Class 1 encompasses multisubunit effector complexes, whereas class 2 effector complexes consist of a single protein. We focus on Type III multiprotein effector complexes belonging to class 2 systems.

For a long time the mechanism of immunity provided by the Type III CRISPR-Cas systems appeared to be inconsistent. Type III-A Csm complexes were thought to target DNA whereas Type III-B Cmr complexes were thought to target RNA. In a collaborative study with the lab of Prof. Virgis Siksnys, we have resolved this long-standing conundrum by showing that the Type III Csm complex is both an RNase and a target RNA-activated DNA nuclease. The immunity is achieved by coupling binding and cleavage of RNA transcripts to the degradation of single-stranded regions of foreign DNA generated during transcription. Once target RNA is cleaved, the DNase activity of the Csm complex is suppressed ensuring that the DNA degradation is tightly controlled both in space and time. We also discovered that the base-pairing potential between the target RNA and the CRISPR RNA (crRNA) 5'-handle plays an important role in discriminating self and non-self nucleic acids. If target RNA is complementary to the 5'-handle of crRNA, indicating that the transcript is derived from the host DNA, the DNase activity of the Csm complex is strongly inhibited. In this way, if antisense transcript with full complementarity to crRNA is generated, Type III systems prevent targeting of self DNA. In addition to revealing the molecular mechanism of immunity by Type III CRISPR-Cas systems, we contributed to the understanding of the evolution of multisubunit CRISPR-Cas complexes. It was known that Type I and type III CRISPR-Cas effector complexes share similar architecture and have homologous key subunits. However, the relationship between the so-called small subunits of these complexes remained uncertain. Using computational analysis we have shown that all small subunits share common structural fold suggesting they

are all related. Moreover, the study has substantiated the hypothesis that the Csm-like complex was an ancestor of extant Type I and III complexes. These findings were published in *Molecular Cell* and *FEBS Letters* (the study was featured on the cover), whereas an overview of recent developments was published in *Trends in Microbiology*.



Computational structural model of** Streptococcus thermophilus **Type III-A (Csm) complex

Small subunits of Type I and Type III CRISPR-Cas complexes share a common fold.

Computational analysis of the nature and distribution of DNA replication proteins in genomes of double-stranded DNA viruses

Genomic DNA replication is a complex process that involves multiple proteins. Cellular DNA replication systems are broadly classified into only two types, bacterial and archaeo-eukaryotic. In contrast, double-stranded (ds) DNA viruses feature a much broader diversity of DNA replication machineries. Viruses differ greatly in both completeness and composition of their sets of DNA replication proteins. To explore whether there are common patterns underlying this extreme diversity, we performed a global computational analysis of viral DNA replication proteins. We identified and analyzed all major functional groups of DNA replication proteins in all available proteomes of dsDNA viruses. Our results showed that some proteins are common to viruses infecting all domains of life and likely represent components of the ancestral core set. These include B-family polymerases, SF3 helicases, archaeo-eukaryotic primases, clamps and clamp loaders of the archaeo-eukaryotic type, phage T7-like SSBs, RNase H and ATP-dependent DNA ligase. We also discovered a clear correlation between genome size and self-sufficiency of viral DNA replication, the unanticipated dominance of replicative helicases and pervasive functional associations among certain groups of DNA replication proteins. Altogether, our results provide a comprehensive view on the diversity and evolution of replication systems in the DNA virome and uncover fundamental principles underlying the orchestration of viral DNA replication. The study has been reported in *Nucleic Acids Research*.

Collaborative interactions

In addition to extensive scientific interactions with our colleagues at the Institute of Biotechnology we are involved in a number of external collaborations:

Prof. Maris Laan, Institute of Molecular and Cell Biology, University of Tartu, Estonia

Dr. Mart Krupovič, Institut Pasteur, Paris, France

Prof. Lajos Haracska, Institute of Genetics, Hungarian Academy of Sciences, Szeged, Hungary

Prof. Martin Kupiec, Tel Aviv University, Israel

Prof. Penny Beuning, North Eastern University, Boston, USA

Prof. Valerie Mizrahi & Dr. Digby Warner, University of Cape Town, South Africa

Publications 2015-2016

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Department of

Eukaryote Gene Engineering

Senior Scientist and Head
Gintautas Žvirblis, PhD
phone: 370 5 223 4358; fax: 370 5 223 4367
e-mail: zvirb@ibt.lt; gintautas.zvirblis@bti.vu.lt
http://www.ibt.lt/en/laboratories/egil_en.html

Scientific staff
Asta Abraitienė, PhD
Rokas Abraitis PhD
Aistė Bulavaitė PhD
Evaldas Čiplys, PhD
Alma Gedvilaitė, PhD
Vaiva Kazanavičiūtė, PhD
Irutė Meškienė, PhD
Rasa Petraitytė-Burneikienė PhD
Raimundas Ražanskas, PhD
Aušra Ražanskienė, PhD
Kęstutis Sasnauskas, Prof., PhD, Dr. Habil.
Alois Schweighofer, PhD
Rimantas Slibinskas, PhD
Monika Valavičiūtė, M. Sc.
Mindaugas Zaveckas, PhD
Danguolė Žiogienė, PhD

PhD students
Edita Bakūnaitė, M. Sc.
Žilvinas Dapkūnas, M. Sc.
Kotryna Kvederavičiūtė, M. Sc.
Justas Lazutka, M. Sc.
Gitana Mickienė, M. Sc.
Milda Norkienė, M. Sc.
Urtė Starkevič, M. Sc.
Paulius Tamošiūnas, M. Sc.
Indrė Širkė, M. Sc.
Rita Vorobjovienė, M. Sc.
Rūta Zinkevičiūtė. M. Sc.
Aliona Špakova, M.Sc.
Neringa Macijauskaitė, M.Sc.
Eimantas Žitkus, M.Sc.
Šarūnas Paškevičius, M.Sc.

Students
Artiomas Akatov
Karolis Goda
Judita Jankauskaitė
Vaida Kurmauskaitė
Kornelija Marcinkevičiūtė
Neringa Macijauskaitė
Juozas Nainys
Jurgita Oželytė
Tautvydas Paškevičius
Severina Marija Pociūnaitė
Juta Rainytė
Jurgita Raškevičiūtė
Sofija Semeniuk
Karolis Simutis
Jomantė Stonytė
Lukas Valančiauskas
Viktorija Versockaitė
Arūnė Verbickaitė
Barbora Bajorinaitė
Gertrūda Pėtelytė
Dominykas Murza
Emilija Vasiliūnaitė
Simonas Pajėda
Marija Fiodorovaitė
Kotryna Markevičiūtė
Živilė Grublytė
Raminta Batiuškaitė
Erik Ilkevič

Technicians
Olga Jančevskaja
Danutė Jaskytė
Genė Vercholamova

Research overview

Department of Eukaryote Gene Engineering is focused on the research directions of recombinant viral and human protein analysis, expression and assembly of proteins into virus-like particles (VLPs) as well as mechanisms of plant signaling and biosynthesis. We are using methods of gene cloning and expression in different host systems, electron microscopy, immunological and functional biochemical assays of proteins, biosynthesis and purification of essential amounts of target recombinant proteins with application area including diagnostics and therapeutics. Please find few examples of our research studies during 2015-2016.

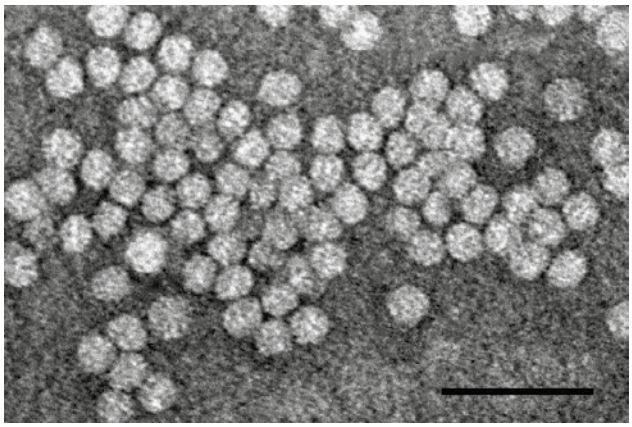


Figure 1. Electron microscopy pictures of VLPs formed by PCV2-Cap protein generated in yeast stained with 2% aqueous uranyl acetate solution and examined by Morgagni 268 electron microscope. Scale bar 100 nm.

Development of porcine circovirus type 2 diagnostics tools

Porcine circovirus type 2 (PCV2)-associated diseases are responsible for significant mortality among pigs and remains a serious economic problem to the swine industry worldwide leading to significant negative impacts on profitability of pork production. The two major groups designated as PCV2a, PCV2b are found worldwide and have been demonstrated to be virulent enough to trigger PCV2-associated diseases. We determined the genomes of PCV2 virus spread in Lithuania pig farms in the samples collected between 2009 and 2013 and confirmed that all isolated virus genomes belonged to the PCV2b genotipe. Three slightly different open reading frame 2 (ORF2) variants were amplified by PCR from native PCV2 genomes as well as ORF2 of one PCV2-Cap gene variant was codon-optimized by *S. cerevisiae* codon usage and used for the expression in yeast *S. cerevisiae*. Recombinant PCV2b capside protein generated in yeast assembled into particles (Cap VLPs) similar to native virions (Fig.1). Yeast-derived PCV2 Cap VLPs were capable to induce the generation of PCV2-specific MAbs that did not show any cross-reactivity with PCV1-infected cells. Moreover, yeast-derived recombinant PCV2 Cap VLPs were used as antigen in newly developed the indirect IgG PCV2 Cap VLP-based ELISA. The high sensitivity and specificity of newly developed the indirect IgG PCV2 Cap VLP-based ELISA clearly suggested that this assay is potentially useful diagnostic tool for screening PCV2-suspected samples [Nainys J et al, 2014]. The new two-step chromatographic purification procedure of PCV2 Cap VLPs from yeast lysate was developed using Q Sepharose XL and cation-exchange CIMmultus SO3 monolith [Zaveckas M et al, 2015]. This work was funded by European Social Fund, grant No VP1-3.1-ŠMM-10-V-02-017.

Production of recombinant human polyomavirus-like particles in yeast

Over recent years eleven new human polyomaviruses (HPyVs) have been identified. According to preliminary serological studies all this variety of HPyVs subclinically infects the general population at an early age. As many HPyVs cannot be easily cultured, major capsid protein VP1 is an ideal protein for generation of virus-like particles (VLPs) which resemble native virions they are derived from in structure, immunogenicity and tropism, but do not contain any viral genetic material. Recombinant VP1 VLPs originated from eleven newly identified HPyVs were efficiently produced in yeast [Norkiene M et al, 2015]. Merkel cell polyomavirus (MCPyV) and trichodysplasia spinulosa-associated polyomavirus (TSPyV) derived VP1 self-assembled into homogeneous in size VLPs. Karolinska Institute polyomavirus (KIPyV), HPyV7, HPyV9, HPyV10 and St. Louis polyomavirus (STLPyV) VP1 proteins formed heterogeneous in size VLPs with diameters ranging from 20 to 60 nm. Fractions of smaller VLPs (25–35 nm in diameter) dominated in preparations of VP1 proteins originated from Washington University polyomavirus (WUPyV) and HPyV6. HPyV12 VP1 VLPs were generated from the second

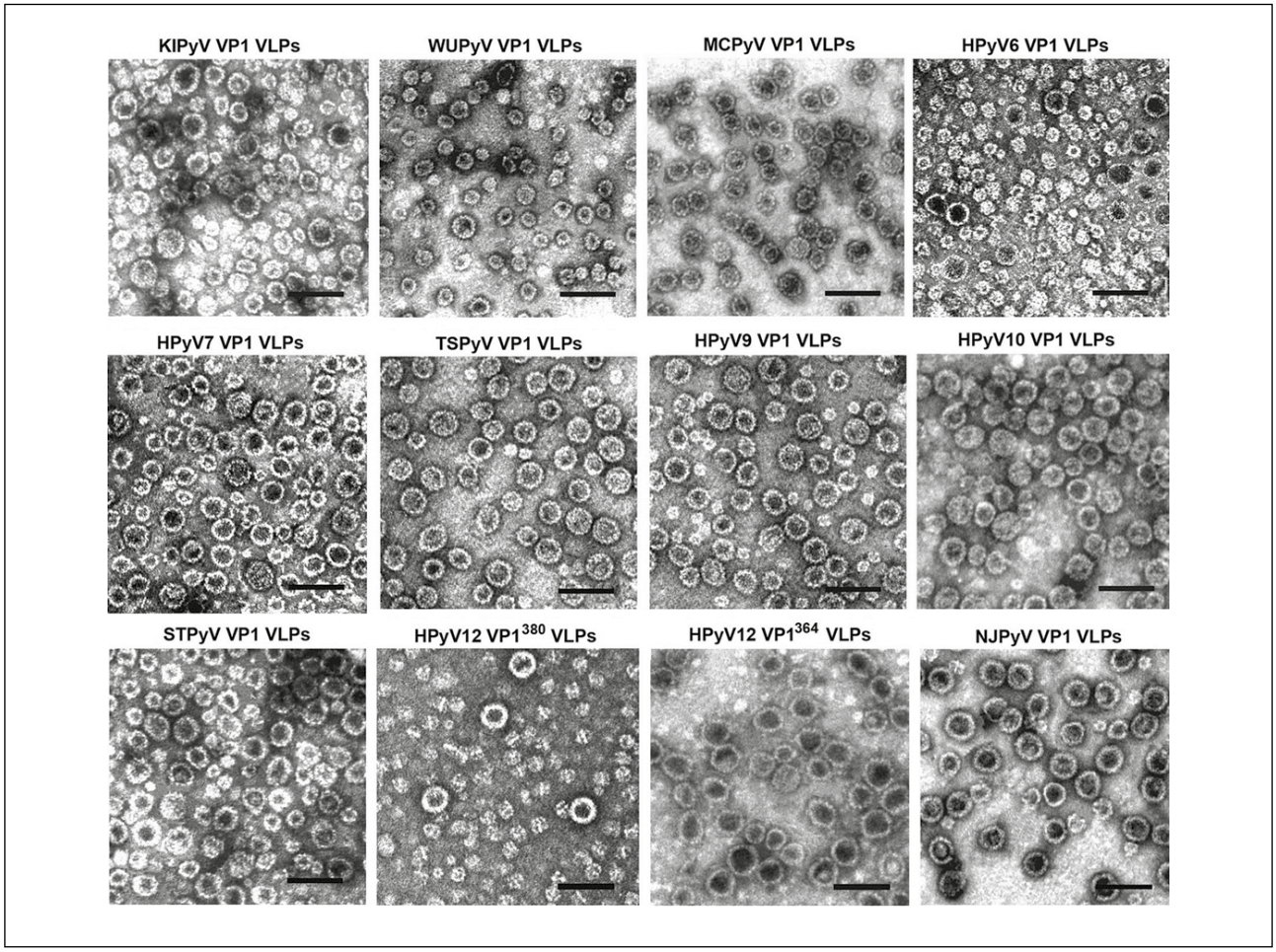


Figure 2. Electron microscopy pictures of VLPs formed by novel human polioma viruses-derived VP1 VLPs generated in yeast stained with 2% aqueous uranyl acetate solution and examined by Morgagni 268 electron microscope. Scal bar 100 nm.

of two potential translation initiation sites in VP1-encoding open reading frame. Yeast-produced recombinant VP1 VLPs originated from different HPyV demonstrated distinct HA activity and could be useful in virus diagnostics and capsid structure studies or investigation of entry pathways and cell tropism of new HPyVs replacing corresponding viruses until cell culture systems for the new HPyVs will be developed (Fig.2). This work was funded by the European Social Fund under National Integrated Program Biotechnology & Biopharmacy, grant No. VP1-3.1-SMM- 08-K01-005.

Polyomavirus-derived virus-like particles as universal carriers of foreign peptides and single chain antibodies

The hamster polyomavirus (HaPyV) major capsid protein VP1 VLPs have been used as carriers for a variety of different foreign peptides, protein segments and entire proteins of different origin including virus- and cancer associated. A major reason for generation and using of chimeric VLPs is to transfer the intrinsic strong immunogenicity of the VLP carrier to per se low immunogenic peptide sequences. HaPyV VP1-derived VLPs were further explored for generation of chimeric VLPs as tools for hybridoma technology. Our approach to use chimeric VLPs was proven useful for the generation of virus-reactive MAb against hantavirus Gc glycoprotein. The generated broadly-reactive MAb #10B8 might be useful for various diagnostic applications [Zvirbliene A et al, 2014].

The localization of potential insertion sites for foreign peptide sequences is essential for the generation of chimeric VLPs as

well as evaluation of foreign peptide size limits that can be inserted. The evaluation of new carrier for construction of chimeric VLP - Trichodysplasia spinulosa-associated polyomavirus (TSPyV) VP1 VLPs - was exploited in comparison to hamster polyomavirus VP1 protein. The insertion sites were selected based on molecular models of TSPyV VP1 protein. Chimeric VLPs harbouring inserted either hepatitis B virus preS1 epitope DPAFR or a universal T cell-specific epitope AKFVAAWTLKAAA were capable to induce a strong immune response in mice, activated dendritic cells and T cells. This demonstrated that TSPyV VP1 protein represents a new potential carrier for construction of chimeric VLPs harboring target epitopes [Gedvilaite A et al, 2015].

HaPyV VP1/VP2 pseudotype VLPs was used as carrier for fused with VP2 protein a surface-exposed functionally active neutralizing antibody specific to hepatitis B virus (HBV) surface antigen (HBsAg). Formation of VLPs was confirmed by electron microscopy. The antigen-binding activity of the purified pseudotype VLPs was evaluated by ELISA and virus-neutralization assay on HBV-susceptible primary hepatocytes from Tupaia belangeri. The pseudotype VLPs were functionally active and showed a potent HBV-neutralizing activity comparable to that of the parental monoclonal antibody. Polyomavirus-derived pseudotype VLPs harbouring multiple functionally active antibody molecules with virus-neutralizing capability may represent a novel platform for developing therapeutic tools with a potential application for post-exposure or therapeutic treatment of viral infections [Pleckaityte M et al, 2015]. This research was funded by the European Social Fund under the Global Grant measure (Grant No. VPI-3.1-SMM-07-K-02-039).

Survey of molecular chaperone requirement for the biosynthesis of hamster polyomavirus VP1 protein in *Saccharomyces cerevisiae*

A number of viruses utilize molecular chaperones during various stages of their life cycle. It has been shown that members of the heat-shock protein 70 (Hsp70) chaperone family assist polyomavirus capsids during infection. However, the molecular chaperones that assist the formation of recombinant capsid viral protein 1 (VP1)-derived virus-like particles (VLPs) in yeast remain unclear. A panel of yeast strains with single chaperone gene deletions was used to evaluate the chaperones required for biosynthesis of recombinant hamster polyomavirus capsid protein VP1. The impact of deletion or mild over-expression of chaperone genes was determined in live cells by flow cytometry using enhanced green fluorescent protein (EGFP) fused with VP1. The results confirmed the participation of cytosolic Hsp70 chaperones and suggested the potential involvement of some Hsp40 co-chaperones and Hsp90 in the biosynthesis of VP1 VLPs in yeast [Valaviciute M et al, 2015]. This work was funded by the European Social Fund under the Global Grant Measure (Grant No. VP1-3.1-SMM-07-K-02-038).

Identification and characterization of polyomaviruses in wild voles and GASH:Sal Syrian golden hamsters suffering from lymphomas

Two novel polyomaviruses (PyVs) were identified in kidney and chest-cavity fluid samples of wild bank voles (*Myodes glareolus*) and common voles (*Microtus arvalis*) collected in Germany. All cloned and sequenced genomes had the typical PyV genome organization, including putative open reading frames for early regulatory proteins large T antigen and small T antigen on one strand and for structural late proteins (VP1, VP2 and VP3) on the other strand. Virus-like particles (VLPs) were generated by yeast expression of the VP1 protein of both PyVs. VLP-based ELISA and large T-antigen sequence-targeted polymerase chain reaction investigations demonstrated signs of infection of these novel PyVs in about 42% of bank voles and 18% of common voles. Phylogenetic and clustering analysis including all known PyV genomes placed novel bank vole and common vole PyVs amongst members of the tentative Wukipolymavirus genus. The finding of novel vole-borne PyVs may suggest an evolutionary origin of ancient wukipolyomaviruses in rodents and may offer the possibility to develop a vole-based animal model for human wukipolyomaviruses [Nainys J et al, 2015]. This work was funded by the European Social Fund under National Integrated Program Biotechnology & Biopharmacy, grant No. VP1-3.1-SMM- 08-K01-005.

Almost 16 % (90 males and 60 females) of the 975 GASH:Sal Syrian golden hamsters (Mesocricetus auratus) were affected during a 5-year period by the development of a progressing lymphoid tumour and exhibited similar clinical profiles,

and a rapid disease progression resulting in mortality within 1 to 2 weeks. A TaqManprobe-based real-time PCR analysis of genomic DNA, immunohistochemical analysis using HaPyV-VP1-specific monoclonal antibodies, indirect ELISA and western blot analysis confirmed the presence of viral proteins in all hamster tumour tissues, the presence of antibodies against the VP1 capsid protein in sera. The HaPyV genome that accumulated in tumour tissues typically contained deletions affecting the noncoding regulatory region and adjacent sequences coding for the N-terminal part of the capsid protein VP2. The analysis of the non-deleted HaPyV genome derived from GASH-Sal hamster tumour tissues (JX416849) in comparison with the sequence of HaPyV isolated from Syrian hamsters in Berlin-Buch (JX036360) revealed 132 nucleotide exchanges (112 nucleotide exchanges were in the coding sequence, which resulted in a total of 23 amino acid exchanges in six viral proteins, suggesting that in the Salamanca new strain of HaPyV was responsible for the infection which did not originate directly from the same source as Berlin-Buch HaPyV strain [Munoz LJ et al, 2013].

Functional analysis of recombinant lipolytic protein encoded in phytoplasma phage based genomic island

Wall-less bacteria known as phytoplasmas are obligate transkingdom parasites and pathogens of plants and insect vectors. These unusual bacteria possess some of the smallest genomes known among pathogenic bacteria, and have never been successfully isolated in artificial culture. Disease symptoms induced by phytoplasmas in infected plants include abnormal growth and often severe yellowing of leaves, but mechanisms involved in phytoplasma parasitism and pathogenicity are little understood. A phage based genomic island (sequence variable mosaic, SVM) in the genome of Malaysian periwinkle yellows (MPY) phytoplasma harbors a gene encoding membrane-targeted proteins, including a putative phospholipase (PL), potentially important in pathogen-host interactions. The present study provided for the first time phytoplasma gene expression investigated and compared in two different protein expression systems: bacterial (*E.coli*) and yeast (*S.cerevisiae*). Despite the low level of expression of rPL in both hosts, a sufficient amount of the enzyme was purified for activity testing. The enzyme activity tests revealed that phytoplasmal rPL from MPY phytoplasma demonstrated only moderate PLA1and low PLA2 enzyme activity on substrates incorporated in liposomes and may be called a phospholipase with low PLA activity. The results obtained in the present study show the existence of an active lipolytic enzyme, encoded in the genomic SVM region of MPY phytoplasma [Gedvilaite A. et al., 2014].

Development of a diagnostic system which detects anti-Schmallenberg virus antibodies in bovine serum, saliva and milk

Schmallenberg virus (SBV), discovered in continental Europe in late 2011, causes mild clinical signs in adult ruminants, including diarrhoea and reduced milk yield. However, fetal infection can lead to severe malformation in newborn offspring. To develop improved reagents for SBV serology a high-level yeast expression system was employed to produce recombinant SBV nucleocapsid (N) proteins with and without hexa-histidine tag. Recombinant SBV N proteins were investigated as antigens in SBV-specific IgG enzyme immunoassay. Both yeast-expressed SBV N proteins were reactive with anti-SBV IgG-positive cow

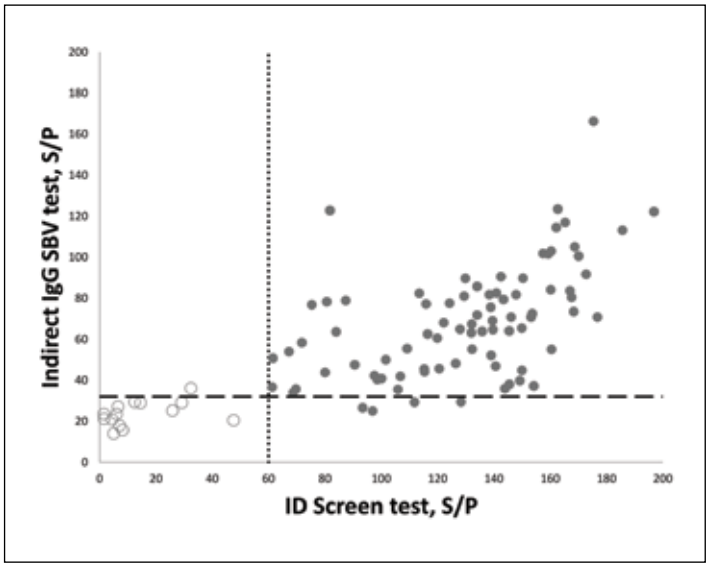


Figure 3. Antibody responses of individual cow serum specimens defined by the newly developed indirect IgG SBV ELISA based on yeast-expressed SBV N protein in comparison to the commercial ID screen test. The S/P ratios of reactivity were plotted. Grey markers represent positive and white negative serum samples obtained by commercial ID screen test. The dotted line represents the cut-off value of the newly developed indirect IgG SBV ELISA.

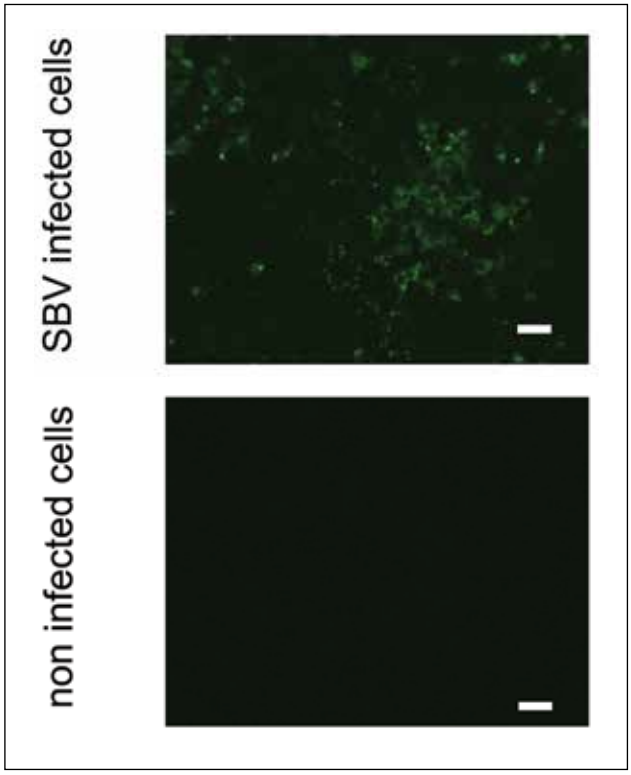


Figure 4. ***Fluorescence microphotographs showing the reactivity of the MAbs 4F3 with BHK cells infected with SBV BH80/11 strain (upper). Noninfected BHK cells were used as a negative control ((lower). Hybridoma culture supernatants were used at a dilution of 1 : 10. Scale bar: 100 μm.***

serum specimens collected from different farms of Lithuania (Fig.3). Additionally, N protein without His-tag reacted with milk and saliva samples of SBV seropositive cows. His-tagged N protein was used for generation of monoclonal antibodies (MAbs) in mice. Four MAbs raised against recombinant SBV N protein were generated and reacted with native viral nucleocapsids in SBV-infected BHK cells in immunofluorescence assay (Fig.4.). In summary, yeast-expressed SBV N proteins and newly developed SBV-reactive MAbs may provide useful reagents for diagnostics and seroprevalence studies of SBV infection. The study demonstrates that yeast expression system is suitable for high-level production of recombinant SBV N proteins and provides the evidence on the presence of SBV-positive antibodies in cow serum, milk and saliva specimens collected in Lithuania [Lazutka J. et al, 2014, Lazutka J. et al, 2015]. This work is supported by Lithuanian Science Council grant MIP-044/2013.

Study of nucleocapsid protein of human parainfluenza viruses 2 and 4

Human parainfluenza viruses 2 and 4 (HPIV2, HPIV4) cause respiratory tract infections. They belong to the genus *Rubulavirus* of the family *Paramyxoviridae*. The research aimed to produce nucleocapsid protein N of HPIV2 and HPIV4 in yeast *S. cerevisiae*. The HPIV2 N gene encoding amino acid sequence RefSeq NP_598401.1 was expressed but the protein did not form nucleocapsid-like particles (NLPs) characteristic to yeast-produced *Paramyxoviridae* N proteins. PCR mutagenesis was carried out to change the encoded specific aa residues to the ones conserved across HPIV2 isolates. The substitution NP_598401.1:p.D331V concluded in NLP formation (Fig. 5). Protein 3D-structure model (ModBase) suggested that this amino acid position was in the hydrophobic core of the C-terminal domain facing the inside of the domain. It is plausible that a negatively charged aspartic acid side chain in place of valine disrupts the packaging of α-helices, resulting in deformed monomers unable to multimerize correctly. Meanwhile, HPIV4 N protein successfully formed NLPs when expressed in yeast.

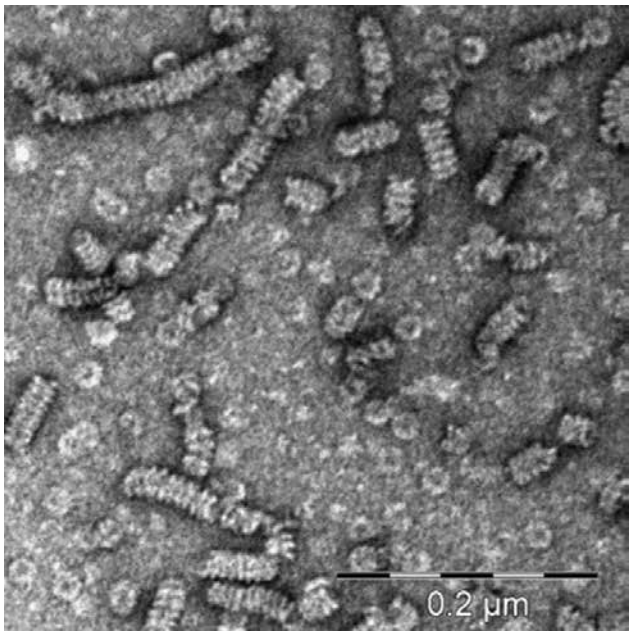


Figure 5. ***Electron micrograph of CsCl density gradient purified HPIV2 N_{VRP} NLPs. Scale bar 200 nm.***

To characterize the antigenic structure of NLP-forming HPIV2 N protein, a panel of monoclonal antibodies was generated after immunizing the mice with the recombinant antigen. The majority of the monoclonal antibodies raised against the recombinant NLPs recognized HPIV2-infected cells, suggesting the antigenic similarity between the recombinant and virus-derived HPIV2 N protein. Fine epitope mapping revealed the C-terminal part (aa 386-504) as the main antigenic region of the HPIV2 N protein. HPIV2 and HPIV4 N proteins were evaluated as antigens in the developing of competitive ELISA for detecting of specific IgG in human sera.

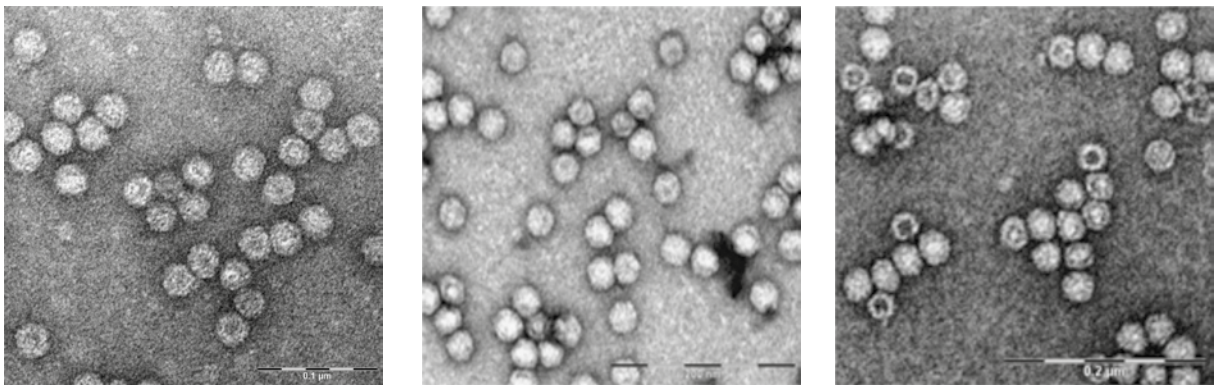
Generation of recombinant capsids of parvoviruses

Parvoviruses are among the smallest and simplest eukaryotic DNA viruses, that infect a wide range of species, both vertebrates and invertebrates. Porcine parvovirus (PPV) is a widespread infectious virus that causes serious reproductive diseases of swine and death of piglets. Human parvovirus 4 (HPARV4) and human bocaviruses (HBoV) 1-4 are newly discovered viruses found around the globe in asymptomatic as well as patients with respiratory (HBoV1) or gastrointestinal (HBoV2-4) symptoms. Recent reports suggest the possible role of these visures in encephalitis. Self-assembled virus-like particles (VLPs) composed of the major parvoviral capsid protein VP2 were generated in yeast *Saccharomyces cerevisiae* [Tamošiūnas et al. 2013; 2014; 2016]. Recombinant VLPs were similar to native parvovirus particles in size and morphology (Fig. 6). A collection of VP2-specific monoclonal antibodies was generated using yeast-synthesised VLPs of PPV, HBoV and HPARV4. Indirect IgG ELISA based on the recombinant VLPs for detection of PPV-specific antibodies in swine sera was developed and evaluated [Tamošiūnas et al. 2014]. The sensitivity and specificity of the new assay were found to be 93.4 % and 97.4 %, respectively. Recombinant HBoV1-4 VP2 VLPs were employed to develop serological assays to detect virus-specific IgG antibodies in human serum specimens [Tamošiūnas et al. 2016]. Competition ELISA format was used in order to eliminate cross-reactivity between bocavirus species. Results revealed that HBoV1 is more prevalent than other HBoV subtypes and majority of tested patient encounter bocaviral infection in early childhood. Recombinant HPARV4 VLPs were employed in evaluation of virus prevalence in Lithuanian population, revealing higher percentage (9.4%) of seropositive low-risk patients than in other European countries. Therefore, yeast *S. cerevisiae* represents a promising expression system for generating recombinant parvoviral VP2 protein VLPs of diagnostic relevance and high antigenicity.

Figure 6. ***Electron micrograph of recombinant PPV (a), HBoV1 (b) and HPAR4 (c) VP2 VLPs generated in yeast. Scale bars 100 nm (a) or 200 nm (b,c).***

Analysis of yeast expression mechanism

Yeast expression mechanism has been investigated by different techniques. Two-dimensional gel electrophoresis (2DE) is one of the most popular methods in proteomics. Research group of Dr. R. Slibinskas employed non-equilibrium pH gradient electrophoresis (NEPHGE)-based 2DE technique for studying stress responses in recombinant protein producing yeast. Comparison of broad range (pH 3-10) gradient-based 2DE



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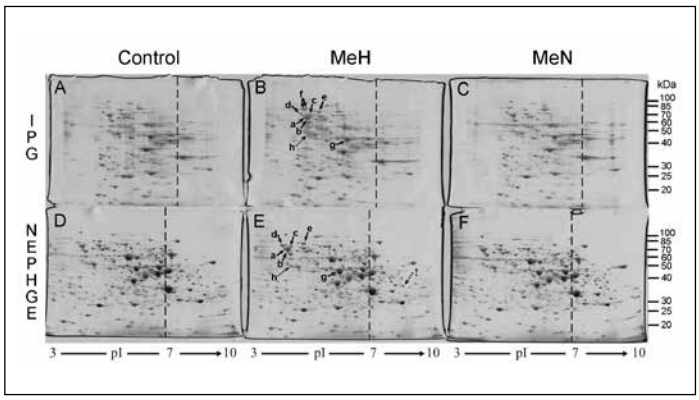

Figure 7. ***2DE of yeast whole cell lysates using IPG (A-C) and NEPHGE (D-F) based methods at high protein load. [Proteome Sci. 2013, 11:36].***

methods suggested that NEPHGE-based method is preferable over commonly used immobilized pH gradient (IPG)-based 2DE method for the analysis of basic proteins (Fig. 7), [Slibinskas R. et all, 2013].

Plant anthocyanin research

Anthocyanins are essential contributors to the fruit coloration, an important quality feature and a breed determining trait of a sweet cherry fruit. It is well established that the biosynthesis of anthocyanins is regulated by the interplay of specific transcription factors belonging to MYB and bHLH families accompanied by the WD40 protein. In this study, we isolated and analysed WD40, bHLH3, bHLH33 and several closely related MYB10 gene variants from different cultivars of sweet cherry, analysed their expression in fruits with different anthocyanin levels at several developmental stages and determined their capabilities to modulate anthocyanin synthesis in leaves of two Nicotiana species. Our results indicate that transcription level of one MYB10 variant correlates with fruit coloration, but anthocyanin synthesis in Nicotiana was induced by another variant, moderately expressed in fruits. The analysis of two fruit-expressed bHLH proteins revealed that bHLH3 enhances MYB-induced anthocyanin synthesis, whereas bHLH33 has strong inhibitory properties (Fig.8). This study is supported by National Science Program "Healthy and safe food" grant No. SVE-11008.

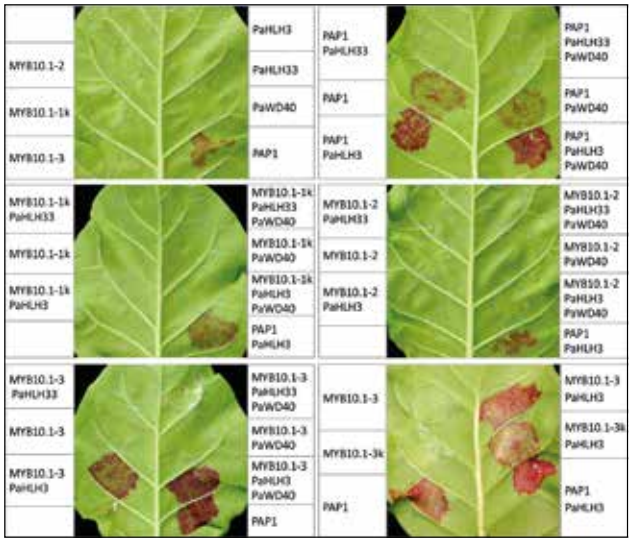

Figure 8. ***Transient expression of putative regulators of anthocyanin synthesis in leaves of Nicotiana tabacum. Infiltrated control cDNAs: PAP1, Arabidopsis thaliana PAP1 (MYB75); GFP, Aequorea victoria GFP. Tested Prunus avium genes isolated from following cultivars: PaWD40, PaHLH33, MYB10.1-1, MYB10.1-3 from 'Irema BS'; PaHLH3 from 'Kitayanka'; MYB10.1-3 from 'Regina'. MYB10.1-1k – cDNA from 'Kitayanka' fruits; MYB10.1-3k – cDNA expressed in N. benthamiana leaves from infiltrated 'Irema BS' gene.***

Viroid evolution in the symptomless host

Viroids are small circular RNA able to survive, propagate and cause disease in the susceptible agricultural crops. There were recently reported the multiple plant species acting as the reservoirs for the further viroid spread without any visible symptom expression. We have investigated the possible impact of such reservoir on viroid evolution under the pressure of increasing anthropogenic pollution. Change in viroid variability caused by chronic, acute and extremely strong oxidative stress induced by an elevated ozone concentration has been assessed (Fig.9). Our studies revealed that strong oxidative stress as well as the genotypic peculiarities of host in the symptomless host could significantly alter viroid adaptability and polymorphism.

Figure 9. ***Visual injury caused by an acute ozone treatment (400 ppb × 6 h) carried out on grown up plants of tomatoe*** *(Lycopersicon esculentum* ***Mill.) cv. Micro-Tom before viroid inoculation.***

Studies of plant signaling mechanisms

During plant responses to stress or developmental cues signaling via mitogen-activated protein kinases (MAPKs) mediates fast, precise and specific responses in cells. The mechanism of this signaling in plants is similar as in other eukaryotes and is relatively well understood, whereas termination of this process by the MAPK phosphatases is less known. The study is aimed to understand the biological roles of PP2C-type MAPK phosphatases in the model plant *Arabidopsis thaliana*. We found that PP2C-type phosphatases regulate MAPKs. PP2C can influence cell fate decisions during stomata development. Stomata are cells essential in water/gas exchange between plant and environment and thus supporting our ecosystem. Gene expression analysis enabled identification of specific MAPKs and a PP2C phosphatase induced during stomata cell development [Fuchs S et al., 2013, Schweighofer A et al., 2014]. This study is supported by Lithuanian Science Council grant No. MIP-003/2014. PP2C-type phosphatases also control plant cell signaling pathways in reponse to pathogens. We have identified a novel PP2C function in signaling induced by bacteria. This study is supported by Lithuanian-Swiss Program grant No. CH-3-ŠMM-01/10.

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Department of

Immunology and Cell Biology

Chief Scientist and Head
Prof. Aurelija Žvirblienė
phone: +370 5 223 4360, fax +370 5 223 4367
e-mail: azvirb@ibt.lt
http://www.ibt.lt/en/laboratories/ilbl_en.html

Scientific staff
Arvydas Kanopka, PhD.
Petras Stakėnas, PhD.
Milda Plečkaitytė, PhD.
Indrė Kučinskaitė-Kodzė, PhD
Daiva Bakonytė, M Sc.
Rita Lasickienė, M.Sc.
Inga Pečiulienė, M.Sc.
Eglė Jakubauskienė, M.Sc.
Aušra Vaitiekaitė, M.Sc.

PhD Students
Indrė Dalgėdienė, M.Sc.
Dovilė Dekaminavičiūtė, M.Sc.
Vaida Simanavičienė, M.Sc.
Miglė Janulaitienė, M.Sc
Milda Zilnytė, M.Sc.
Laurynas Vilys, M.Sc
Vilija Rubinaitė, M.Sc.
Martynas Simanavičius, M.Sc
Asta Lučiūnaitė, M. Sc

Students
Aistė Imbrasaitė, B. Sc
Pijus Andrejauskas
Karolina Juškaitė, B. Sc
Daina Pamedytytė,
Jovita Tamulytė
Enrika Leiputė

Technician
Leokadija Diglienė

Department of Immunology and Cell Biology comprises of three research groups. In 2015-16, the research was focussed to the following topics: development of monoclonal and recombinant antibodies; investigation of virus-like particles as diagnostic tools and carriers for target molecules, investigation of human papillomavirus (research group of A.Žvirblienė), regulation of gene expression by alternative splicing (research group of A.Kanopka), molecular epidemiology of tuberculosis (research group of P.Stakėnas).

Development and characterization of monoclonal antibodies against viral antigens

The Department has long-term experience in development and characterization of monoclonal antibodies against various targets. During 2015-2016, a large collection of monoclonal antibodies against viral antigens has been generated.

Previous studies have shown that recombinant viral structural proteins with their intrinsic capacity to self–assemble to highly-organized structures - virus-like particles (VLPs) or nucleocapsid-like particles (NLPs) – are highly immunogenic and represent promising antigens for developing virus-specific antibodies. In collaboration with the Department of Eukaryote Gene Engineering, novel monoclonal antibodies against recombinant yeast-expressed viral antigens have been generated and employed for diagnostics and structural studies. Those include antibodies against capsid protein VP2 of human bocaviruses (*Tamošiūnas et al., 2016*), parainfluenza type 2 nucleocapsid protein (*Bulavaite et al., 2016*) and neutralizing antibodies against hepatitis B virus (HBV) surface antigen (*Kucinskaite-Kodze et al., 2015*). Development of neutralizing antibodies against HBV surface antigen allowed identification of a highly conserved epitope involved in HBV neutralization (*Kucinskaite-Kodze et al., 2015).*

Human bocaviruses (HBoV) are emerging pathogens associated with pneumonia and/or diarrhea in young children. Six monoclonal antibodies against hBoV1 were used to identify both strain-specific and conserved conformational epitopes on the HBoV1 capsid using cryo-electron microscopy and image reconstruction (*Kalisan et al., 2016*). Structural studies of hBoV1 capsid-antibody complexes were performed at Florida University (USA). Human BoV1-specific antibodies were also employed in a rapid antigen detection test developed at ArcDia (Finland) for laboratory diagnostics of acute hBoV1 infection (*Bruning et al., 2016*).

A large collection of neutralizing monoclonal antibodies against DNA polymerases has been developed (PCT/EP2017/050648, contract with Thermo Fisher Scientific, No AP5-560000-1032).

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Virus-like particles as carriers for functionally active antibody molecules

Virus-like particles (VLPs) can be efficiently produced by heterologous expression of viral structural proteins in yeast. Due to their repetitive structure, VLPs are extensively used for protein engineering and generation of chimeric VLPs with inserted foreign epitopes (*Diederich et al., 2015*). However, insertion of large sized protein sequences may interfere with VLP self-assembly competence. The co-expression of polyomavirus capsid protein VP1 with minor capsid protein VP2 or its fusion protein may result in pseudotype VLPs where an intact VP1 protein mediates VLP formation. We have used this approach to generate pseudotype VLPs displaying large-sized antibody molecules.

Pseudotype VLPs harbouring a surface-exposed functionally active neutralizing antibody specific to hepatitis B virus (HBV) surface antigen (HBsAg) were constructed. The pseudotype VLPs consisting of an intact polyomavirus major capsid protein VP1 and minor capsid protein VP2 fused with the anti-HBsAg molecule were efficiently produced in yeast and purified by density-gradient centrifugation. Formation of VLPs was confirmed by electron microscopy. Two types of pseudotype VLPs were generated harbouring either the single-chain fragment variable (scFv) or Fc-engineered scFv on the VLP surface. The antigen-binding activity of the purified pseudotype VLPs was evaluated by ELISA and virus-neutralization assay on HBV-susceptible primary hepatocytes from *Tupaia belangeri.* Both types of the pseudotype VLPs were functionally active and showed a potent HBV-neutralizing activity comparable to that

of the parental monoclonal antibody. The VP2-fused scFv molecules were incorporated into the VLPs with higher efficiency as compared to the VP2-fused Fc-scFv. However, the pseudotype VLPs with displayed VP2-fused Fc-scFv molecule showed higher antigen-binding activity and HBV-neutralizing capacity that might be explained by a better accessibility of the Fc-engineered scFv of the VLP surface (*Pleckaityte et al., 2015*).

In conclusion, polyomavirus-derived pseudotype VLPs harbouring multiple functionally active antibody molecules with virus-neutralizing capability may represent a novel platform for developing therapeutic tools with a potential application for post-exposure or therapeutic treatment of viral infections.

This work was supported by the Lithuanian Science Council (Global Grant No. VPI-3.1.-SMM-07-K-03-039) and performed in collaboration with Giessen University (Germany).

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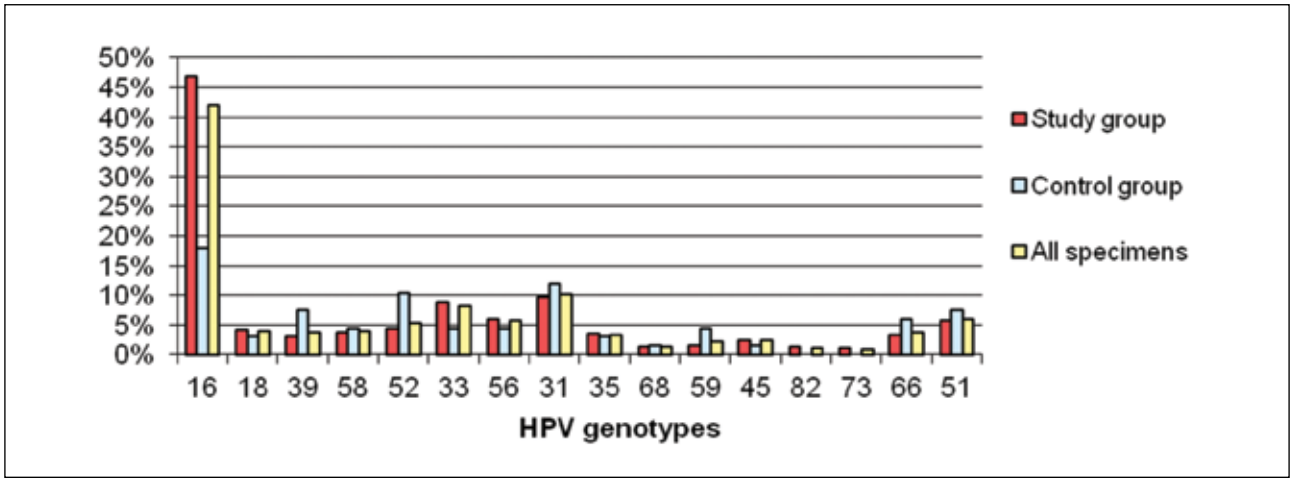
Investigation of human papillomavirus

Human papillomaviruses (HPVs) are human pathogens that infect cutaneous or mucosal epithelia and may cause benign and cancer diseases. High-risk (HR) HPV genotypes are linked to the development of cervical cancer which is the *third* most prevalent *cancer* in women worldwide. Therefore, studies on the prevalence of HR HPV genotypes are important in predicting cervical cancer.

To estimate the prevalence of 16 carcinogenic and potentially carcinogenic HPV types in Lithuania, the in-house developed multiplex PCR-based systems for HPV genotyping were employed and the prevalence of HR HPV genotypes in cervical specimens (n= 824) collected from women with various grades of cervical pathology was investigated. In addition, the frequency of HPV16 variants and the status of HPV16 integration in selected groups of clinical specimens were studied. As expected, the prevalence of HPV infection and HR HPV genotypes correlated with the severity of cervical pathology. HPV 16 was the most common HPV genotype identified in 42.3% of HPV-positive specimens. A relatively high prevalence of other HR HPV genotypes - HPV31 (10.1%), HPV33 (8.2%), and HPV56 (5.7%) was determined. In contrast, the frequency of HPV18 was lower as compared to other countries. (*Simanaviciene et al., 2015a*) (Fig. x). Analysis of HPV16 variants showed that most clinical specimens were attributed to the European Phylogenetic Line L83V variant (52.1%) (*Gudleviciene et al., 2015a*). The study of HPV16 integration demonstrated the presence of both – episomal and integrated – HPV 16 forms in cervical cancer (CC) and in carcinoma *in situ* (CIS). The complete HPV E2 gene deletion was detected in 9.4% of CC specimens and in 2.2% of CIS specimens (*Gudleviciene et al., 2015b*). To investigate the role of epigenetic factors in the development of HPV-induced cancer, DNA methylation pattern at 3' parts of the L1 gene and the LCR sequence of HPV16, HPV18 and HPV51 was analysed. For the first time, DNA methylation of HPV51 was investigat-

Figure. ***The distribution of 16 analysed HR HPV genotypes among all HPV DNA-positive specimens. The study group represents women with cervical pathology and the control group represents healthy women with normal cytology.***



ed. The methylation of cytosines at CpG sites was more prevalent in the L1 gene than in the LCR sequence of all analysed HR HPV genotypes. Moreover, the frequency of HPV DNA methylation correlated with the severity of cervical neoplasia (*Simanaviciene et al., 2015b*).

Investigation of HPV was performed in collaboration with the National cancer institute.

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Hypoxia-dependent regulation of alternative pre-mRNA splicing

RNA splicing takes place in the nucleus and occurs either co- or post-transcriptionally. Noncoding sequences (introns) in nuclear mRNA precursors (pre-mRNA) are removed by dedicated splicing machinery. The coding sequences (exons) are joined to generate the mature mRNA that is exported to the cytoplasm and translated into protein. Splicing events are tissue-specific. This process plays an important role in cellular differentiation and organism development. The splicing machinery heavily contributes to biological complexity and especially to the ability of cells to adapt to different developmental stages and altered cellular conditions.

A striking change has been observed in alternative splicing pattern of genes and alterations in splicing factor expression under pathologic conditions, especially in human cancers. Cancer cells are often confronted with a significant reduction in oxygen availability, which is a major reason for the changeover of major cellular processes. Hypoxic regions have been identified within all solid tumors and their presence has been linked to malignant progression, metastasis, resistance to therapy, and poor clinical outcomes after treatment. Cellular responses to hypoxia are mainly mediated by hypoxia inducible transcription factors (HIFs).

Our goal is to elucidate the mechanism and factors involved in hypoxia-dependent splicing regulation (Fig).

We found that essential splicing factors - the SR proteins - are hyperphosphorylated in hypoxic cells. They are more efficiently recruited to pre-mRNA molecules, where they attract other splicing factors to the sites which are not used under normoxic conditions. This leads to production of mRNAs, from which synthesized products are needed for cell adaptation to changed surrounding conditions (*Jakubauskiene et al., 2015*).

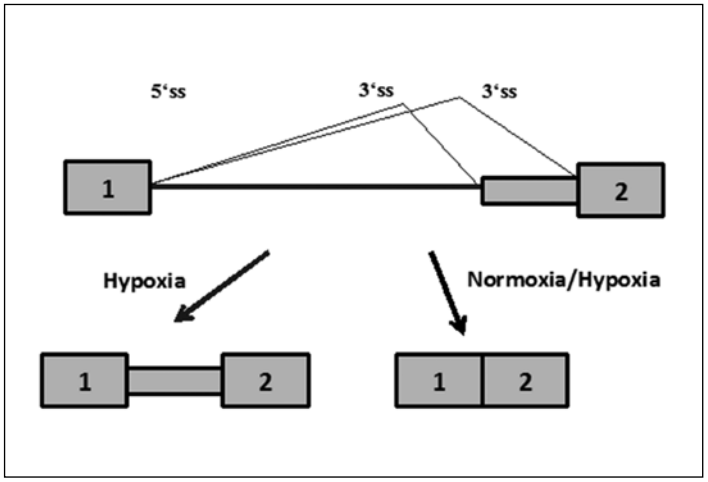

Figure. ***Schematic presentation of an alternative hypoxia-dependent pre-mRNA splicing.***

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Molecular epidemiology of *Mycobacterium tuberculosis*

Tuberculosis that is caused by *Mycobacterium tuberculosis* complex bacteria remains to be a leading bacterial infection causing mostly death worldwide. The epidemiological tuberculosis situation is a serious health problem in Lithuania. The incidence of tuberculosis, particularly multidrug-resistant tuberculosis is one of the highest in the European Society. The aim of our study is to characterize genotypes of *M. tuberculosis* strains, especially those of drug-resistant strains and to identify the mutations associated with drug resistance. Aiming to verify additional targets involved in resistance to pyrazinamide we searched for polymorphisms in several putative genetic regions of *M. tuberculosis*. However, the mutations significant for development of drug resistance were not detected. We continued a retrospective study on genetic diversity of *M. tuberculosis* by using MIRU-VNTR typing. The data on the genotypes of *M. tuberculosis* from Lithuania have been valuable to describe

in detail the most widespread *M. tuberculosis* Beijing lineage and lineage 4. Almost all of the strains from Lithuania belong to these genetic lineages. The analysis of the Beijing strains revealed that the clonal complexes CC1 (Central Asian) and CC2 (European-Russian) predominate in Lithuania. These two sublineages more frequently associated with multi-drug resistant genotypes started to spread throughout central Asia and Russia concomitantly with the collapse of the public health system in the former Soviet Union (*Merker et al., 2015*). It was demonstrated recently that lineage 4 of *M. tuberculosis* comprises globally distributed (generalist) and geographically restricted (specialist) sublineages (*Stucki et al., 2016*). Out of 10 genetic sublineages of lineage 4 three generalist sublineages (L4.1.2/Haarlem, L4.3/LAM, and L4.10/PGG3) and L4.2/Ural are most prevalent in Lithuania. Interestingly, a significant number of drug-resistant clinical strains from Lithuania belong to the L4.2/Ural sublineage that is prevalent in particular countries in Eurasia and Africa only.

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Collaboration

Dr. R.Ulrich, Friedrich-Loeffler Institute, Greifswald-Insel Riems, Germany

Dr. D.Glebe, Giessen University, Germany

Dr. W.Michalski, Australian Animal Health Laboratory, Australia

Prof. K. Gadin, Karolinska Institute, Stockholm, Sweden

Dr. J.Makino, Tokyo University, Tokyo, Japan

Prof. E. Pettersen, Oslo University, Oslo, Norway

Prof. P.Ebbesen, Aalborg University, Aalborg, Denmark

Prof.A.C. Cato, Karlsruhe University, Karlsruhe, Germany

Dr.D. M. Cirillo, Dr. P. Miotto, San Raffaele Scientific Institute, Milan, Italy

Prof. A. Gori, Dr. G. Lapandula, San Gerardo Hospital, Monza, Italy

Dr. J. L. de Beer, National Institute for Public Health and the Environment (RIVM), Bilthoven, The Netherlands.

Dr. E. Davidavičienė, Dr. E. Pimkina, Infectious Diseases and Tuberculosis Hospital, affiliate of public institution Vilnius University Hospital Santariskiu Klinikos (Vilnius)

Dr. Ž.Gudlevičienė, National Cancer Institute (Vilnius)

Grants

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Contracts

UAB Fermentas/Thermo Scientific, Vilnius, Lithuania

Abcam Ltd, Cambridge, UK

Santa Cruz Biotechnology, USA

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Patent

Dovilė Dekaminavičiūtė, Milda Plečkaitytė, Daumantas Matulis, **Aurelija Žvirblienė**. Novel recombinant antibody against human carbonic anhydrase XII. LT patent No. 6331, issued 10-11-2016.

Contacts:

azvirb@ibt.lt, *pstak@ibt.lt*, kanopka@ibt.lt

Department of

Biothermodynamics and Drug Design

Chief Scientist and Head

Prof. Daumantas Matulis, PhD

phone: 370 5 2691884; fax: 370 5 2602116

e-mail: matulis@ibt.lt; daumantas.matulis@bti.vu.lt,

http://www.ibt.lt/en/laboratories/laboratory-of-biothermodynamics-and-drug-design.html

Scientific staff

Lina Baranauskienė, PhD
Edita Čapkauskaitė, PhD
Virginija Dudutienė, PhD
Vaida Juozapaitienė, PhD
Jurgita Matulienė, PhD
Vytautas Petrauskas, PhD
Vilma Petrikaitė, PhD
Asta Zubrienė, PhD
Jelena Jachno, M.Sc.
Darius Lingė, M.Sc.
Vilma Michailovienė, M.Sc.
Aurelija Mickevičiūtė, M.Sc.
Audrius Zakšauskas, M.Sc.

PhD students

Egidijus Kazlauskas, M.Sc.
Justina Kazokaitė, M.Sc.
Vaida Morkūnaitė-Linkuvienė, M.Sc.
Alexey Smirnov, M.Sc.
David Timm, M.Sc.
Agnė Vegytė-Janonienė, M.Sc.
Joana Smirnovienė, M. Sc.

Students

Sandra Bakšytė-Satkūnė,
Denis Baronas,
Brigita Bartkutė,
Mikalojus Brazdžiūnas,
Aistė Dagytė,
Dovilė Daunoraitė,
Danielius Dvareckas,
Marius Gedgaudas,
Linas Jezepčikas,
Lina Kačenauskaitė,
Mindaugas Kostikovas,
Guoda Makarevičiūtė,
Goda Milinavičiūtė,
Vaida Paketurytė,
Edvinas Paliulis,
Virginijus Ruibys,
Kotryna Semėnaitė,
Gediminas Skvaranavičius,
Marija Vaitkevičienė

Technicians

Dalia Černiauskaitė,
Leokadija Davidian,
Jurgita Revuckienė

The Laboratory of Biothermodynamics and Drug Design (LBDD) was established in 2006 based on the former Laboratory of Recombinant Proteins. The LBDD designs novel chemical compounds for therapeutic purposes. The efficiency of both naturally occurring and synthetic compounds is evaluated by biothermodynamic and structural methods.
The laboratory's personnel consist of five teams according to their research goals and activities:

The Team of Molecular and Cell Biology, headed by Dr. Jurgita Matulienė (Ph. D. in cell biology from the University of Minnesota, USA, 2003), prepares target proteins by gene cloning, expression in *E.coli*, insect, or mammalian cells, and chromatografic purification of large quantities of active proteins sufficient for biothermodynamic measurements of compound interaction with target proteins. Several projects involve the design of mutants and truncated protein domain constructs. Live human cancer cells are cultured for the evaluation of compound anticancer activity. Dr. Vilma Petrikaitė has a Ph. D. in pharmacy and performs compound testing in mice xenografts. The team collaborates with the Laboratory of Immunology and Cell Biology in antibody design and diagnostic markers.

The Team of Organic Synthesis, headed by Dr. Virginija Dudutienė (Ph. D. in organic synthesis from the Vilnius University, 2005), synthesizes compounds that are designed to bind carbonic anhydrases and other drug target proteins. Compounds are designed by computer docking, molecular modeling, and comparison with naturally occurring or previously synthesized compound functional groups. Compound identity and purity is verified by NMR and HPLC-HRMS.

The Team of Biophysics, headed by Prof. Daumantas Matulis (Ph. D. in biochemistry, molecular biology and biophysics from the University of Minnesota, USA, 1998), measures compound binding to target proteins by isothermal titration calorimetry (ITC), fluorescent thermal shift assay (DSF, ThermoFluor®), pressure shift assay (PSA), and conventional enzyme inhibition methods. The team determines the *intrinsic* Gibbs free energies, enthalpies, entropies, heat capacities and volume of binding and measures protein stability in the presence of various excipients.

The Team of Computer Modeling, headed by Dr. Vytautas Petrauskas (Ph. D. in physics from the Vilnius University, 2008), is responsible for the application of computational methods, database management, *in silico* docking of large compound libraries and the analysis of X-ray crystal structures of synthetic compound – protein complexes solved in collaboration with Dr. Saulius Gražulis group at the Laboratory of Protein – DNA interactions. Molecular modelers collaborate with the Laboratory of Bioinformatics and use their methods to model protein structures that are not solved by X-ray crystallogphy. The group, together with several collaborating scientists is developing the software that estimates the energies of compound binding to a protein when only the crystal structure of the free protein is available.

The Team of Amyloid Research. Recently a new team has started upon the return of Dr. Vytautas Smirnovas (Ph. D. from the Technical University of Dortmund, 2007) to Lithuania in 2011. The team is described below.

Research Projects

The Laboratory of Biothermodynamics and Drug Design performs fundamental and applied research focused on protein-ligand interactions and drug design. The state of the art in today's industrial drug design is still based on high-throughput approaches due to the lack of fundamental understanding of physical forces underlying such processes as protein folding and protein-ligand interactions. It is still impossible to predict and computer-model the compounds that would exhibit desired affinity and selectivity profiles towards their target proteins.

Carbonic anhydrases as drug targets

Carbonic anhydrases (CAs), a group of zinc containing enzymes, are involved in numerous physiological and pathological processes, including gluconeogenesis, lipogenesis, ureagenesis, and tumorigenicity. CAs catalyze the conversion of CO_2 to the bicarbonate ion and protons. In addition to the established role of CA inhibitors as diuretics and drugs used to treat glaucoma and high-altitude sickness, it has recently emerged that CA inhibitors could have potential as novel anti-obesity, anticancer, and anti-infective drugs.
There are 12 catalytically active CA isoforms in humans. CAs I, II, III, VII and XIII are cytosolic, CAs IV, IX, XII and XIV

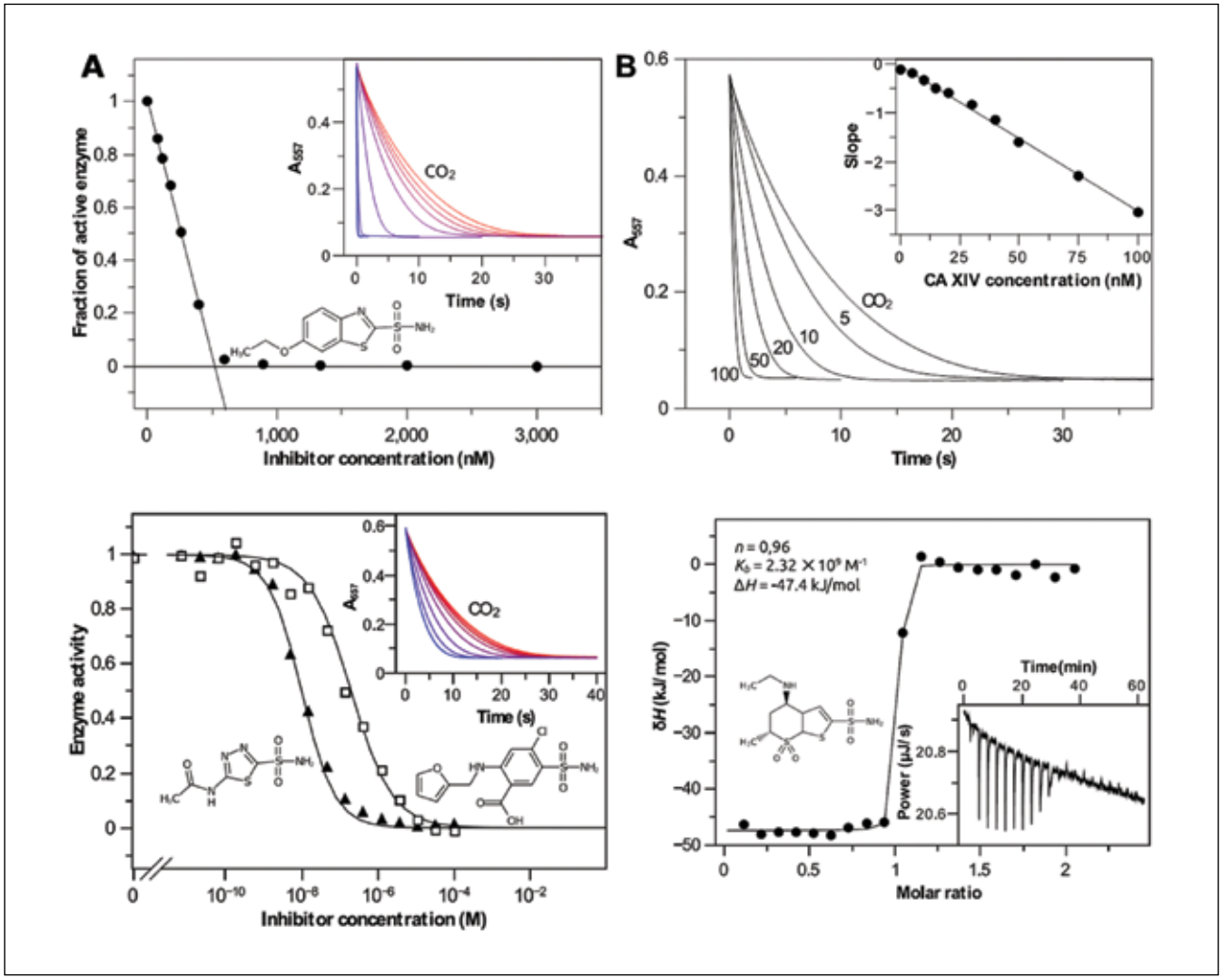


Figure 1. ***Characterization of CA XIV by the stopped-flow activity inhibition assay (A, B), drug dosing curves (C), and the purity demonstration by ITC (D). Published in Juozapaitiene et al 2016.***

are membrane-attached and located on the outside of the cell, CAs VA and VB are found in the mitochondria, and CA VI is the only secreted isoform found in saliva and milk. A number of CA inhibitors, mostly aromatic sulfonamides, have been designed and developed into drugs. However, most inhibitors possess low selectivity towards the target CA isoforms. It is especially important to develop highly selective inhibitors towards the novel anticancer target isoforms, CA IX and XII that are highly over-expressed in numerous tumors and increase cancerous cell survival and metastatic invasiveness.

We have cloned and purified all human CA catalytic domains in bacterial or mammalian cells. Over 700 novel compounds were designed and synthesized that bound CAs with micromolar to picomolar affinities. Six CA isoforms were crystallized in complex with numerous inhibitors and solved to high resolution thus providing structural insight into compound affinity and selectivity. A series of fluorinated CA inhibitors exhibited high affinity and great selectivity towards CA IX isoform. Several other series of compounds were determined to bind various CA isoforms.

However, there are several linked reactions that occur simultaneously with the binding reaction. Such linked reactions greatly influence the observed thermodynamic parameters of binding. For example, affinities are greatly dependent on pH, the enthalpies of binding – on the buffer in solution. Therefore, we determine the intrinsic thermodynamic parameters of binding that are independent of experimental conditions such as buffer and pH and may be directly correlated with the crystal structures.

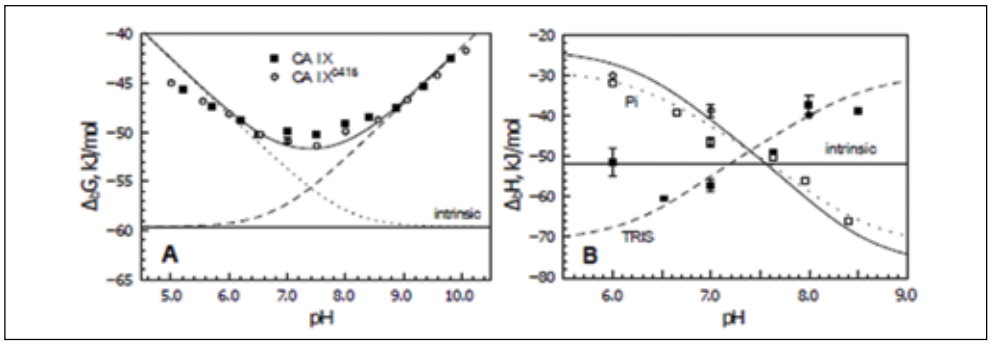


Figure 2. Characterization of CA IX. Binding of ethoxzolamide as a function of pH and determination of intrinsic binding affinity (A), and determination of intrinsic binding enthalpy (B). Published in Linkuviene et al 2016.

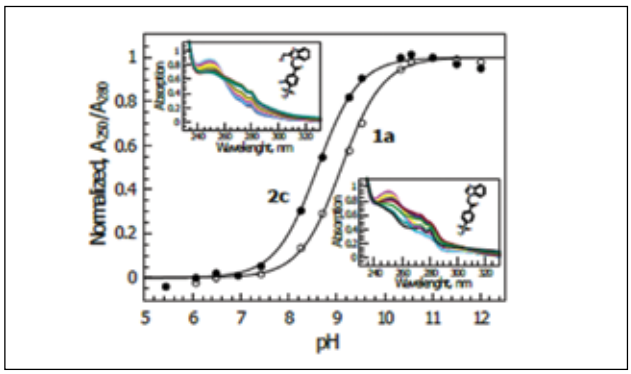
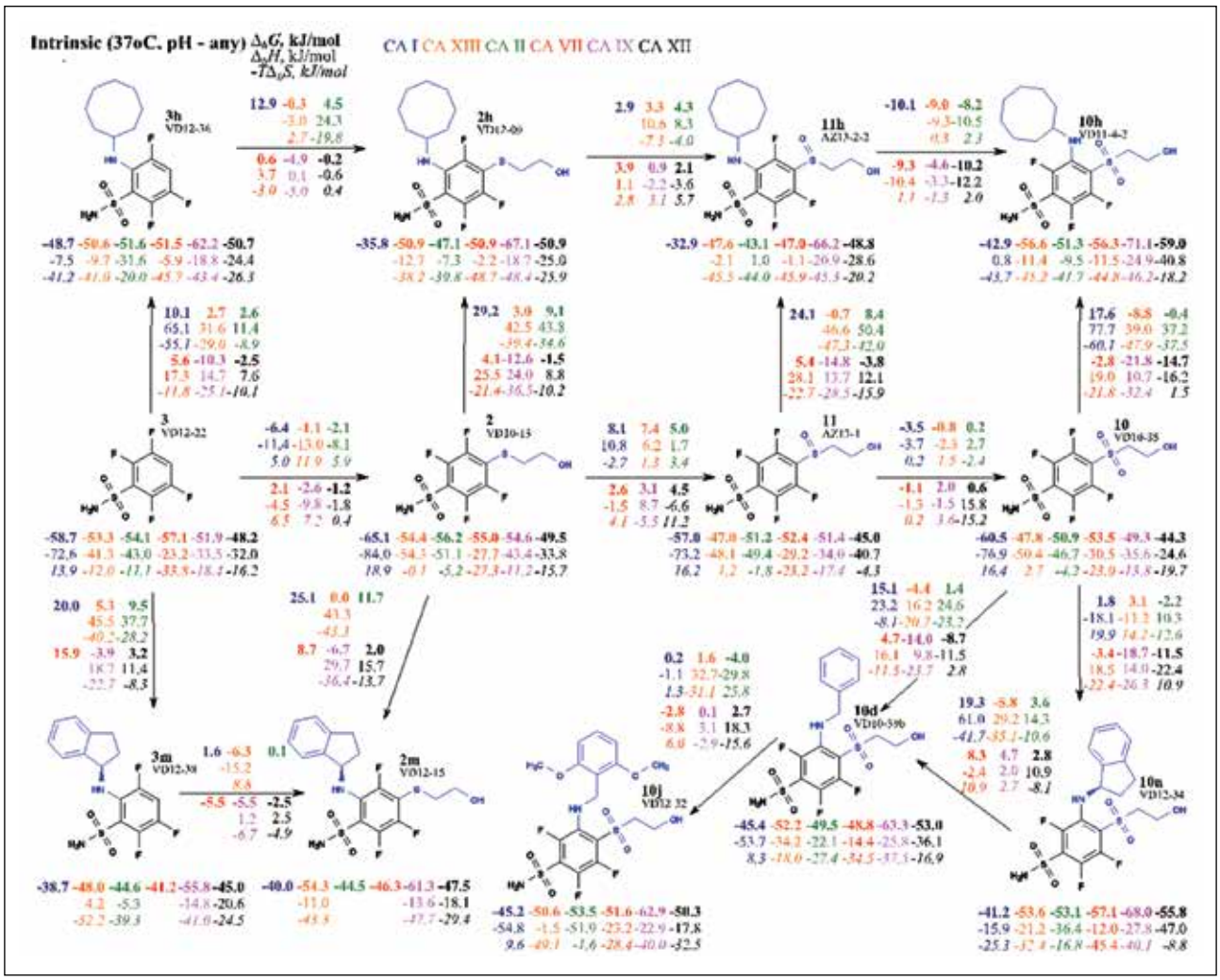


Figure 3. Determination of the inhibitor sulfonamide deprotonation pKa by spectrophotometry. Published in Linkuviene et al, 2016.

Figure 4. A map of intrinsic Gibbs energies and enthalpies of compound binding to 6 CA isoforms. Published in Zubriene et al 2016.



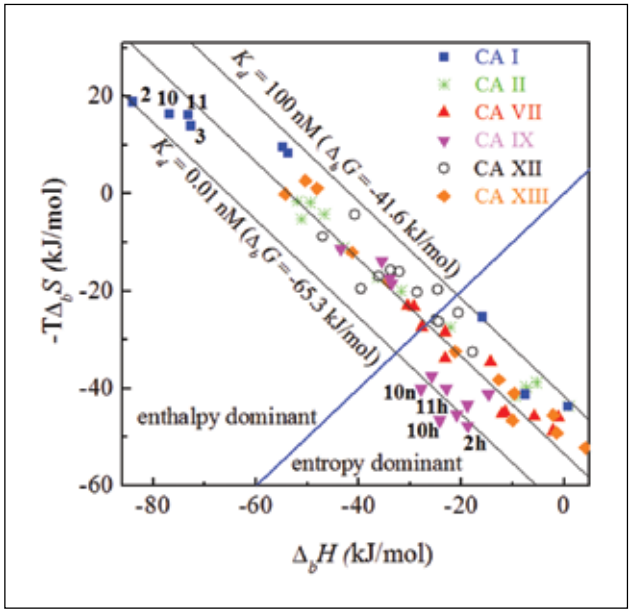


Figure 5. The enthalpy-entropy compensation plot of the fluorinated sulfonamide inhibitor binding to 6 CAs. Published in Zubriene et al 2016.

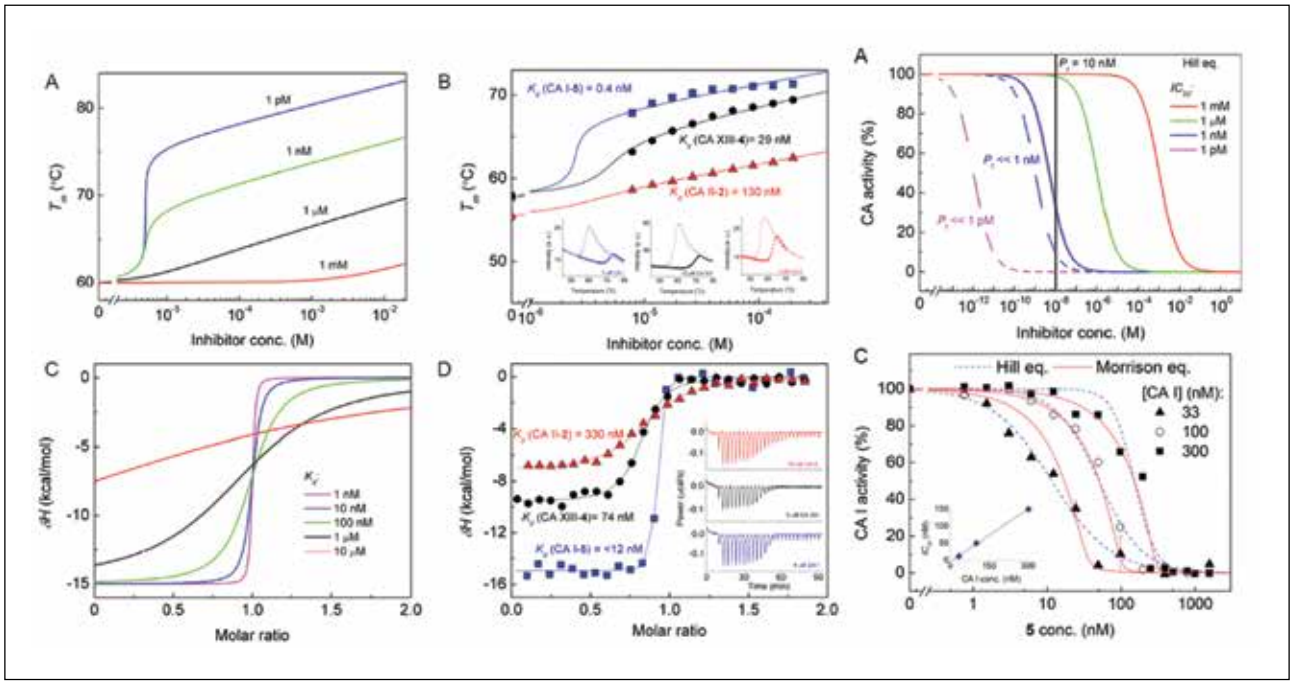


Figure 6. Comparison of theoretical and experimental binding curves of the stopped-flow inhibition assay, isothermal titration calorimetry and thermal shift assay. Published in Smirnoviene et al 2016.

The Team of Amyloid Research

Group leader senior scientist
Vytautas Smirnovas, PhD
Phone: +370 670 69929
Email: vytautas.smirnovas@bti.vu.lt

PhD students
Ričardas Mališauskas
Tomas Šneideris

Students
Gerda Blantaitytė
Artūras Blažaitis
Miglė Čiurinskaitė
Domantas Dargužis
Martynas Grigaliūnas
Lukas Gudiškis
Elžbieta Kulicka
Jurgis Kuliešius
Ieva Kunigėlytė
Fausta Labanauskaitė
Akvilė Leckienė
Greta Musteikytė
Mai Nguyen Ngoc
Valdemaras Petrošius
Artūras Polita
Andrius Sakalauskas
Dovilė Skujienė
Karina Sluckaitė
Romuald Stanilko
Algirdas Šerėnas
Izabelė Šuikaitė
Darius Šulskis
Mantas Žiaunys

Protein aggregation into amyloid structure 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 an increasing evidence of amyloid nature of proteinaceous infectious particles – prions. One of possible ways of prion spreading is self-replication of amyloid-like fibrils, thus there is a chance of all amyloid-associated diseases to be potentially infective.

We study effects of environmental factors such as temperature, pressure, pH, ions, macromolecular crowding, and the presence of different organic solvents, ligands and biomolecules on aggregation kinetics, thermodynamic stability, and structural properties of amyloid-like fibrils. We believe only comprehensive knowledge of all factors may give genuine understanding of mechanisms of amyloid self-replication and thus proteinaceous infectivity.

Services

The LBDD is seeking to license out the compounds described in patents and patent applications. The LBDD is interested in collaborations where our expertise in recombinant protein production and the determination of compound – protein binding thermodynamics and recombinant protein stability characterization could be applied. Protein – ligand binding constants and protein thermal stability profiles at hundreds of conditions may be determined in a single experiment by consuming microgram quantities of protein.

Conferences

The LBDD regularly participates in many international conferences and symposiums, including:

International Conference on the Carbonic Anhydrases.

International Conference on High Pressure Bioscience and Biotechnology.

European Biophysics Congress.

Biophysical Society Annual Meeting.

International Conference of Lithuanian Biochemical Society.

Since 2014 when Prof. Daumantas Matulis became the chairman of the Lithuanian Biochemical Society, we have organized the "XIVth International Conference of Lithuanian Biochemical Society", 2016 06 27 – 30, Druskininkai, Lithuania.

Funding

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Punlications 2015-2016

Journal Articles

1. Petrauskas, **V.**, **Maximowitsch**, **E.**, **Matulis**, **D**. 2015. Thermodynamics of Ion Pair Formations Between Charged Poly(Amino Acid)s. *J. Phys. Chem. B.* **119:** 12164-12171.

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Patent Applications

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Sector of

Applied Biocatalysis

Research Associate and Head
Inga Matijošytė, PhD
phone: +370 5 223 4371, fax +370 5 223 4367
e-mail: inga.matijosyte@bti.vu.lt
http://www.bti.vu.lt/en/departments/sector-of-applied-biocatalysis

Scientific staff
Rimantas Šiekštelė, M.Sc.
Aurelija Sirvydaitė, M.Sc.
Aušra Veteikytė, B.sc.

PhD students
Milda Šulcienė
Veslava Matikevičienė

Students
Inga Šileikaitė
Povilas Matusevičius
Marija Danilocevaitė
Mantas Baliukynas
Žymantas Venckus
Justinas Babinskas
Gabija Aleknavičiūtė

Affiliated scientist
Prof. Gervydas Dienys, PhD, Dr.

Sector of Applied Biocatalysis was established in 2007 as a group of Industrial Biotechnology in conjunction with the start of the National Programme for the Development of Industrial Biotechnology in Lithuania 2007-2010. In 2010 the group was transformed into the Sector of Applied Biocatalysis and is headed by Inga Matijošytė (Ph.D. in biochemistry and biocatalysis from the Delft University of Technology, The Netherlands, 2008).

The limited number of suitable and well-characterized biocatalysts delays the progress in the application of enzymes in the synthesis of compounds for materials, pharmaceuticals, and chemicals. Sector's research is directed towards the search for enzymes with new functionalities and their development towards applied biocatalysis. The research focuses on developing of biocatalytic systems employing oxidative, lypolytic, hydrolytic, proteolytic enzymes. We strive to meet scientific challenges in combination with application-oriented research towards the discovery of new/novel biocatalytic routes for high-added value products from bio-based raw materials.

Screening for new enzymes

The demand for enzymes with new/novel or specific characteristics and functionalities is constantly increasing. Functional analysis of strain collection, which we have in hands, indicated the presence of lipoxygenase, laccase, lipase, peroxidase, secondary alcohol oxidase, cytochrome C oxidase activities, hydrolase with distinguished features, which currently are explored for further development as biocatalysts.

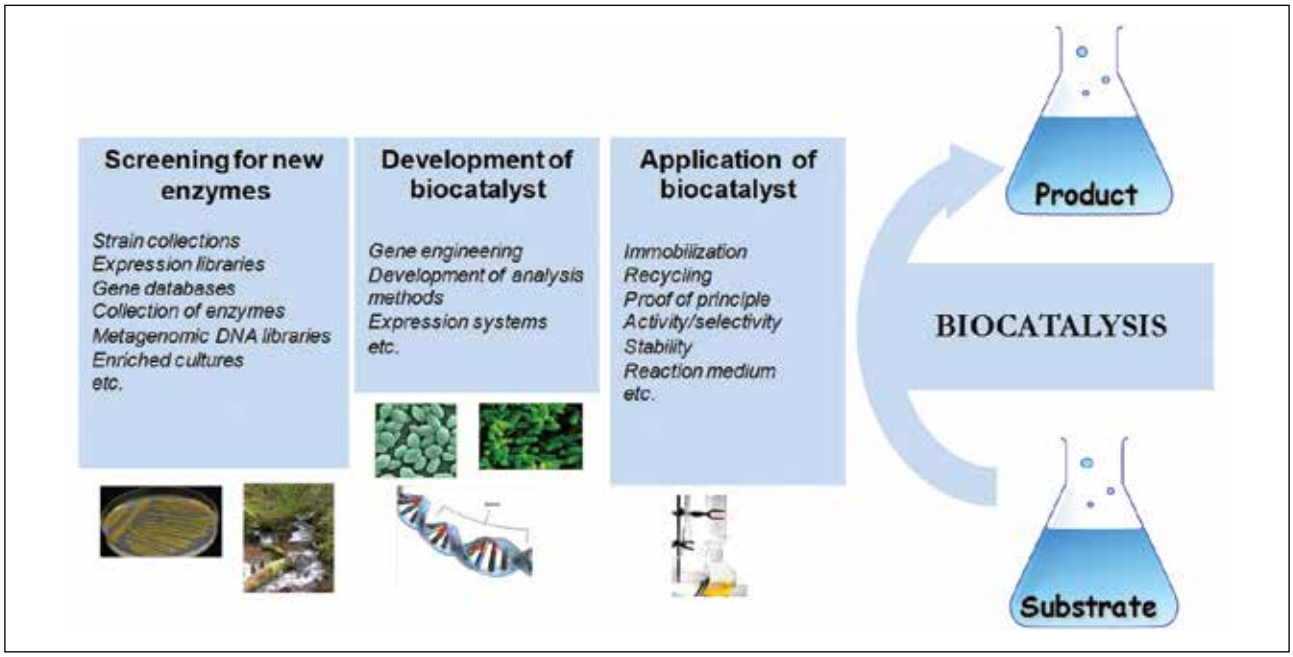
We have the possibility to explore the expression of the newly cloned genes in a number of *E. coli* strains and vectors, in yeasts such as *Saccharomyces cerevisiae*, *Kluyveromyces lactis* and *Kluyveromyces marxianus*. For the last two, we use our own expression system.

We have successfully constructed metagenomic DNA libraries originated from sludge, soil and polluted water samples.

Development of biocatalysts

Many microbial lipases have been successfully expressed in yeasts, but not in industrially attractive *Kluyveromyces lactis,* which among other benefits can be cultivated on the medium supplemented with the whey - cheap and easily available industrial waste. A new bacterial lipase from *Serratia* sp. was isolated and for the first time expressed into the yeast *Kluyveromyces lactis* by heterologous protein expression system based on a strong promoter of *Kluyveromyces marxianus* triosephosphate isomerase gene (TPI1) and signal peptide of *Kluyveromyces marxianus* endo-polygalacturonase (EPG1) gene. Following studies for estimation of optimal cultivation conditions showed that *K.lactis*

Figure 1. ***Flowchart of R&D at Sector of Applied Biocatalysis. Sector of Applied Biocatalysis seeks to identify biocatalysts with novel activities by screening for enzymes, development of biocatalysts and application of biocatalysts.***



can be successfully grown in the medium containing whey – a cheap raw material. Both variants of lipases (mG1 and sG1) expressed in *K.lactis* showed promising characteristics for application in biocatalytic systems.

Further, we are also working on the improvement of the heterologous proteins expression systems in the yeast *Kluyveromyces*, which are provided with specific dominant markers.

Enzyme collections are typical of environmental origins, such as microbial strain collections and/or metagenomic libraries. We are keen on the development of screening systems for growing cells aiming to search for targeted enzymes: enzyme assays based on colored substrates or products can be applied to identify colonies producing active enzymes which can be then be picked for future studies. We have developed such assays for epoxidation, hydrolysis, lypolytic, oxidation (laccase) enzyme activities.

Also, we are developing direct and indirect assays for determination of soluble and immobilized enzyme activities. Rapid and easy to handle enzymatic assays were established for xylanase, cellulase, glucanase, mannanase and pectinase activities.

Application of biocatalyst

There is still a great demand for sustainable oxidative systems for the conversion of alcohol to carbonyl compound, a pivotal reaction in daily organic synthesis, in the fine chemical and pharmaceutical industries. In the past, several attempts were made to apply alcohol oxidase (AO) for oxidations, which is known to catalyze the oxidation of short aliphatic alcohols, but its poor stability has limited the comprehensive applications of this enzyme. We have investigated the stabilization of AO by CLEA method, which to best of our knowledge up to date, was applied for AO immobilization for the first time, and which was showed to be a proper technique, suited particularly for that purpose, due to AO's structural complexity. It was demonstrated that the process was not limited by the stability of enzyme and that further development strategy of this biocatalytic oxidation system should be directed towards creating engineering systems with an immediate elimination of the formed reaction product (aldehyde).

Our investigated technology was based on a continuous exploitation of the whey, industrial waste product. Primarily, whey was used as a nutrition source for the cultivation of *Kluyveromyces lactis* with the aim to produce the targeted biocatalyst – lipase. During cultivation, the whey was transformed into the hydrolyzed form, which was further successfully applied as a protein feeder (external linker) for immobilization of lipase by CLEA (cross-linked enzyme aggregate) method. The first time use of whey as a co-feeder for immobilization of enzymes by CLEA method has shown promising results and increased the stability of lipases for temperature and organic solvents. Hydrolysis of rapeseed oil catalyzed with immobilized derivatives was obtained with 45-96 % efficiency at non-optimized conditions.

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Agency of Science, Innovation and Technology

COST Action 1303

Research contracts

Bioenergy LT

Pienas LT

Baltijos enzimai

Naujoji Ringuva

IMD technologies

Publications

A. Veteikytė, R. Šiekštelė, B. Tvaska, **I. Matijošytė**. Sequential application of waste whey as a medium component for *Klyuveromyces lactis* cultivation and a co-feeder for lipase immobilization by CLEA method. *Appl. Microbiol. Biotechnol.* 2017, 101: 3617-3626.

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International collaboration

Dr. Boris Kovenbach**,** University of Applied Sciences, Muttenz, Switzerland

Prof. Dr. Phillipe Corvini, University of Applied Sciences, Muttenz, Switzerland

Dr. Frank Hollman, Delft University of Technology, the Netherlands

Dr. Linda Otten, Delft University of Technology, the Netherlands

Prof. Isabel Arends, Delft University of Technology, the Netherlands

Prof. Marcellus Ubbink, Leiden University, the Netherlands

Dr. Susanne Zibek, Fraunhofer Institute IGB, Stuttgart, Germany

Prof. Vytas Švedas, Laboratory of Biocatalysis and Enzyme Engineering, Belozersky Institute

Sector of

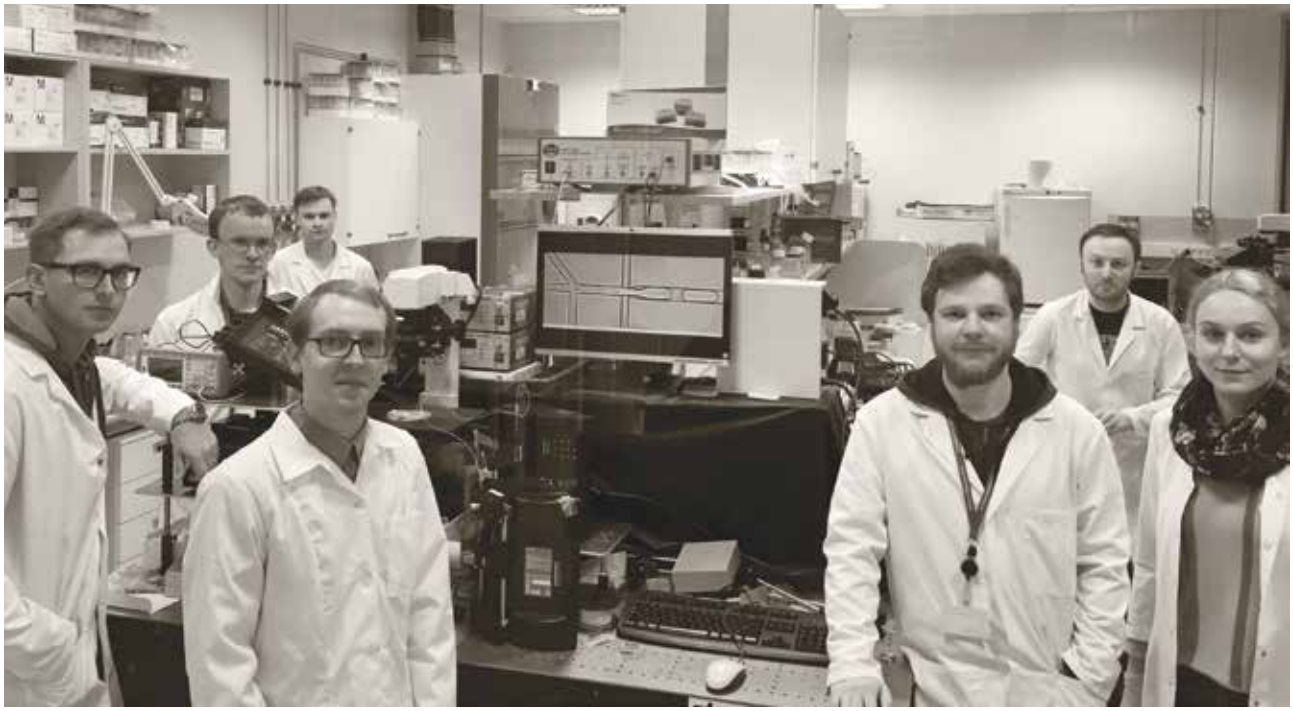
Microtechnologies

Senior Scientist and Head

Linas Mažutis, PhD

phone: +370 693 19333, +370 5 223 4356, fax +370 5 223 4367

e-mail: linas.mazutis@bti.vu.lt



Scientific staff

Linas Mažutis, PhD

Karolis Leonavičius, M.Sc

PhD students

Robertas Galinis

Justina Rutkauskaitė

Valdemaras Milkus

Juozas Nainys

Rapolas Žilionis

Students

Karolis Goda

Dalius Kučiauskas

Karolis Simutis

Jūratė Skerniškytė

Greta Stonytė

Research Overview

Droplet microfluidics technology offers a powerful tool to isolate and quantify biological molecules and individual cell in a massively parallel manner. The use of droplets in biological assays provides not only significant savings for cost of the reagents but also increased analytical sensitivity and ultra high throughput capabilities. The basic principle of droplet microfluidics is relatively simple: highly uniform aqueous droplets are generated in inert carrier oil on a microfluidics 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 droplet is roughly a thousand to a million times smaller. Different microfluidic modules can be employed to manipulate droplets in sophisticated, yet highly controllable manner. Large numbers of droplets (>10^9) can be generated at astonishingly high rates (>20,000 droplets per second), their size tuned precisely, new reagents introduced into pre-formed droplets at defined time points, droplet split and sorted, therefore opening new opportunities for molecular and cell biology. Our group is developing and applying droplet microfluidic tools for a diverse set of quantitative experiments.

1. Microfluidic tools for biological and biomedical applications
2. Single-cell barcoding and sequencing
3. High-throughput screening of antibody secreting cells *in vitro* directed evolution using artificial cells
4. Drug delivery systems
5. DNA-Mg-PP particle biosynthesis and their use in synthetic biology

Our largest efforts in the past 2 years have been dedicated to single-cell RNA-Seq applications. Deciphering the unbiased composition of heterogeneous cell populations requires innovative techniques that could capture not tens or hundreds but thousands of single-cells and to do so in a high-throughput manner and affordable cost. In collaboration with Harvard University we have developed a platform to isolate and barcode the transcriptome of thousands of single-cells (Klein*, Mazutis*, et al., ***Cell***, 2015 and Zilionis et al., ***Nature Protocols***, 2017). This work is built on idea of isolating individual cells into microfluidic droplets carrying RNA/DNA barcodes, and assay reagents. Because individual cells are compartmentalized in drops, cell genetic make up and contents can be accessed. For single-cell studies our approach provides unprecedented scalability, significantly increased throughput, minimal sample loss, reagent savings and large experimental flexibility. Using this technology we are interested in understanding the genetic and epigenetic factors that are responsible for cellular heterogeneity and inheritance.

For more information please refer to:

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

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 * - joint first author

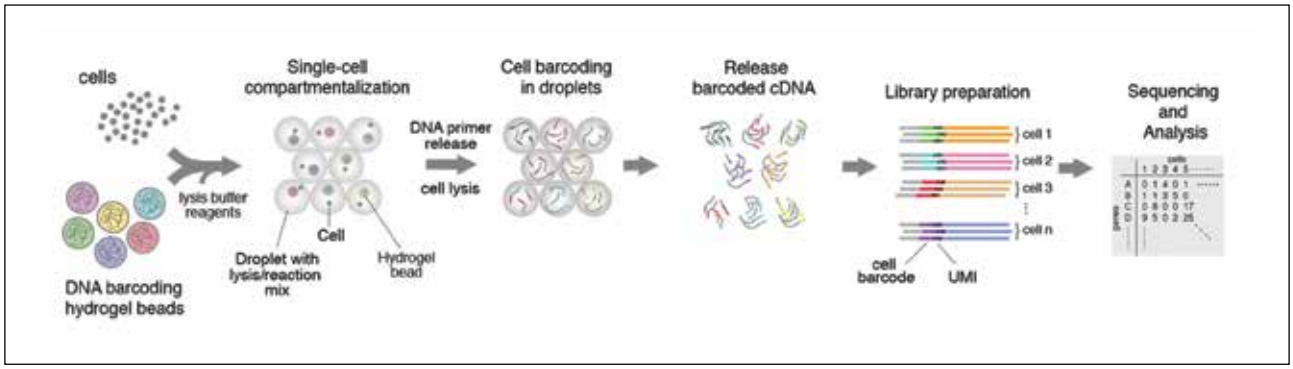


Figure 1. Single-cell barcoding and sequencing principle. Heterogenious mix of cells is encapsulated into droplets together with reverse-transcription enzyme, lysis mix, and hydrogel beads carrying barcoding primers. After encapsulation cells are lysed, primers are released from the beads and cDNA is tagged with a barcode during reverse transcription reaction. Once cDNA synthesis is complete, droplets are broken and barcoded material from all cells is amplified for next-gen sequencing.

In 2015 we have developed droplet microfluidics approach for production of nano- and pico-liter volume particles in which inner part of the vesicle contains preloaded biological or chemical molecules and outer part (shell) is composed of biodegradable polymer. We use alginate as a biomaterial due to its biocompatibility and ease of polymerization.

For more information please refer to following work:

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

Vasiliauskas R, Liu D, Cito S, Zhang H, Shahbazi MA, Sikanen T, Mazutis L, Santos HA (2015) A Simple Microfluidic Approach to Fabricate Monodisperse Hollow Microparticles for Multidrug Delivery, ***ACS Appl. Mater. Interfaces***, 7 (27), pp 14822–14832.

Finally, in collaboration with ETH Zurich researchers (Prof. Donald Hilvert lab) we have discovered the reaction conditions under which DNA molecule amplification leads to formation of condensed DNA nanoparticles of >1000 nm size. The DNA particles comprise up to ~100.000 copies of clonally amplified DNA template (e.g. gene encoding enzyme of interest). Surprisingly, the inorganic pyrophosphate, produced during isothermal DNA synthesis, and magnesium ions induce DNA condensation into the crystalline-like globular structures. This process is enhanced when the DNA amplification reaction is performed inside droplets, which can be attributed to the confined volumes and spatial accumulation of the reaction products. We have demonstrated the biological functionality of the DNA nanoparticles, by applying them in in vitro transcription-translation reactions and observed improved protein expression yields relative to standard assay conditions.

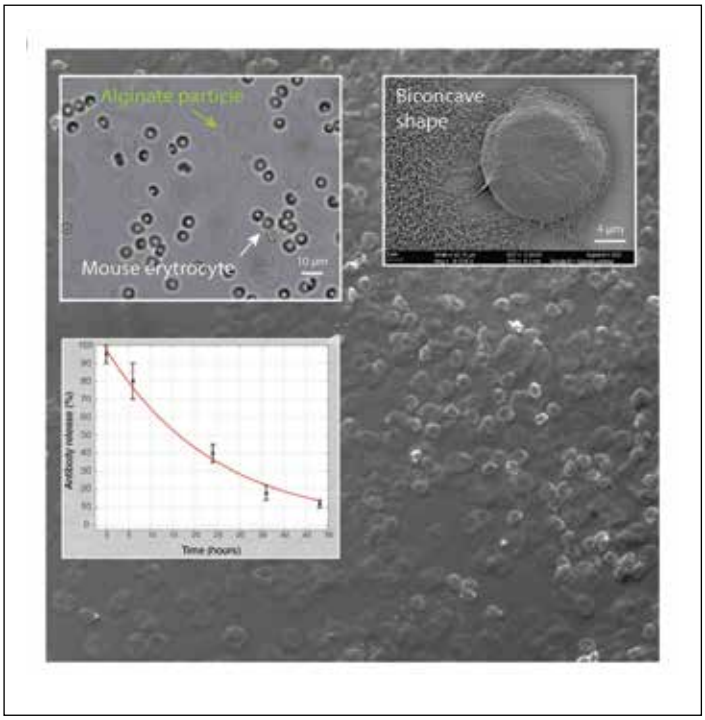


Figure 2. Production of hydrogel particles for biomedical applications. We have developed a microfluidic approach for production of hydrogel particles having the unique biconcave shape and the size of a mammalian cell (~10 μm). The release of encapsulated antibodies was largely affected by the presence of phosphate ions that chelate calcium (a cross-linker of alginate polymer) and cause the dissolution of hydrogel. In addition, the image shows the biocompatibility of alginate particles in the whole blood.

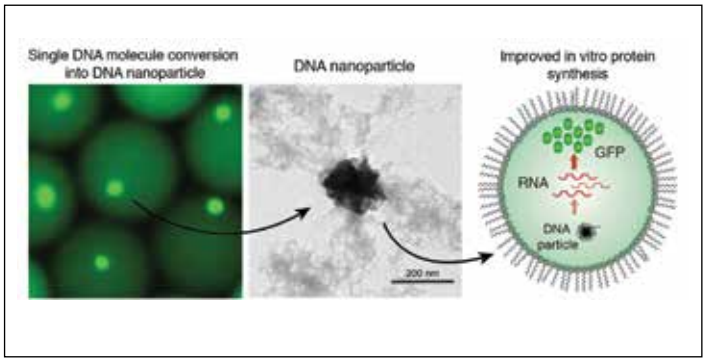


Figure 3. Generation of DNA-Mg-PP particles and their use. DNA nanoparticle formation induced by inorganic pyrophosphate (PP) and magnesium ions during a phi29-catalyzed DNA polymerization reaction. TEM image of the DNA-Mg-PP particle. The use of DNA for efficient gene expression and protein synthesis in vitro.

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2. Einav T., **Mazutis L,** and Phillips R, (2016) Statistical Mechanics of Allosteric Enzymes, ***J. Phys. Chem. B,*** 120 (26), pp 6021–6037.

3. Wagner O, Thiele J, Weinhart M, **Mazutis L**, Weitz D, Huck W Haag R. (2016) Biocompatible fluorinated polyglycerols for droplet microfluidics as an alternative to PEG-based copolymer surfactants, ***Lab on a Chip***, 16, 65-69

4. 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 * - joint first author

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

6. **Vasiliauskas R**, Liu D, Cito S, Zhang H, Shahbazi MA, Sikanen T, **Mazutis L**, Santos HA (2015) A Simple Microfluidic Approach to Fabricate Monodisperse Hollow Microparticles for Multidrug Delivery, ***ACS Appl. Mater. Interfaces***, 7 (27), pp 14822–14832.

7. Bender M, Thon JN, Ehrlicher AJ, Wu S, **Mazutis L**, Deschmann E, Sola-Visner M, Italiano JE Jr, Hartwig JH. (2015) Microtubule sliding drives proplatelet elongation and is dependent on cytoplasmic dynein, ***Blood***, 125(5): 860-868

DNA Sequencing Center

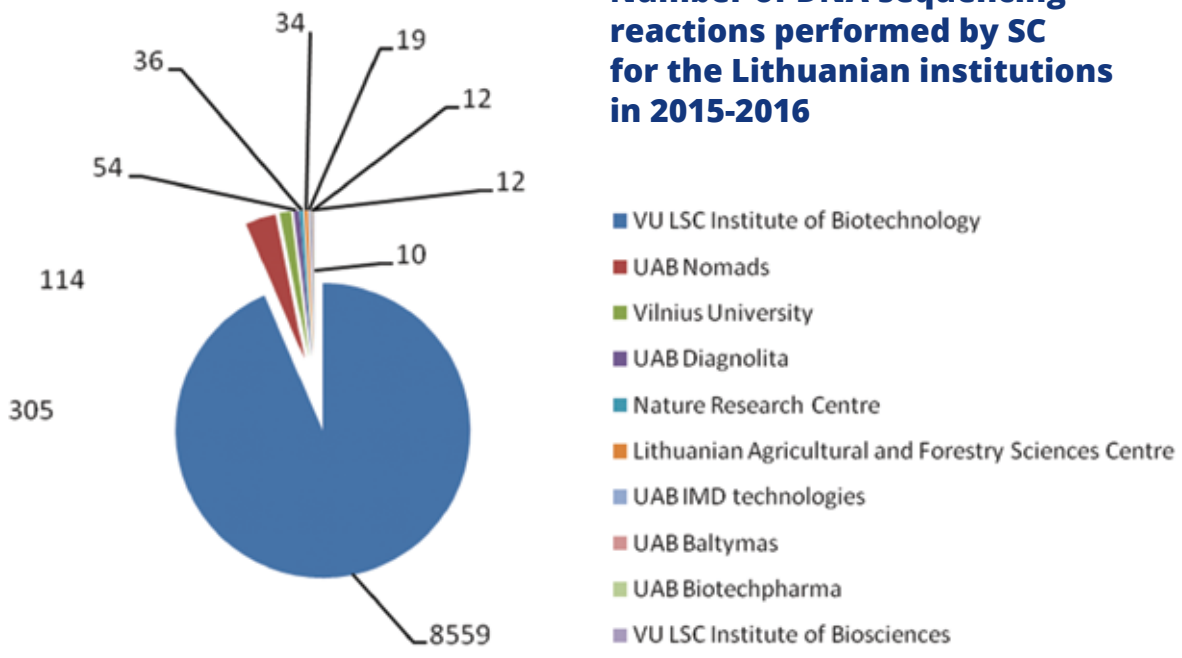
Eglė Rudokienė, M.Sc.
Phone: 370 5 2234370
e-mail: egru@ibt.lt

DNA Sequencing Center (SC) of the Institute of Biotechnology (IBT) is successfully running since March 27 of 2003. SC was founded to help researchers, both at IBT as well as other institutions in Lithuania, process DNA samples in an efficient and economical manner. The Center is equipped with the Applied Biosystems 3130*xl* 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 the user's reactions. Usually, turnaround time takes 2-3 days after the receipt of samples. Sequencing of the larger samples may take longer.

Services provided by the DNA SC include:

- Custom DNA Sequencing
- Sequencing, PCR troubleshooting and training workshops

We are committed to giving every user satisfactory sequence.



Number of DNA sequencing reactions performed by SC for the Lithuanian institutions in 2015-2016

COD: Crystallography Open Database

The COD project (*abbrev. from* "Crystallography Open Database", http://www.crystallography.net/) aims at collecting in a single open access database all organic, inorganic and metal organic structures [1] (except for the structures of biological macromolecules that are available at the PDB [2]).

The database was founded by Armel Le Bail, Lachlan Cranswick, Michael Berndt, Luca Lutterotti and Robert T. Downs in February 2003 as a response to Michael Berndt's letter published in the Structure Determination by Powder Diffractometry (SDPD) mailing list [3]. Since December 2007 the main database server is maintained and new software [4; 5] is developed at the Institute of Biotechnology, Vilnius University by Saulius Gražulis and Andrius Merkys. In 2014 data and software from CrystalEye project, managed by Peter Murray-Rust, were integrated into the COD [6]. Currently, the COD curates over 375 thousand records describing structures published in major crystallographic and chemical peer-reviewed journals [7].

The COD project aims not only at preserving data, but also at becoming a collaboration platform for scientists. The database presents itself on the Internet as a website (Fig. 1a) with basic data entry, search and retrieval capabilities. Registered researchers can upload their structures to the COD using automatic data deposition system, and curate the existing COD data. The uploaded data may come directly from experiments (so called prepublication data), from previous publications or as personal communications using the deposition website. The deposition software performs rigorous checks of syntax and semantics, thus ensuring high quality of records deposited in the COD. Searches can be carried out by entering basic crystallographic and chemical parameters or by providing a structural formula. The retrieved COD records can be viewed on-line (Fig. 1b) or downloaded for further processing. Data search and retrieval does not require any registration, nor is limited in any other way. The addition of functionality of public discussions and structure annotation is planned in the near future.

A great interest is also shown in relating COD crystal structures to their chemical information. In the beginning of 2014 Open Babel [8] toolbox was used to create 35 thousand reciprocal links between the records of the COD and ChemSpider [9], an open database of chemical structures; in the following year, an algorithm to restore stoichiometric molecular composition of a crys-

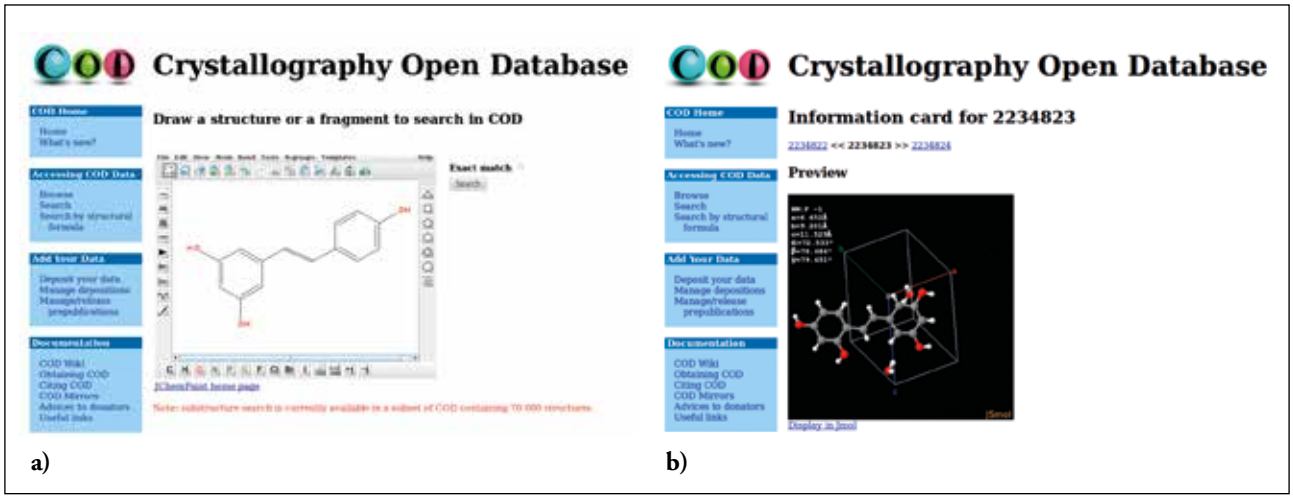


Fig. 1 a) Website and search interface of the Crystallography Open Database (COD) permits searches of crystallographic data by a range of crystallographic and chemical parameters, bibliography, as well as by structural formula. b) Data can be viewed on-line in the J(s)Mol applet or downloaded for further processing, either record-wise or in bulk.

tal [4] was created and implemented. By utilizing the before mentioned algorithm and Open Babel toolbox, it is expected to automate link building between the COD and PubChem, an open database of biological activities of small molecules.

The open nature of the COD permitted numerous mirrors around the globe [10-13] and specifically tailored COD database variants [14]. For massive data mining, the COD permits downloads and updates of the whole database using Subversion, Rsync or HTTP protocols. The ease of access to COD data has spurred the use of this resource for software testing, teaching [14], and research [15]. COD software codebase also facilitated the creation of two additional open access databases PCOD (*abbrev. from* "Predicted COD", http://www.crystallography.net/pcod) and TCOD (*abbrev. from* "Theoretical COD", http://www.crystallography.net/tcod). PCOD, founded in 2008, now stores over a million records of first-principles-predicted crystal structures; TCOD, launched 5 years later in 2013, now stores a few hundred theoretically calculated or refined crystal structures. Even though TCOD is quite small at the moment fast database growth is expected in collaboration with various partners such as the work group of AiiDA [16] framework for atomistic simulations led by Nicola Marzari or the OQMD database [17] of DFT models of quantum materials supervised by Chris Wolverton.

Over the last 14 years the COD has earned the recognition of the scientific community. After the integration of CrystalEye into the COD in 2014, Saulius Gražulis was presented with the Blue Obelisk [18] award for achievements in promoting open data, open source and open standards. During the same year the COD was ranked 5th in accordance to the number of records by the analysis of Thomson Reuters data citation index [19]. The increasing popularity is noticeable among the academic publishers as well – more and more peer-reviewed journals [20; 21] or even entire publishing groups [22] are recommending the COD as the preferred repository for the long term scientific data storage. At present, the COD is the most comprehensive open resource for small molecule structures, freely available to all scientists in Lithuania and worldwide.

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SOLSA project

The main goal of the SOLSA project ("Sonic Drilling coupled with Automated Mineralogy and chemistry On-Line-On-Mine-Real-Time
– SOLSA" is funded by European Union's Horizon 2020 program, grant agreement No. 689868) is to create a novel system for identification of drill core characterization using combined XRD, XRD and spectroscopic techniques. One of the main components of this new system are open databases of experimental structural data. It is planned that the COD database, curated by Saulius Gražulis, will provide the collected open access descriptions of crystal structures that will enable rapid identification of sample composition in real time using the X-ray powder diffraction technique. The COD will also collect, preserve and disseminate data that will be determined by the SOLSA project. This data will be useful later for mineralogy, crystallography, geology and palaeontology, to name just a few areas of application. The SOLSA data will be unique since it will, for the first time, record comprehensive geospatial, stratigraphic, crystallographic and spectral information about the sample.

Two new databases based on COD software were launched for storage of SOLSA data in 2017: Raman Open Database (ROD, http://solsa.crystallography.net/rod/, Fig. 1) and Hyperspectral Open Database (HOD, http://solsa.crystallography.net/hod/). In order to homogenize the data and ensure its quality, Antanas Vaitkus and Yassine El Mendili initiated the development of an ontology for Raman spectroscopy in the format of CIF dictionary [1]. The dictionary is supported by International Union of Crystallography (IUCr, http://iucr.org), which will continue its development after the end of SOLSA project.

Analogous CIF dictionary for hyperspectral data is planned as well as software for the validation of data against the compiled CIF dictionaries. Validating software will be employed for the quality control of data, automatically deposited into the open databases. These developments will allow scientists from the whole world contribute to the spectral databases.

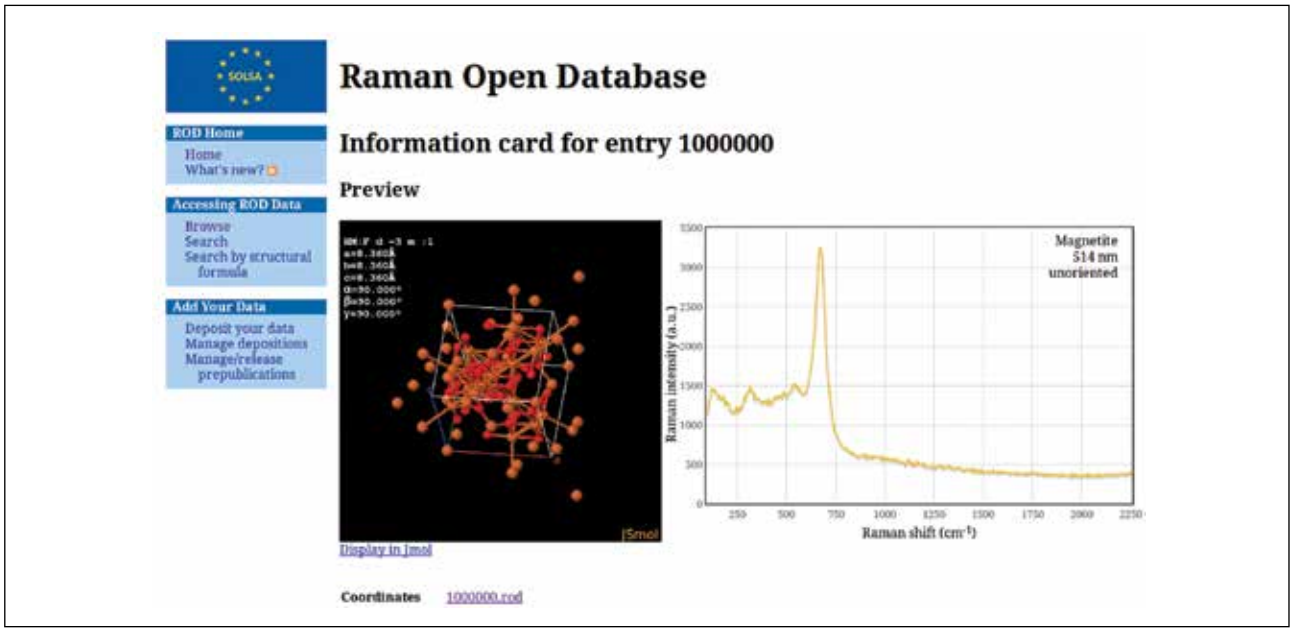

Fig. 1. The preview of a crystal structure and its associated Raman spectra in the ROD website.

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Start-ups

Sekos MB is a small company established in 2015 by scientists of Department of Eukaryotic Gene Engineering, Institute of Biotechnology, Vilnius University, Lithuania. The initial focus of the company is the production of several recombinant viral proteins in yeast by using technology developed at Institute of Biotechnology. In the future, the company will seek to expand its product line by developing new recombinant proteins and synthesizing them in a wider host range. The produced proteins are primarily used in commercial immunodiagnostic tests, but can also be applied in other areas of industry as well as in scientific research.

Another area of company's activity is bioinformatics and general programming. We seek to exploit our expertise in programming, especially in analysis of next generation sequencing data and other biological data by providing services to interested parties. The company is always open to emerging opportunities and collaborations, and can adapt available expertise by entering new areas of activities.

Contact:

info@sekos.lt

UAB "Droplet Genomics" was founded to solve one of the fundamental challenges with biological analytics - heterogeneity. Every cell is different and looking at sample averages is not representative of the underlying processes. Up to now, analyzing single cells was an intensive task in terms of financial resources and time. However, thanks to the pioneering microfluidics research and unique bioengineering approaches, single-cell experimentation is now analogous to other standard biological equipment. We supply a combination of microfluidic hardware, consumables and consulting services, which make it possible to perform single cell experiments for all academic laboratories. We leverage the latest methodology from microfluidics, Next-Gen Sequencing and 3D image analysis to reveal the differences between single cells in terms of their DNA, RNA and Protein composition. DNA is the biological blueprint that defines an organism. Single-cell sequencing allows to look at cell differences in heterogeneous populations. RNA determines which portions of DNA are turned into functional proteins. This makes it possible to simultaneously look at 1000s of genes, which are activated in individual cells. Proteins are the fundamental building blocks of life. Single-cell protein analysis allows to characterize cell populations in 3D tissues and cell suspensions.

UAB "Droplet Genomics" is a start-up company that aims to commercialize various single-cell isolation, sequencing and analysis assays using droplet microfluidics technology. We offer services ranging from data analysis to fully-integrated solutions covering experimental design, equipment, data analysis and everything in-between.

Contact:

+370 693 19333
M. Daukšos g. 4-10, LT-02101, Vilnius, Lithuania
support@dropletgenomics.com

Vilnius University
Institute of Biotechnology
Saulėtekio al. 7
LT-10257 Vilnius, Lithuania
Phone +370 (5) 223 4365
Fax +370 (5) 223 4367
E-mail: office@bti.vu.lt
www.bti.vu.lt