COINS

Conference of Natural and Life sciences.

2014

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CONTENT

ABOUT CONFERENCE

The ninth international conference of life and natural sciences The Coins'14, organized by Vilnius University Students' Representation, gathers both students and scholars to discuss, learn and share their scientific experience in the Faculty of Natural Sciences. The Coins '14 is organized for BA and MA students and doctorates, who are doing their scientific research and want to present it to a larger audience, get constructive criticism and useful advice for defending Your final thesis. Senior year school students interested in the newest research in the fields of natural and life sciences are also invited to the conference. The conference will include presentations of students' research, lectures and visits to Lithuanian scientific centers and companies (Institutes of Biochemistry, Biotechnology, Geology and Geography, Thermo Fisher Scientific).

During scientific presentations it becomes evident what scientific problems are analyzed by young scientists in Lithuania and Europe, and during lectures participants will get acquainted with scientific innovations and perspectives in the fields of biotechnology, genetics, biophysics, biochemistry and ecology.

Well-known and accomplished Lithuanian scientists working and living not only in Lithuania but also abroad will be participating and doing presentations about their research.

The Coins'14 is a scientific student-oriented event based on curiosity, constructive criticism and a wish to improve. It's an open scientific space full of youthful enthusiasm that gathers students interested in natural and life sciences who want to discuss about scientifically relevant topics and socialize.

You can find more information about the conference, lecturers, participants and the whole programme in this publication or online: www.thecoins.eu.

Spread the news and science!

FOREWORDS

Dear Colleagues,

I am very pleased to welcome participants of the 9th International Student's Conference of Life Sciences (COINS) on behalf of Vilnius University and Faculty of Natural Sciences. This is nice opportunity to meet young colleagues from different scientific areas and different countries as well as prominent senior scientists and to exchange ideas, results, experiences.

I wish you interesting, productive conference and good time in old but attractive Vilnius!

Prof. Osvaldas Rukšėnas
Dean Faculty of Natural Sciences
Vilnius University

I am very happy to welcome all attendees of the Coins 2014, the 9th International Conference of Natural and Life Sciences. The conference slogan "Spread the News and Science!" has never been more relevant for today's students. Having transcended their role as listeners in university lecture halls, now they are taking an increasingly active part in research and contribute to scientific developments and advances. Therefore, this conference is a great opportunity for them to share their knowledge and achievements.

I am particularly delighted that this event is being held at Vilnius University which is the Alma Mater for many renowned scientists currently involved in scientific projects both in Lithuania and abroad in the leading science centers.

Once more, I would like to welcome everyone to The Coins 2014 conference and I hope that it will inspire you for new discoveries both during the conference and beyond.

Algimantas Markauskas
Vice President/General Manager
Thermo Fisher Scientific Baltics

Dear community of scientists,

I am deeply honored to have taken part in the organization and realization of ninth international conference of life and natural sciences The Coins '14. A few moths a go I was anxious and afraid of how everything is going to happen, how will we gather the scientists, whether students will come to the conference and even whether it's important for them..

But, today I cannot believe my eyes, but I'm saying what I see. Well-known scientists from abroad, accomplished Lithuanians who've made their career abroad and in Lithuania have come here to present their scientific research.

Natural and life sciences are one of the most up-and-coming and rapidly developing fields in the whole world. And You, students are brave and ambitious! It's admirable that even in your student years you are participating in such a conference because you're actively working in science. You are our future, you will come with new ideas and you will be able to do much amazing work, create something incredible. You are the potential scientists. I'm sure, that among you there are future well-known scientists, who will win many awards, maybe you'll even be laureates of the Nobel or other prizes. You will be invited to conferences of this kind in the whole world! It is not only I and Lithuania, that is proud of you, but the whole world of science is too.

This week, let's be together, share experience and ideas, socialize and get acquainted with colleagues from all over the world.I wish for you to enjoy this conference and take all you can from it and even more.

I am tremendously proud of you!
The Coins 2014 coordinator
Ieva Vėbraitė

PROGRAMME

MARCH 4

08:30-09:00	Registration
09:00-10:00	Opening Ceremony Speakers: Ambassadors
10:00-11:00	Plenary by **Jonas Cicėnas** NEXTPROT: A KNOWLEDGE PLATFORM FOR HUMAN PROTEINS
11:00-12:00	Plenary by specialists from LITHUANIAN HYDROMETEOROLOGICAL SERVICE UNDER THE MINISTRY OF ENVIRONMENT
12:00-12:30	Coffee break
12:30-13:30	Plenary by **Urtė Neniškytė** GARDENING THE BRAIN: SYNAPTIC PRUNING BY MICROGLIA CELLS
13:30-14:30	Plenary by **Ieva Sliesoraitytė** VISION RESTORATION POTENTIAL IN BLIND HUMANS
14:30-15:30	Lunch

15:30-16:30	Plenary by **Konstantin Kotliar** : NON-INVASIVE IN-VIVO ASSESSMENT OF ARTERIAL STIFFNESS IN THE CENTRAL MICROCIRCULATION
16:30-17:30	Plenary by **Valdas Laurinavičius** BIOTERRORISM: SITUATION TODAY AND TRENDS FOR TOMORROW
17:30-20:00	Poke'r'Brain

MARCH 5

08:30-18:00	Company Visits
08:30-09:00	Registration
09:00-10:30	Company/Institute nr. 1
10:30-12:00	Institute of Biochemistry
12:00-13:30	Institute of Geology & Geography
13:30-15:00	Institute of Biotechnology
15:00-16:30	Thermo Fisher Scientific

16:30-18:00	Company/Institute nr. 6
20:00-22:00	Social Programme Vilnius Night Tour

MARCH 6

08:30-09:00	Registration
09:00-09:30	Session Opening: Ambassadors
09:30-10:00	Plenary by Daumantas Matulis CARBONIC ANHYDRASES AS ANTICANCER DRUG TARGETS
10:00-18:00	Oral Presentations Session
10:00-10:30	**Aksana Kirsanava** (Belarus) Structural Diversity of RNA Transcripts of Fusion Oncogene RUNX1/RUNX1T1
10:30-11:00	**Karolis Leonavičius** (United Kingdom) New Techniques to Investigate Mechanics of Mammalian Development
11:00-11:30	Coffee break

11:30-12:30	**Laurynas Šiliauskas** (Lithuania) Paleoproterozoic Volcanic Massive Sulphides (VMS) in the Lithuanian Crystalline Basement: Evidences for a Back-arc Tectonic Setting
13:00-13:30	**Dalia Kitavičiūtė** (Lithuania) The Application of Probalistic Uncertainty Analysis for Modelling of Tornado
13:30-14:00	**Ilona Šeputytė** (Lithuania) Probabilistic Assessment of Extreme Temperatures and Relative Humidity
14:00-15:00	Lunch
15:00-15:30	**Laura Gedminienė** (Lithuania) Palynology as a Bridge between Ecosystems and Paleoecosystems. Late Glacial Sedimentary Environment of the Ula River Basin: an Example from Ula 2 Outcrop
15:30-16:00	**Aleksejs Fjodorovs** (Latvia) Importance of Trehalose in the Desiccation Stress Resistance of Baker's Yeast S. cerevisiae
16:00-16:30	**Artis Linars** (Latvia) In vitro Evaluation of Alphaviral RNA Delivery to Different Cancer Cell Cultures.

16:30-17:00	**Agnese Ezerta** (Latvia) Differentiation Potential and Surface Marker Expression in Adipose-Derived Stem Cells at Different Passages
17:30-20:00	Excursion in Faculty of Natural Sciences

MARCH 7

09:30-10:00	Registration
10:00-11:00	Plenary by **Giedrius Gasiūnas** Cas9 - a Programmable RNA - guided DNA Endonuclease from the Bacterial Adaptive Immune System
11:00-11:30	**Robertas Galinis** (Lithuania) Microfluidics for Protein Evolution: Preparatory Issues
11:30-12:00	**Gediminas Drabavičius** (Lithuania) On Construdtion of Dimeric Cytokines, Penguins and the Origin of Life
12:00-12:30	Coffee break
12:30-14:30	Poster session
14:30-15:30	Lunch

16:00-17:00	Final Conference Plenary by **Juozas Vidmantis Vaitkus** New and Re-newed Physical Methods in Biomedicine
17:00-18:00	**Awards**
20:00-23:00	**Closing Event**

AMBASSADORS

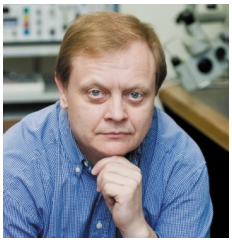
Professor dr. Benediktas Juodka – lithuanian biochemist, Member of Seimas of Republic of Lithuania, Chair of Committee on Foreign Affairs. Former rector of Vilnius University, president of Lithuanian Academy of Science and winner of Lithuanian Science Prize.

Algimantas Markauskas – "Thermo Fisher Scientific Baltics" general manager, vice-president for Baltic region. Alumni of Vilnius University.

Rolandas Maskoliūnas – doctor of biology, science popularization shows presenter, Lithuanian National Radio and Television TV Programming Director.

Inga Kalpakovaitė – Vilnius University Students' Representation president, 4th year student of molecular biology.

Prof. dr. Osvaldas Rukšėnas



A Lithuanian neurobiologist, biophysicist, the dean of the Faculty of Natural Science in Vilnius University, the head of the Department of Neurobiology and Biophysics, professor, doctorate of biomedicine.

He is one of the originators and developers of neuroscience in Lithuania. The first study programmes of neurobiology in Lithuania, which produced a large group of specialists, going hand in hand with global neuroscience development, were organized with the initiative of Professor (pavardė). Winner of Lithuanian Science Prize.

SPEAKERS

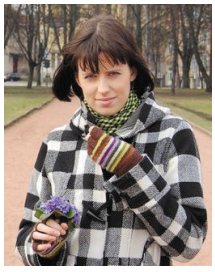
neXtProt: a knowledge platform for human proteins

Jonas Cicėnas

In the last several decades, enormous resources have been organized to understand the molecular components and processes of human and animal cells, both for basic and research uses. Though this work has been first directed toward the sequencing of the genome, it has now moves more toward the studies of the major players in cells, proteins. The molecular and functional complexity of proteins is perplexing and

requires bioinformatics means specifically directed at capturing, incorporating and maintaining the existing knowledge. Data are much easier to produce than knowledge. Considerable amount of undiscovered knowledge is concealed in large sets of diverse and not always reliable data disseminated across a multitude of publications, web sites and other resources. Thus, the best tools to gather, systemize and store data and convert it to the knowledge, are biological databases. neXtProt (http://www.nextprot.org/) is a new database and a knowledge platform, dedicated to human. Developed at the Swiss Institute of Bioinformatics, it aims to help researchers answer questions related to human proteins. To achieve this goal, neXtProt is built covers both curated knowledge mainly transferred from the UniProtKB/Swiss-Prot knowledgebase and carefully selected and filtered high-throughput data relevant to human proteins. This talk presents an overview of the database and the data integration process.

Lithuanian Hydrometeorological Service LHMS



Ms Inga Stankūnaitė – Senior Specialist of Weather Forecasting Division. Meteorological radar and severe weather.

Ms Janina Brastovickytė – Hydrologist of Hydrology Division. The change of hydrological regime in Lithuanian rivers.

Mr Justinas Kilpys – Specialist of Climatology Division. Snow cover detection using meteorological satellites.

Lithuanian Hydrometeorological Service under the Ministry of Environment of the Republic of Lithuania (LHMS) is a state budgetary institution responsible for meteorological (including agrometeorological, aeronautical and marine) and hydrological observations and forecasts. LHMS provides hydrometeorological information for Lithuanian institutions, enterprises and organizations, observes rules and regulations of the World Meteorological Organization (WMO), participates in international projects and programmes, carries out scientific research, compiles and publishes reference books, annals, and surveys.

LHMS represents Lithuania in WMO, EUMETSAT and ECMWF (European Centre for Medium-range Weather Forecasts) and participates in various international projects and programmes like EUMETNET, HIRLAM, CLIPS, NAVTEX safe navigation programme and many others.

Lithuanian hydrometeorological observations network, which covers all Lithuanian territory, is composed of 16 meteorological stations with observers and 9 automated meteorological stations, 43 automated agrometeorological stations, 93 water-gauging stations – most of them automated, and 3 aeronautical meteorological stations. Meteorological stations measure main meteorological parameters: air temperature, soil temperature, atmospheric pressure, precipitation, humidity, wind speed and direction, snow cover, cloudiness, solar radiation, visibility, observe weather phenomena. While water-gauging stations carry out hydrological measurements, many of them are also measuring some meteorological parameters like precipitation and air temperature.

Gardening the Brain: Synaptic Pruning by Microglia Cells

Urte Neniskyte

Mouse Biology Unit, European Molecular Biology Laboratory, Italy

Brain immune cells called microglia are known to play an important part in the removal of dead neurons, their debris or unwanted cellular material in injured or diseased brain. In contrast, their role in healthy brain remained concealed for many years. Recent evidence suggests that these motile cells continuously monitoring brain environment are in fact required for postnatal brain development as well as adult plasticity. The development of a complex nervous system is accompanied by a generation of superfluous neuronal connections that are removed when neural circuits mature. Permanent elimination of immature synapses is an activity-dependent process that leads to selective maintenance and strengthening of a subset of synapses. The maturation of synaptic circuits is tightly related to the presence of microglia cells that actively contact and engulf unnecessary synaptic terminals. Aberrant or impaired microglial function leads to abnormal synaptic densities and dysfunctional connectivity that causes morphological, functional and behavioral deficits. The elimination of immature synapses is dependent on microglial phagocytic pathways, implicating microglial phagocytosis as a mechanism of synaptic pruning. A couple of pathways have been suggested to mediate the removal of synapses, including immune complement cascade proteins, apoptosis-related secreted opsonins and corresponding microglial receptors. The question remains which neuronal surface signals label weak synapses for elimination or mark strong synapses for strengthening thus ensuring proper brain development and maintenance.

Vision restoration potential in blind humans

Dr. Ieva Sliesoraityte, MD Ph.D. upon the completion of her studies in electronical engineering and medicine, qualifying as a certified ophthalmologist, defended her PhD thesis in technical sciences. Since 2009, she has been working as a senior scientist in the Institute for Ophthalmic Research, supervised doctoral students and has been teaching at School of Neural & Behavioural Sciences, International Max Planck Research School in Tuebingen, Germany. Dr. Sliesoraityte serves as a reviewer for multiple scientific journals, such as Molecular Vision, British Journal of Ophthalmology, IEEE PECON, IEEE ISIEA and others. Dr. Sliesoraityte recieved many distinguished international scientific prizes and has been awarded by the Lithuanian Academy of Sciences twice. In 2010, she was an invitee to the Lindau Nobel Laureate Meeting. In 2012, she was awarded by a European Union grant for a Eur-USH project based at the Institute of Vision in Paris, France. A key focus of her research is rare diseases, with the ambitious goal of establishing the largest Europe-wide database and to assess cures for blindness. It is expected that biosensors will be extremely successful and will dominate the market for elderly population. Biosensors are anticipated to find applications in diverse areas, not only in biological structures, but also in various industrial fields.

Non-invasive in-vivo assessment of arterial stiffness in the central microcirculation

Prof. Konstantin Kotliar, Ph.D., Dr.Sc.

Currently from 08/2012 Professor for Mathematical Modelling in Biomedical Engineering at Aachen University of Ap-

plied Sciences, Germany. Till 07/2012 senior research associate and group leader at Institute of Micro Technology and Medical Device Technology (MIMED), Munich University of Technology, Garching, Germany. 1/2010 – 12/2011: Senior research assistant, group leader "Retinal vessel analysis" at Department of Nephrology, Munich University of Technology, Munich, Germany. 11/1999 – 10/2010: Research assistant and later on from 2005 senior research assistant, group leader "Retinal vessel analysis" at Department of Ophthalmology, Munich University of Technology Munich, Germany. 1/1996 – 06/1998: research assistant, lecturer at St.Petersburg University of Technology, St.Petersburg, Russia.

Research interests include biomedical engineering in ophthalmology including biomedical imaging, retinal vessel analysis, retinal microcirculation and ocular biomechanics, particularly: dynamic retinal vascular research, biomechanics of intraocular pressure regulation, aqueous flow and accommodation.

Non-invasive in-vivo assessment of arterial stiffness in the central microcirculation

Blood vessels become stiffer because of the combined effects of aging, high blood pressure, and other factors. These alterations occur primarily in small vessels. Methods to investigate microvascular stiffness are scarce and require at present invasive generation of vascular material.

A novel clinical methodology is proposed, which characterizes in-vivo arterial stiffness in the central microcirculation. Pulse wave propagation in retinal arteries is assessed and a parameter "retinal pulse-wave velocity" (rPWV) is measured non-invasively using an optical approach.

Bioterrorism: situation today and trends for tomorrow

Laurinavicius Valdas. Master of Science in organic chemistry from Chemistry faculty of Vilnius University, 1972. Professor in biochemistry, 1996. Member of Lithuanian Academy of Science. Head of the department of Bioanalysis, Director of the Institute of Biochemistry. Main field if interests- biochemistry, bioengineering, biosensors, analytical chemistry, bioelectrochemistry. Mathematical modeling of the heterogeneous bioelectrochemical systems.

Three main topics will be discussed. Review of the main technologies associated with bioterrorism. Review of main challenges we can expect during the next 5-10 years. What measures community should apply trying to keep under control the development of new technologies in life sciences? Responsibility of the Government and scientific community will be discussed as well.

Carbonic anhydrases as anticancer drug targets

Chief Scientist and Head **Daumantas Matulis**, PhD

Carbonic anhydrases (CAs), a group of zinc containing enzymes, are involved in numerous physiological and pathological processes, including gluconeogenesis, lipogenesis, ureagenesis, tumorigenicity and the growth and virulence of various pathogens. In addition to the established role of CA inhibitors as diuretics and antiglaucoma drugs, it has recently emerged that CA inhibitors could have potential as novel anti-obesity, anticancer, and anti-infective drugs (Supuran, 2008, 2012). CAs catalyze the conversion of CO_2 to the bicarbonate ion and protons. There are 12 catalytically active CA isoenzymes in humans. A number of CA inhibitors, mostly unsubstituted sulfon-

amides, have already been designed.

However, most present inhibitors are insufficiently selective for targeting CA isozymes, such as hCAIX and hCAXII, which are anticancer targets. Here at the DBDD (Department of Biothermodynamics and Drug Design) we have cloned and purified most cytoplasmic CAs and catalytic domains of transmembrane CAs. The organic synthesis team, together with collaborators, designed and synthesized over 500 novel compounds that bind CAs with submicromolar to subnanomolar affinity. Several novel series of CA inhibitors exhibited extremely tight affinity and an appreciable selectivity towards selected CA isozymes.

Lithuanian energy institute research activities

Darius Laurinavicius has completed the following studies: applied physics bachelor, thermal engineering master (Kaunas University of Technology), energetic and thermal-engineering PhD studies (Lithuanian Energy Institute). D. Laurinavičius holds the position of a Junior Research Associate in the Laboratory of Nuclear Installation Safety of Lithuanian Energy Institute. The main fields of interest are nuclear, thermal-engineering, and thermal-hydromechanics. D. Laurinavičius is a member of the Nuclear Energy Association, Lithuanian Society of Young Researchers, Lithuanian Energy Institute Union of Young Scientists, Students' Corporation TauTiTo, Alumni Association of Kaunas University of Technology.

Lithuanian energy institute perform research and develop innovative technologies in the fields of energy engineering, thermal engineering, measurement engineering, material science and economics, in conducting fundamental and applied research, participating in studies' processes, applying the results of applied scientific research results to industry and business, as well as pro-

viding consultations to state, public and private institutions/enterprises on the issues related to Lithuanian sustainable energy development. The main scientific research activities of the institute are:

1. Research in the fields of thermal physics, fluid and gas dynamics, and metrology;
2. Materials, processes and technologies research for the management of renewable energy sources, hydrogen energy, effective use of energy resources and reduction of environmental pollution;
3. Safety and reliability of nuclear, thermonuclear energy and other industrial objects;
4. Radioactive waste management and decommissioning of the Ignalina Nuclear Power Plant;
5. Modeling and control of energy systems; energy economy.

Lithuanian energy institute is membership and corporate with 18 national and internationals organizations.

Lithuanian energy Institute, Kaunas University of Technology and Lithuanian University of Health Sciences implements the projects of Integrated science, studies and business center (valley) Santaka.

About LEI:

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Cas9 – a programmable RNA-guided DNA endonuclease from the bacterial adaptive immune system

Giedrius Gasiunas, Tautvydas Karvelis, Virginijus Siksnys

Institute of Biotechnology, Vilnius University Vilnius, Lithuania;

Clustered regularly interspaced short palindromic repeats (CRISPR) together with CRISPR associated (cas) genes form an adaptive prokaryotic immune system which provides acquired resistance against viruses and plasmids. CRISPR consists of arrays of short conserved repeat sequences interspaced by unique DNA sequences called spacers. The CRISPR-Cas system functions by acquiring short pieces of foreign DNA as new spacers and subsequently uses them as templates to generate specific small RNA molecules (crRNA) which combined with Cas proteins into effector complexes that trigger degradation of foreign nucleic acid. In Type I and Type III CRISPR systems, nucleoprotein complexes involved in crRNA-mediated silencing of foreign nucleic acids are comprised of large multisubunit aggregates. We show that in Type II systems, the silencing complex consists of a single Cas9 protein [1], which binds to crRNA:tracrRNA duplex to mediate sequence-specific cleavage of invasive dsDNA.

We isolated the Cas9-crRNA-tracrRNA complex of the S. thermophilus CRISPR3-Cas system and demonstrated that it generates

in vitro a double strand break at specific sites in target DNA molecules that are complementary to crRNA sequences and bear a DNA motif. We show that DNA cleavage is executed by two distinct active sites (RuvC and HNH) within Cas9, to generate site-specific nicks on opposite DNA strands. Sequence specificity of the Cas9-crRNA-tracrRNA complex is dictated by the 42 nt crRNA. All together our data demonstrate that the Cas9-crRNA complex functions as an RNA-guided DNA endonuclease [2]. The simple modular organization of the Cas9-crRNA complex, where specificity for DNA targets is dictated by a small crRNA and the cleavage machinery consists of a single, multidomain Cas protein, provides a versatile platform for the engineering of programmable RNA-guided DNA endonucleases. These findings pave the way for the development of novel molecular tools for RNA-directed DNA surgery [3].

[1] Sapranauskas R, Gasiunas G, Fremoux C at al. (2011)The Streptococcus thermophilus CRISPR/Cas system provides immunity in Escherichia coli. Nucleic Acids Res 39, 9275–82.
[2] Gasiunas G, Barrangou R, Horvath P et al. (2012) Cas9-crRNA ribonucleoprotein complex mediates specific DNA cleavage for adaptive immunity in bacteria. Proc Natl Acad Sci USA 109, E2579–2586.
[3] Horvath P, Gasiunas G, Siksnys et al. (2013) Applications of the Versatile CRISPR-Cas Systems. In CRISPR-Cas Systems (Barrangou R & van der Oost J, eds), Springer-Verlag, Berlin Heidelberg.

New and re-newed physical methods in biomedicine

Juozas Vidmantis Vaitkus, Prof.-emeritus & Chief researcher at Vilnius University, Member of Lithuanian Acad. Sci.

Studies: VU, Diploma in Physics 1963, PhD 1967 (a part of research performed in laboratory of Nobel laureate N.Basov's laboratory (1965) in Moscow.
Work carrier all at Vilnius University: starting from laboratory assistant via Head of Dept. & Prof., Director of Institute of Applied Research, Prorector for research positions, now Prof. & Chief Researcher. Since 1985 – members of Lithuanian Acad. Sci. Since 1996 collaborates in CERN programs (at the beginning as visiting Prof. of Glasgow U, since 2002 as VU Prof.).

Scientific achievements: Main activities in semiconductor physics, optoelectronics, laser application for materials research: designed: a first laser in Lithuanian (1966) and since this time lasers are used for materials research and technology; the „eyes" for computer (a gold medal at USSR national exhibition, 1968); new methods for material research gave a National Award (1981): the "eyes" for satellite - the USSR State Award (1988). The results of investigation of semiconductors, including nanostructures, were recognized by Lithuanian National Science Awards (1996, 2005).

Activity in a field of this event: now there are attempts to design the equipment for the diagnostics of cancer and pre-cancer , the development of detectors for ionizing radiation imaging. (Earlier the devices for registration of cardiogram of fetus during delivery, the human body parts temperature imaging, a method for the X-rays imaging of blood vessel hidden under the bone were proposed). Now – General Secretary of International Society of Laser Surgery and Medicine, the invited lectures were delivered

and the Congresses in Japan, Taiwan, Italy.

This lecture presents the examples how physicists and biomedical researchers have created or now are on a way to design different methods for investigation and treatment of different pathologies of different tissues.

The methods involving different radiations and different particles, including antiparticles, are discussed. The examples of techniques are presented.

It is shown a few cases what is done or can be done if the new structures – nanotechnology or sub-micron technology products are or would be used.

ORAL PRESENTATIONS

Structural diversity of RNA transcripts of fusion oncogene RUNX1/RUNX1T1

Aksana D. Kirsanava, Alexandr A. Migas, Vasily V. Grinev

Translocation t(8;21)(q22;q22) which leads to availability of RUNX1/RUNX1T1 fusion gene is a common cytogenetic marker in the cases of acute myeloid leukemia. Together with full-length transcript an unprecedented level of alternative transcripts was detected. Such forms can generate truncated proteins and thus significantly influence disease development and progression. Therefore, the aim of the study was to evaluate the variability of transcripts with termination in alternative exons 12a, 15a and 17a, as well as uncover patterns of combinations of their structural elements. Total RNA was extracted from Kasaumi-1 cells and peripheral blood of 3 acute myeloid leukemia patients. Further cDNAs were prepared using Oligo-dT and SuperScript III reverse Transcriptase and heterogeneous PCR products were obtained using forward RUNX1 specific primers and reverse RUNX1T1 primers

to the target exons. Amplicons were either sequenced from gel-purified products or the PCR reaction mix was cloned in pTZ57R/T vector. Ligation mixture was used directly for Escherichia coli XL-I Blue transformation. Then the plasmid DNA was extracted and sequenced. Total 154 transcripts were analyzed, 114 were full-length and 40 expressed sequence tags, some of them contain unique elements combination, which indicates great heterogeneity signalizes that real heterogenity of alternative transcripts is greater. In analyzed transcripts 98 unique exons, which form 21 group of overlapping exons were identified. Using Cytoscape v.2.8.3 the exons' network we tried to find out combinatorial patterns of exons. We have shown that exons differ by the number of pairwise combinations with other exons (from 1 to 37). The greatest variety of overlapping exons was found in recombination area. These exons are found only in fusion oncogene. Bioinformatic analysis using the ASSP indicated a large number of alternative splicing sites, the activation of which apparently occurs only in the presence of translocation. Although alternate forms of RUNX1/RUNX1T1 transcripts have been described our work significantly extends these results in terms of the variety of these forms. Moreover, combinatorial patterns of exons were analyzed.

New techniques to investigate mechanics of mammalian development

Karolis Leonavičius, Dr. Shankar Srinivas

Development is a time of rapid change, when genetic information is transformed into tissues of complex shapes and functions. Such changes usually create mechanical stresses, which need to be controlled for robust reproduction and feedback mechanisms must exist, to couple mechanical stimuli to genetic signalling. Such signalling pathways have been implied to be important in controlling stem cell behaviour and are collectively known as mechanotrans-

duction. My research is focused on developing tissue engineering techniques to alter cell shapes and stresses in order to investigate how mechanics affect development, especially in the context of Hippo signalling. As this pathway has been shown to not only control organ size, but also respond to cell shape changes, I use YAP/TAZ proteins to monitor mechanotransduction in developing mouse embryonic stem cells and preimplantation embryos.

Paleoproterozoic Volcanic Massive Sulphides (VMS) in the Lithuanian crystalline basement: evidences for a back-arc tectonic setting

(1)Laurynas Šiliauskas and (2) Gražina Skridlaitė

(1) Faculty of Natural sciences, Vilnius University, Lithuania (juniorlaurynas@gmail.com), (2) Institute of Geology and Geography, Nature Research Center, Vilnius, Lithuania (skridlaite@geo.lt)

In the southwestern part of East European Craton several Paleoproterozoic volcanic arcs were recognized. These include TTG type 1.89 Ga and 1.86-1.84 Ga suites. Volcanic rocks have been metamorphosed under amphibole to granulite conditions. In the southern and middle Lithuania, sedimentary – volcanic suite were recognized. The later was affected by green schist to amphibolite metamorphism.

The upper part of Lazdijai_13 drill core, from southern Lithuania, at 493 m depth, consists of exhalative sediments, mixed with andesitic volcanic rocks. These comprise cherts, impregnated by magnetite, which forms replacements around calcite. Most of magnetite is overgrown by kovelite dendrites. It is thought that kovelite was formed while magnetite was still in the aqueous surrounding. Other accessory phases include apatite, xenotime, zircon and Sr-Ba sulfate. Cherts are underlain by a metaandezites

which volcanic structures were obscured by hydrothermal alteration, i.e. porphyritic plagioclase was replaced by clay minerals and quartz, or muscovite in many places. Thin mudstone layers were metamorphosed into garnet+chlorite±biotite rocks. These rocks were affected by silicification, chloritization, argilitization and carbonatization. The composition of the rocks, macro and micro alterations and absence of breccia, resembles the outer part of VMS stockwork. The lower boundary (at 526 m) is sharp, marked by quartz veins, bellow which lies sandstone comprised of quartz, biotite (±chlorite) and minor tremolite. It was intensely affected by silicification, and enriched in Na, K and Ca. The accessory phases include monazite, xenotime, apatite and detrital zircon. Sandstone forms fine grained schist, which exhibits fine mineral foliation. At 654 m depth, a 4 m thick granitic vein crosscuts sediments, bellow which lies ampibolites with layers or lenses of marble and skarns. Some amphibolites resembles porphyritic basalts. These might be basaltic dikes, which are common in back arc VMS surroundings. The volcano-clastic rocks from Lazdijai_13 core yielded 1.83-1.80 Ga age. The whole rock Sm-Nd isotopic composition points towards juvenile origin of the rock (TDM=2.08 Ga, εNd(1.9) +1.8).

After comparing obtained data with VMS deposits from different environments, it is most likely, that Lazdijai_13 volcano – clastic sequence was formed in back arc tectonic setting. Volcano – clastic sequence can be correlated with the 1.83 Ga Oskarshamn-Jönköpling belt and the volcano-sedimentary Vetlanda formation in southeast Sweden. The 1.83-1.80 Ga volcanic arc and back arc system continues from southeastern Sweden through the Baltic Sea to Lithuania.

The application of probabilistic uncertainty analysis for modelling of tornado

Dalia Kitavičiūtė

Extreme Tornado events in Lithuania are not common, but can have serious and damaging effects on humans' society and infrastructure, as well as on ecosystems and wildlife. Furthermore tornado is the local extreme meteorological event, which can cause huge losses and significant destruction in a particular territory. Also, tornado could pose a risk for energy objects, such as nuclear power plant, hydro power plant or wind power plant. Due to the following reason estimating the probabilities of extreme weather events is crucially important for avoiding weather-related disasters. However, in general, mathematical models can not completely or without any error reflect the reality; therefore it is important to perform the uncertainty and sensitivity analysis of the model results. The main aim of the research work is to analyze the characteristics of extreme tornado probabilistic assessment methods, tornado hazard probability models, identify uncertainties of model inputs noting their distribution and variation range. Also, apply uncertainty and sensitivity analysis to the model. For this purpose SIMLAB and GLUE software packages were used. In this research the statistical data regarding the Lithuania tornadoes documented cases during the period of 1961 – 2011 was analyzed. Using this data a map of tornadoes events in Lithuania was composed. In addition, a methodology of probabilistic models application for tornado impact assessment in a local region was developed and used in the analysis. Then, the methodology of uncertainty and sensitivity analysis was applied for results of this model. Applying uncertainty analysis the tolerance limits for tornado model results were given. Using SIMLAB and GLUE software packages the tolerance interval for probability estimate that tornado wind speed equal or higher 70m/s is: (3.78E-09; 2.68E-

06). Sensitivity analysis was also used in order to determine the parameters that have the greatest influence on the model results. This analysis enabled to determinate that the uncertainty of data regarding the tornado damage area has the greatest influence on tornado hazard probability assessment estimate.

Probabilistic assessment of extreme temperatures and relative humidity

Ilona Šeputytė, Robertas Alzbutas

Extreme temperatures are fairly common natural phenomenon in Lithuania. They have mainly negative effects both on the environment and humans. Thus there are important to perform probabilistic and statistical analyzes of possibly extreme temperature values and their time-dependant changes. This is especially important in areas where objects (sensitive to the extreme temperatures) are foreseen to be constructed. In order to estimate the frequencies and consequences of possible extreme temperatures, the probabilistic analysis of the event occurrence has been performed: statistical data have been collected and analyzed. The main objective of performed work was the probabilistic assessment of extreme temperature and relative humidity occurring in whole Lithuania and specifically Dūkštas region. In addition, the other purpose of this work was to analyze the changes of extreme temperatures. The probabilistic analysis of extreme temperatures in Lithuanian territory was based on the historical data from 1961 up to 2012. The probabilistic assessment was focused on the application and comparison of Gumbel, Weibull and Generalized Value (GEV) distributions, enabling to select a distribution, which has the best fit for data of extreme temperatures.

Importance of trehalose in the desiccation stress resistance of baker's yeast S. cerevisiae

A. Fjodorovs, A.Kokina

Since in the literature there are contradictory assessments of trehalose role in the desiccation stre ss tolerance, in this work the role of trehalose was studied by using bakers yeast grown in various carbon sources and different physiological conditions of cell growth. To ensure variation of accumulated trehalose amount, several strains with switched off genes for trehalose synthesis were used, as well as adenine auxotroph strain with increased trehalose accumulation abilities . Methods used in this work helped to determine the amount of trehalose in the yeast cells, the amount of surviving cell water dess iccation , as well as to create primers with which it is possible to create one's own TPS1 deletion and TPS2 deletion strains. It was found that the role of trehalose in the cell survival is greater in the medium with glucose than in the medium with galacto se or glycerol.

In vitro evaluation of alphaviral RNA delivery to different cancer cell cultures

A. Linars, J. Vasilevska, T. Kozlovska, A. Zajakina

Semliki Forest virus (SFV) is a positive-strand RNA-enveloped virus belonging to the Alphavirus genus. In recent years, high potential of recombinant SFV based vector systems has been shown in a lot of preclinical oncology trials, demonstrating high expression level and strong cytophatic effect of cancer cells trough the induction of p53-independent apoptosis. Despite the promising results, the infectivity of recombinant SFV particles varies depending on cell type, and manipulation with alphaviral vector targeting is complicated, since the mechanism of alphavirus infection is not fully understood. Therefore, it is important to develop new strategies

for specific alphavirus RNA delivery to cancer cells using non-viral vectors. In this study we have tested four methods for SFV/Ds-Red RNA delivery in vitro, such as standard alphavirus infection, electroporation with naked RNA, transfection with RNA packaged into liposomes and magnetofection, using positively-loaded nanoparticles with absorbed RNA. The efficacy of RNA transport, gene expression and cytopathic effect was evaluated in four cell cultures: baby hamster kidney cells (BHK-21), mouse metastatic melanoma (B16-F10), mouse mammary carcinoma (4T1) and mouse hepatoma (GM-22A). To determine the toxicity of the delivery method itself, transduction of cells without viral RNA also was tested. Our results showed significant differences in alphaviral RNA transfection efficiency and cytopathic effect in tested cell cultures. Infection with recombinant SFV particles provided the highest cell transduction in majority of cell lines (BHK-21, 4T1 and GM-22A). In contrast, high number of transgene expressing B16-F10 cells was detected using only lipofection or electroporation RNA delivery methods, demonstrating inability of recombinant SFV particles to entry these cells. Optimization of viral genome delivery methods is important for development of novel cancer therapy strategies.

Differentiation potential and surface marker expression in adipose-derived stem cells at different passages

A. Ezerta, A. Bogdanova, U. Berzins, S. Nikulshin, T. Kozlovska, and A. Zajakina

Nowadays the potential use of mesenchymal stem cells (MSC) in medicine for treatment of different diseases is growing rapidly. Recently MSC were implicated as potential agents in many cellular therapies, regenerative medicine and gene therapy. However, using MSC in cellular therapies requires a high quality stem

cell preparation, expansion and characterization. In purpose of MSC application in medicine for therapeutic aims, they should express certain cell markers and differentiate into adipocytes, osteocytes and chondrocytes in vitro. The aim of this study was to examine adult human adipose-derived mesenchymal stem cells (ADSC) surface marker expression from passage 2 (P2) to P8 and differentiation potential from P3 to P6. Using RT-PCR and q-RT-PCR, the obtained results showed that ADSC expressed all characteristic positive marker genes as CD29, CD44, CD73, CD90 and CD105, but negative marker genes as CD14, CD19, CD34, CD45 and HLA-DR had low level of expression. The expression level of positive marker genes CD29, CD44 and CD73 increases until P5 or P6 and diminishes afterwards but in case of CD90 and CD105 the increase of the expression level was not observed. Expression of ADSC surface markers in different passages was examined using flow cytometry analysis. Also, at the protein level, positive markers of MSC such as CD29, CD44, CD73, CD90 and CD105 were expressed, but MSC negative markers CD14, CD19, CD34, CD45 and HLA-DR were not detected. ADSC from P3 to P6 can efficiently differentiate into adipocytes and chondrocytes, however their osteogenic differentiation potential is limited. In conclusion, the most optimal passages in medicine for treatment of different diseases are from P3 to P6.

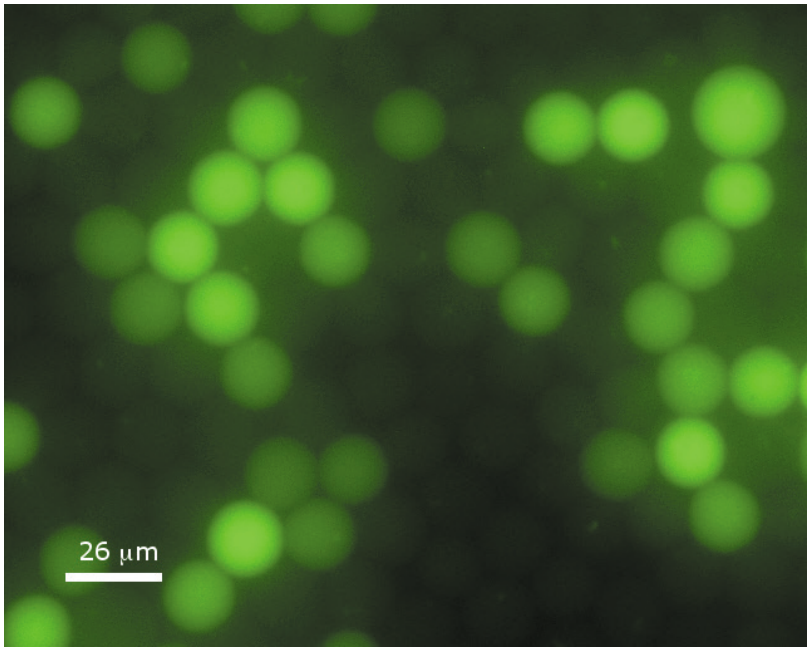
Microfluidics for protein evolution: preparatory issues

Robertas Galinis

Over the last decade, using proteins for different applications in biomedicine, biotechnology or pharmaceutical industry has experienced a strong growth. However, because the conditions at which industrial enzymes must operate are often incompatible with the delicate catalytic properties of enzymes. In such cases various protein engineering techniques serve for improving the

catalytic and biochemical properties of enzymes. In our project, we are combining the latest advances in droplet-based microfluidics technology with directed evolution to optimize computationally designed enzymes. Microfluidic droplets, resembling the population of artificial cells, are used to perform in vitro protein expression under strictly controlled conditions and screen for improved enzyme variants at hight-throughput rates. We use naive protein scaffolds designed in silico as a starting point to evolve enzymes from the mild catalysts into efficient ones.

Protein expression in droplets is performed with in vitro transcription translation system using single DNA molecule as a template. However, low template amount often results in low protein yields, therefore we are pre amplifying single DNA molecule in droplets using phi29 DNA polymerase. This polymerase is well known for its processivity and high DNA yields and thus is widely used in different whole genome amplification strategies. My first experimental data shows promising results indicating the formation of DNA microstructures visible with optical microscope.



Palynology as a bridge between ecosystems and paleoecosystems. Late glacial sedimentary environment of the ūla river basin: an example from ūla 2 outcrop

Laura Gedminienė1, Miglė Stančikaitė2, Petras Šinkūnas1, Eugenija Rudnickaitė1, Giedrė Vaikutienė1

The main aim of the conducted investigations was to analyze environmental changes of the Lateglacial in south-eastern Lithuania. Multidisciplinary investigations (pollen, diatom, LOI, isotope 14C (AMS)) were applied for organic enriched deposits, covered with the aeolian sand, in the outcrop located on the left bank of Ūla River, the left tributary of Merkys River, 250 m downstream from Zervynos train station (54006`34,1``N, 24028`44,4``E). Collected data, including the results of radiocarbon dating from Poznan Radiocarbon Laboratory, indicate sedimentation started at the beginning of Lateglacial Interstadial, the warmest period of the investigated interval. Deposition of the gyttja started at about 15200-14650 cal. yr BP, or during GI-1e stage (Lowe et al., 2008), and sedimentation was interrupted at 13630-13300 cal. yr BP, the GI-1b stage. Pollen data shows Betula predominating forests with the grasses underbrush beneath it existed during the GI-1e climatic event, when amount of organic matter in the sediments increased. Later an increase in Pinus representation is observed. Approaching the upper part of sediments some instability in pollen diagram is seen. Abundance of the cold-tolerant plants suggests colder and dryer climate which determined a thinning of the forest cover and expansion of different herbs. According to our new data these sediments were deposited during the GI-1b/Gertsenzee "climatic event". Infilling of this sedimentation basin ended during this climatic event, much earlier, than it was thought before.

On Construction of Dimeric Cytokines, Penguins and the Origin of Life

Gediminas Drabavičius, Gitana Žvirblytė, Indrė Petreikytė, Gintautas Žvirblis, VU IBT

As the world population continues to grow and expectancy of life to increase, cancer is one of the most pressing problems that humanity is faced with. In clinical settings, said disease is treated with radio- and/or chemotherapy, which leaves patient virtually without immunity. To restore it, recombinant versions of cytokines, such as Granulocyte-colony stimulating factor, Stem cell factor and others (that are important in the immune system signaling and differentiation and proliferation of white blood cells [1] [2]), are used. Several of these cytokines are known to work in synergy with one another[3]. Therefore, we thought that making various fusion cytokine proteins, could possibly increase their circulation time and effectiveness. That would decrease the number and frequency of drug injections needed and subsequently would lead to an increase in the quality of life of the patient.

We have genetically constructed and purified several such fusion proteins in our laboratory and demonstrated the increased activity and circulation time, compared to analogous first generation biopharmaceuticals. This has lead us to expect similar effect with different dimeric cytokines and we are dedicating our time and effort to construction, purification and characterization of such constructs.

Additionally, two mathematical models are introduced in this presentation. One on the dynamics of Emperor penguin (Aptenodytes forsteri) huddling: a mechanism by which they keep warm during hatching period fasts. Other on the proposed division mechanism of a primitive protocell (a lipid vesicle), by which first protocells could have divided influenced by thermodynamic and

physicochemical forces alone.

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POSTER PRESENTATIONS

Lysozyme-induced changes in lactococcus lactis and relative cell envelopes

Agnė Žukauskaitė and Rimantas Daugelavičius

Lysozyme is a muramidase founded in all eukaryotic organisms. This protein is the first component of immune system fighting the invaded pathogenic bacteria. Lysozyme has two different activities - enzymatic and membrane-active, but the latter one needs to be better explored. Lactococcus lactis cells were used for studies of the mechanisms of lysozyme action. These nonpathogenic bacteria are widely used in milk and meat industry. Three forms of lysozyme - intact, heat-treated and chemically-inactivated - were studied. It was shown that all these forms of lysozyme induce leakage of K+ ions from cytosol and decrease the respiration rate of L. lactis, Streptococcus thermophiles and Streptococcus agalactiae cells. High concentrations of lysozyme (1 mg/ml and higher) caused an immediate increase of the cell membrane permeability. At lower concentrations of lysozyme the increase in permeability

was observed only after a certain lag period. Some differences in the responses of L. lactis cell to the modified lysozyme were observed. Intact and heat-treated lysozyme caused a faster permeabilization of the cell envelope and a sharper decrease of the respiration rate than chemically-inactivated one. The response to lysozyme of L. lactis mutant strains was also examined. A later leakage of K+ from the cell and a slower decrease of respiration rate were observed in the cases of L. lactis mutants resistant to lysozyme. St. thermophilus wild-type and mutant strains were not sensitive to lysozyme but St. agalactiae mutants were more sensitive to this enzyme than wild-type cells.

Investigation of methylation status of long non-coding rna h19 in prostate cancer

Agnieška Mackoit, Sonata

In recent studies it has been found that the H19 gene has a significant influence on tumor development process. The transcripts expressed by this gene have the ability to enable tumor cells to survive under stress conditions of the environment in which cancerous cells most commonly develop.

H19 gene encodes a long non-coding RNA and functions as a tumor suppressor. It is located in an imprinted 11p15 locus near the insulin-like growth factor 2 (IGF2) gene and its promoter contains differentially methylated region (DMR). Epigenetic alterations in DMR sequences of H19/IGF2 gene cluster lead to the malignant various types of cancer including prostate carcinoma. It is vital to find a reliable molecular markers that are able to accurately distinguish cancerous alterations and identify early stages of the disease.

The main goal of our investigation was to evaluate appropriateness of H19 as epigenetic marker for prostate cancer detection.

In this work we analyzed H19/IGF2 DMR methylation status in 54 samples of prostate carcinoma, compared them to 6 benign tissues and evaluated relations with clinical and demographic data. The DNA from prostate cancer cells was extracted, modified with bisulfite for bisulfite conversion of genomic DNA and amplified using methylation - sensitive PCR (MSP) method for methylation studies.

The results of MSP reactions showed 4 cases with hypermethylated DMR sequences. Final examination revealed no significant correlation between the H19 promoter methylation status and patient age or clinical parameters.

Mutations in EGFR, BRAF and KRAS genes in NSCLC patients' blood

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INTRODUCTION

The treatment of non-small cell lung cancer (NSCLC) has been changed by the development of drugs targeting tumors with specific genetic mutations. For cancer patients with mutations in tumors drugs are administered by tumor mutation status. EGFR (epidermal growth factor receptor) mutations in tumor can predict the response to EGFR-TKIs (tyrosine kinase inhibitors). BRAF (v-raf murine sarcoma) mutation (V600E) is found in 1%–3% of NSCLC. KRAS (Kirsten rat sarcoma) mutations in tumors are mostly localized in codons 12 and 13. The aim of this study was to detect mutations in EGFR, BRAF and KRAS genes in NSCLC patients' blood and compare them with the results obtained from

the tumor tissue.

MATERIAL AND METHODS

22 patients, treated at Department of Thoracic Surgery and Oncology at Institute of Oncology, Vilnius University, were included in the study. DNA was extracted from whole blood using "GeneJET Genomic DNA Purification Kit" (Fermentas, Lithuania). The EGFR mutations status was analyzed by pyrosequencing (PyroMark, QIAGEN); BRAF and KRAS mutations were analyzed using INFINITI Plus Analyzer platform (AutoGenomics).

RESULTS

In this study mutation status of EGFR, BRAF and KRAS were tested. 50 % of patients were positive for EGFR mutations in exon 18. However, only one patient had the same mutation (G719X in exon18) in blood and tumor. 2 patients (9,09%; 95% 2,96-15,22) had V600E1 mutation in BRAF gene in blood. Three mutations (G12A, Q61R, Q61P) in KRAS gene were detected in 5 patients' (22,73%; 95% 13,79-31,66) blood.

CONCLUSIONS

The results demonstrate that mutations in EGFR, BRAF and KRAS genes can be detected in NSCLC patients' blood. Blood samples may be used as an alternative testing material to tumor tissue samples. However studies with larger population should be done to evaluate these mutations in blood as biomarkers for NSCLC patients' treatment decisions.

Better living through biocatalysis: Synthesis of biopolyols from natural oils

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Petrochemical based polyols, which are currently used for polyurethane production [1], depend directly on the petroleum and its price. In addition, the petrochemical based products increase CO_2 production which is claimed to cause global warming. Therefore, bio-based polyol is an excellent alternative which is inherently sustainable, renewable and biodegradable. The aim of our current research is to develop eco-friendly polyols form natural oil – rapeseed oil, which is a cheap and abundant raw material in Lithuania [2].

We perform the synthesis of polyols in a two stage process: chemo-enzymatic epoxidation of the double bonds and latter nucleophilic cleavage of the epoxide ring to form polyols, in this case - hydrolysis of the epoxide ring. By this current research we investigated temperature and reaction time influence for the oxirane oxygen content formation during enzymatic epoxidation process [3]. Further, the hydrolysis of epoxidized rapeseed oil was investigated to determine optimum reaction parameters. The overall profile of hydrolysis reaction at a set reaction parameters is presented in Fig.1.

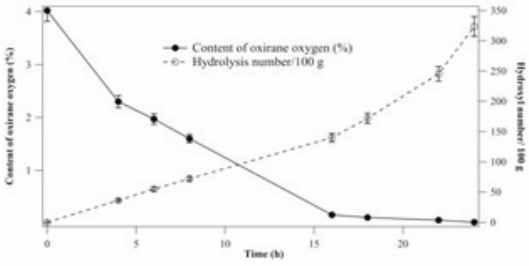

Fig. 1. Reaction profile for hydrolysis of epoxidized oil by Amberlyst-15 at a set of reaction parameters

The obtained results conclusively demonstrated a sustainable chemo-enzymatic process for the synthesis of polyol from rapeseed oil. The results will be presented in more detail during the conference at the poster session.

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The Study of Electroporation Influence of Various Impulses on Cell Morphology

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Introduction

Electroporation is usually used in molecular biology, biomedicine and biotechnology as a way of introducing some substance into a cell [1, 2]. During electroporation the lipid molecules shift position, opening up nm-scale water-filled holes in the membrane. To preserve the membrane integrity and proteins, which are located in cells membrane, the monolayer electroporation method was selected for the study [3]. The aim of our study was to identify the electroporation influence of different strength or shape of electrical impulse on cell morphology.

Materials and methods

A method and apparatus for cell culture wherein cultured cells adhere and divide, in a monolayer, on an optically transparent planar electrode. Photolithography was used to create a gold-coated electrode with 100-200 μm transparent sites. Photolithography was performed using UV light to transfer an interlacing electrode template to a light-sensitive chemical positive photoresist on the substrate. Electroporation was initially developed for the intro-

duction of calcein into Chinese hamster ovary (CHO) cells.

Results

The presence of a fluorescent dye after electroporation caused its penetration into the CHO cells and it was microscopically observed under fluorescence illumination. Two types of electrical impulses were used for the study: rectangular and exponentially fading. We discovered that exponentially fading electrical impulses under the same intensity of electrical impulses influenced more intensive fluorescence.

Conclusions

We identified that electroporation using exponentially fading electrical impulses strongly influenced cell morphology.

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Two-photon absorption cross sections measurements of toluene soluble cdse/zns quantum dots

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Near infrared excitation within the tissue transparency window in

the region 650 nm-1350 nm provides deeper penetration in tissues for imaging and sensitization applications. Biological molecules, photosensitizers, biomarkers and photo-drugs as a rule have no absorption in this spectral region, therefore, cannot be effectively excited. This can be overcome by the use of two photon excitation. In case of quantum dots (QDs) two photon absorption (TPA) cross sections are superior to those observed in conventional photosensitizers. However photophysical properties upon two-photon excitation of quantum dots are yet poorly understood.

The CdSe/ZnS core shell quantum dots of different size were used for the experiments. The two photon excitation of samples was performed by using home-made optical parametric amplifier (λ = 650 - 2500 nm) pumped by femtosecond "PHAROS" laser system. Two-photon absorption cross sections were evaluated by a comparative method with Rhodamine B as a reference compound [1].

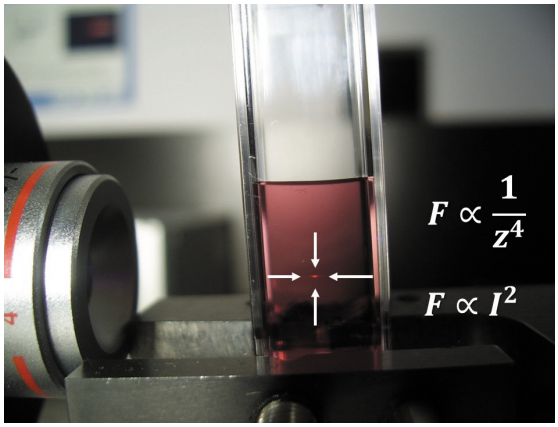

Fig. 1. Two photon excited fluorescence

Our results confirm that QDs have high enough TPA cross sections compared to organic dyes. This is paramount for applications and further optimization of two-photon properties of such nanoparticles. Such large TPA cross-sections make them efficient absorbers for multiphoton biological imaging and other nonlinear optical applications.

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Coformer selection in Pharmaceutical Cocrystal Engineering: a Case Study of a Pentoxifylline Aspirin Cocrystal

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The diversity of pharmaceutical solid-state forms of the same molecules is based on repertoire of non-covalent interactions that allows for control of chemical stability, dissolution, solubility, and in some cases bioavailability of the active pharmaceutical ingredient1. Among those forms pharmaceutical cocrystals are emerging as a new class of solid-state drugs with improved physicochemical properties, which has attracted increased interest from both industrial and academic researchers2.

Pentoxifylline is indicated to treat intermittent claudication resulting from obstructed arteries in the limbs3. In our previous studies4 it was found that in crystal pentoxifylline forms acid-base heterosynthon. Our further studies were focused on discovering ...sing carboxylic acid-aromatic ni-

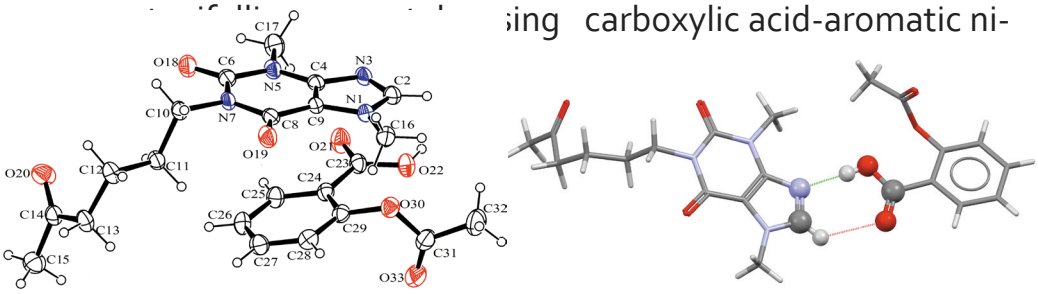

Figure 1. The asymmetric unit of the pentoxifylline aspirin (left) and the same structure showing supramolecular synthon; atoms, which participate in synthon formation are highlighted as spheres (right)

The pentoxifylline aspirin cocrystal (1:1) (Fig.1.) was prepared at room temperature by slow evaporation from dichloromethane. Cocrystal crystallizes in the triclinic system, space group P with one molecule of pentoxifylline and one molecule of aspirin in asymmetric unit. Aspirin is hydrogen-bonded with imidazole ring of pentoxifylline via O–H...N and C–H...O. The atoms participating in synthon lie in one plane (Fig.1.). π– π stacking interactions were detected between purine systems of pentoxifylline with centroid–centroid distance of 3.49 Å and between purine system of pentoxifylline and benzene ring of aspirin with centroid–centroid distance of 3.72 Å. The conformation of 5-oxohexyl group in pentoxifylline molecule is anti-anti-gauche with corresponding values of the torsion angles 170.3(2), 176.0(2) and -75.9(3)°, respectively. The cocrystal has been also prepared in bulk by liquid

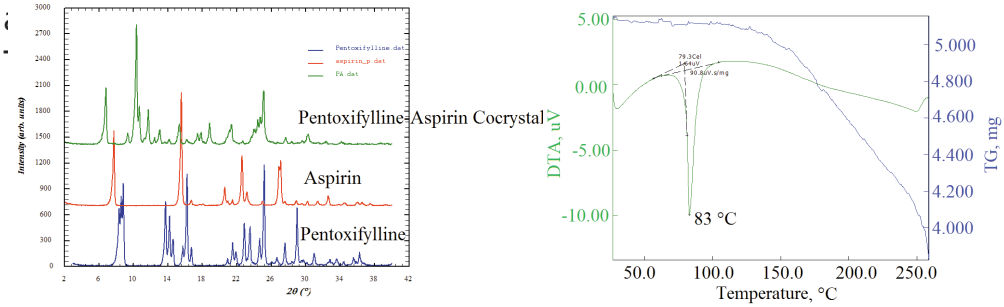

Figure 2. PXRD of starting powders and cocrystal after liquid assisted grinding (left) and DTA/TG patterns of the pentoxifylline aspirin cocrystal (right)

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This work was supported by the European Social Fund (No. 1DP/1.1.1.2.0/13/APIA/VIAA/011).

Value of cardiac magnetic resonance imaging in diagnostics of cardiac amyloidosis

Prof. Nomeda Valevičienė, resident dr. Karolina Lušaitė, Dominyka Rimšaitė, Marius Polianskis

Objectives: To find out potentiality of cardiac magnetic resonance imaging in diagnostics of cardiac amyloidosis. To compare collected data between patient and control groups. To determine delayed contrast enhancement type and alterations of myocardium specific to cardiac amyloidosis found by magnetic resonance imaging.

Method: In 2008 – 2013 prospective trial was conducted in Vilnius University hospital Santariškių clinic Radiology and nuclear medicine center. 14 patients with possible cardiac amyloidosis were selected. Data was compared with control group, which was formed of 12 persons without cardiac pathology. Correlation between cardiac magnetic resonance imaging and histologic analysis was also evaluated. Results: The group consisted of 4 (57,14%) men and 3 (42,86%) women. Age average of control group was 35,83 ±15,34 years We compared the size of interventricular septum (IVS) and left ventricle posterior wall (LVPW) between control and patient groups and we found statisticaly significant ($p<0,05$) increase in IVS and LVPW size in patients with cardiac amyloidosis group. Mean of left ventricle ejection fraction (RV_EF) in group with cardiac amyloidosis was 59,43 % ±12,52 and in control group 64,25% ±6,64. While comparing these findings statistical significance was not found ($p>0,05$). Accumulation of fluid in pericardium and pleura was found to all persons with cardiac amyloidosis, in control group these singns were not observed. In patient group statisticaly significant correlation between IVS and LVPW size was found ($p<0,05$; $p=0,039$; $r=0,78$). Correlation between left ventri-

cle end diastolic volume and left ventricle size in diastole was also spotted in patient group (p=0,02; p<0,05; r=0,882). Conclusion: In group with cardiac amyloidosis IVS and LVPW size increases while left ventricle ejection fraction stays unimpaired. To all patients with cardiac amyloidosis fluid in pericardium and pleura was observed. Incresed left ventricle size in diastole prompts increased left ventricle end diastolic volume and associated heart disorders.

Characterization of vB_ArS-ArV2 – first Arthobacter sp. infecting bacteriophage with completely sequenced genome

Šimoliūnas Eugenijus, Stasilo Miroslav, Kalinienė Laura, Truncaitė Lidija, Zajančkauskaitė Aurelija, Meškys Rolandas

Arthrobacter sp. strains are very widely distributed in the environment and have been found to be among the predominant members of cultivable aerobic soil bacteria. Although Arthrobacter species have been the subject of much study, relatively little is known about their predators in nature – bacteriophages. Only a limited number of reports on Arthrobacter phages have been made. Interestingly, most papers concerning these phages were published in the Seventies and the Eighties of the last century, and none of the previously described Arthrobacter phages was investigated regarding the genome organization or sequence similarity to already known viruses. Moreover, to date, neither partial nor complete genome sequences of Arthrobacter phages have been deposited in databases. In this study, a novel Arthrobacter bacteriophage vB_ArS-ArV2 (ArV2) was characterized. Phage was isolated from soil samples using Arthrobacter sp. 68b strain for phage propagation. The host range determination test revealed that out of 28 Arthrobacter strains tested, only 68b was sensitive to ArV2. Based on transmission electron microscopy, ArV2 belongs to the family Siphoviridae and has an isometric head (~63 nm in diameter), a non-contractile flexible tail (~194 x 10 nm) with short tail

fibers. ArV2 possesses a genome of double stranded DNA molecule (37.372 bp) with a G+C content of 62.73%. The genome is close packed: with an average ORF size of 535 bp, about 96% of the ArV2 genome is coding. A total of 68 ORFs were identified, but no tRNA genes were detected. The genome analysis revealed that 28 out of 68 ArV2 ORFs code for unique proteins that have no reliable identity to database entries. In the case of ArV2 ORFs that encode proteins with matches to those in other sequenced genomes, the percentage of amino acid identity ranged from 25% to 66% and, in most cases (19 out of 40), from 30% to 40%. In keeping with the typical genome architecture of members of the family Siphoviridae, ArV2 genome appears to have a modular organization, with genes for DNA packaging, structure/morphogenesis, host lysis, lysogeny and replication/regulation clustered together. LC-MS/MS analysis of the structural ArV2 proteins led to the experimental identification of 14 virion proteins, including 9 that were predicted by bioinformatics approaches. Comparative phylogenetic analysis, based on the alignment of amino acid sequences of conserved proteins, showed that ArV2 is phylogenetically distant from other phages and represents an evolutionarily distinct branch within the family Siphoviridae. Undoubtedly, genomic sequences and bioinformatics analysis of other Arthrobacter phages are needed to gain further insight into the evolution of this particular group of bacterial viruses. Nevertheless, characterization of Arthrobacter bacteriophage ArV2 is the first step towards understanding of Arthrobacter phage genomics and their evolutionary relationships with other organisms.

Low Concentration of 17β-Estradiol Decreases Frequency of Postsynaptic Currents in GnRH Neurons of Metestrous Mice via Endocannabinoid Signaling

Flóra Bálint, Imre Farkas, Zsolt Liposits

Gonadotropin-releasing hormone (GnRH) neurons regulate re-

production centrally. In vivo, 17β-estradiol (E2) controls GnRH release in concentration and estrus cycle dependent manner. In vitro, patch-clamp electrophysiological data on GnRH neurons of ovariectomized female mice demonstrated that low concentration (10 pM) of E2 decreased spontaneous firing rate which was eliminated by blocking fast synaptic neurotransmission (Chu et al., J.Neurosci., 29:5616, 2009). In the present study, we examined the effect of low concentration of E2 on postsynaptic currents (PSCs) in GnRH neurons of acute brain slices obtained from metestrous female mice and analyzed the putative involvement of endocannabinoid signaling in the evoked effect of E2. Whole-cell patch clamp recordings revealed that 10 pM E2 significantly diminished frequency of the PSCs (50±14.5% of the control) in 9 from 18 GnRH neurons. Pretreatment of the brain slices with the cannabinoid receptor type-1 (CB1) antagonist, AM251 (1 μM) significantly attenuated the effect of E2 on the PSCs (93±8.2%). The PSCs were blocked by application of picrotoxin demonstrating that the PSCs were GABAergic via GABAA-receptor. These data suggest that endocannabinoids are involved in the mediation of the effect of E2 on suppression of fast neurotransmission onto GnRH neurons. The interaction of estradiol and endocannabinoid signaling mechanisms represents novel regulatory machinery in the execution of the negative estrogen feedback to GnRH neurons.

Differences in thermodynamics of inhibitor binding to carbonic anhydrase vi purified from e. Coli, mammalian cells and human saliva

Justina Kazokaitė, Goda Milinavičiūtė, Joana Gylytė, Virginija Dudutienė, Jurgita Matulienė, Daumantas Matulis

The main function of carbonic anhydrases (CAs) is the maintenance of pH homeostasis catalyzing the reversible hydration of carbon dioxide. There are 12 human catalytic CA isoforms differ-

ing from each other in expression patterns, tissue localization and cellular distribution. CA inhibition has been exploited clinically for decades in various classes of diuretics, anti-glaucoma agents, anti-epileptics and anti-tumor drugs. CA VI is the only secreted isoenzyme of the human CA family. This enzyme is found in saliva, tears and milk. It is responsible for antireflux defense, protection from carcinogens and taste function. Gene comparison tells that CA VI is closely related to membrane associated isoenzymes CA IV and CA IX. CA IV is a target for several pathologies, including glaucoma, retinitis pigmentosa and stroke. CA IX is a marker for hypoxic tumors. This association links CA VI with certain cancers which might be associated with salivary glands. Therefore, it is important to analyze the affinity of sulfonamide inhibitors for CA VI. Human recombinant CA VI was expressed in E. coli and in mammalian cells. Also, a half-liter of saliva was collected from twenty volunteers. CA VI from these sources was purified by affinity chromatography. Thermal stability of the protein and thermodynamics of interaction between the enzyme and inhibitors, were determined by the fluorescence thermal shift assay (TSA) and isothermal titration calorimetry (ITC). It was found that CA VI was most stable at pH 6. However, CA VI expressed in human saliva and mammalian cells were more stable than the E. coli form at all pHs. The affinity of commercial compounds and inhibitors designed and synthesized in our laboratory for CA VI from saliva was similar to the affinity for CA VI from E. coli and mammalian cells. This study shows that CA VI purified from bacteria is a perfect model to analyze reactions between human CA VI and inhibitors and the absence of glycosylation in E. coli has no significant effect on thermodynamics of inhibitor binding to this enzyme.

Spectroscopic study of chlorin e6 and bovine serum albumin complexes

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Chlorin e6 (Cle6) is one of the most common photosensitizers used in photosensitized tumor therapy. It is believed that the tumor-localising property of Cle6 may be related to its tendency to bind to serum albumins. As a model we used bovine serum albumin (BSA), whose properties and structure are similar to human serum albumin. The interaction of Cle6 with BSA in aqueous solution has been investigated by means of a steady state absorption spectroscopy. By using a typical absorption difference spectra obtained for Cle6 titration with BSA and a Scatchard plot the number of Cle6 binding sites in BSA molecule was obtained. Also the affinity of Cle6 to BSA was established by using the binding isotherm method and a Scatchard plot. The results of the study are discussed. Addition of BSA to the solution of Cle6 at molecular ratio of 1:1 shifts the absorption maximum to the longer wavelength about 10 nm. The same result was obtained for Cle6 titration with HAS in pr nt of BSA no chang

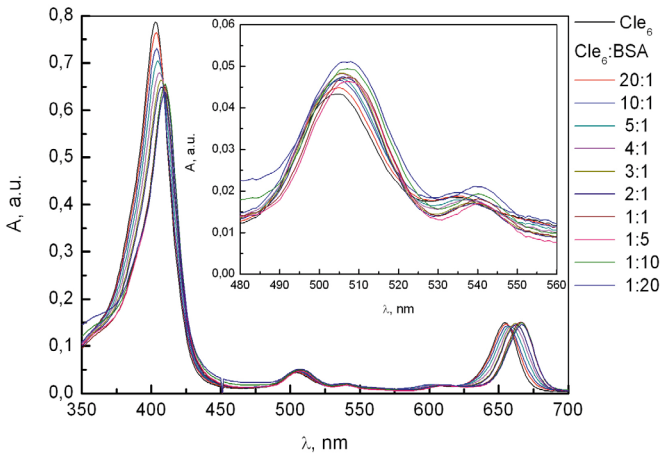

Fig. 1. Absorption spectra of Cle6 titration of BSA.

The experiments were carried out in the Laboratory of Biomedical Physics, Institute of Oncology, Vilnius University using absorption spectrophotometer Cary 5E, Varian

Evaluation of a Risk of Developing Complications in Community Acquired Pneumonia Patients: A Prospective Study

This study has been conducted by **Kotryna Ragaleviciute** and **Ieva Siaudinyte**, Vilnius University, Faculty of Medicine. Vilnius, Lithuania, 2013.

Background. The objective of this study is to identify blood inflammatory markers, respiratory failure and pneumonia severity indicators to predict the risk of developing complications of community-acquired pneumonia.

Methods. This was a prospective study which involved 36 patients hospitalised with community-acquired pneumonia (CAP). Monitoring the course of disease, patients were divided into two groups: uncomplicated and complicated (parapneumonic pleural effusion, empyema, bilateral pneumonia, sepsis). To prognosticate CAP complications, the following criteria were chosen: gender, age, total hospitalization time, blood leukocyte quantity, C-reactive protein's and procalcitonin's concentrations in arterial blood, PaO2, SaO2 of the first hospitalization day. The severity of pneumonia was assessed under the PSI and CURB65 scales.

Results. 23 (64%) out of 36 patients with pneumonia developed complications. Development of complications did not correlate with gender, age, PaO2, SaO2, blood leukocyte quantity, C – reactive protein's and procalcitonin's concentrations in arterial blood, PSI and CURB65 score results. There is significant correlation between: PSI and CURB65, PSI and age, PaO2 and procalcitonin's

concentration in arterial blood, SaO2 and CURB65.

Conclusion. Inflammation markers in arterial blood test (C – RP, white blood cell count, procalcitonin's concentration), respiratory failure indicators (PaO2, SaO2), pneumonia severity scores (PSI, CURB65) correlate, but they are not applicable to predict development of complications on patients with community aquired pneumonia.

Research on growing some species of energy plants fertilized with sewage sludge compost

Eugenija BAKŠIENĖ, Jelena TITOVA

There is a big interest in renewable energy resources worldwide. It's energy of solar, wind, geothermal, hydro, biomass. These resources for energy production is environmentally friendly and contributes to climate stabilization (Lithuanian Energy Institute and others. 2008) . European Union countries each year, more and more grow energy crops. The scientific research suggests that in addition to trees and woody plants are many annual and perennial grasses, whose biomass can accumulate quite a lot of energy. (Lithuanian Energy Institute. 2009) Sewage sludge treatment and disposal is a global problem. In the end it is important to investigate the possibility of using sewage sludge and sewage sludge compost as a fertilizer for growing bioenergy crops. The investigation has been performed at the Vokė Branch of the Lithuanian Research Centre for Agriculture and Forestry. Artemisia dubia, Sida hermafrodita and Miscanthus sp. – were grown in light textured soil in the climatic zone of southeastern region of Lithuania in 2012 – 2013. Sida hermafrodita and Miscanthus sp. planted at a rate of 2 plants 1 m-2, Artemisia dubia – planted at a rate of 4 plants 1 m-2. Length of the field – 3 m, width – 3 m, area – 9 m2. Reps – 4. The number of fields – 20. Plants were fertilized with mineral fertilizers and sewage sludge compost under

the scheme: control (without fertilizer), N90P60K90, 20, 40 and 80 t/ha of dry compost. Mineral fertilizers N90P60K90 rate was poured annually, beginning the year. Sewage sludge compost soil was fertilized once in the spring of 2012, for three years. Tasks: to investigate the change in biomass yield, height of stem and the number of stem different species depending on different fertilization rates. Artemisia dubia rhizomes took root approximately 90 %, Miscanthus sp. – 50 %.

Polymorphisms in DNA repair genes and non-small cell lung cancer

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INTRODUCTION

Non-small cell lung cancer (NSCLC) is a leading cause of cancer death worldwide. Platinum-based drugs (gemcitabine, cisplatin etc.) are used as a first-line therapy for NSCLC patients. However, not for all patients such treatment is effective. Biomarkers that could predict efficiency of the platinum-based treatment should be identified. Single nucleotide polymorphisms in DNA repair genes have been investigated as a potential predictive biomarkers of tumor sensitivity to chemotherapy drugs. The aim of this study was to investigate polymorphisms in DNA repair genes and their association with NSCLC.

MATERIALS AND METHODS

The study included 39 NSCLC patients treated at Department of Thoracic Surgery and Oncology at Institute of Oncology, Vilnius University. DNA was extracted from whole blood using „GeneJET Genomic DNA Purification Kit" (Fermentas, Lithuania). Five polymorphisms, ERCC1 (118 codon C→T), RRM1 (-37 C→A), RRM1 (-524 C→T), XRCC1 (399 A→G) and XPF (-357 A→C) were analyzed by polymerase chain reaction restriction fragment length

polymorphism (PCR-RFLP) method. Final products were electrophoresed onto agarose gel. A $\chi 2$ test was used to analyze polymorphisms in relation to clinicopathological parameters. The results were considered as statistically significant when $P \leq 0{,}05$.

RESULTS

All five polymorphisms in DNA repair genes were detected. Polymorphisms were evaluated according disease stage, tumor differentiation grade, lymph node status and tumor histological type. Only significant association (p=0,01) between disease stage and ERCC1 (118 codon C→T) was found. There were no statistical correlations between other polymorphisms and clinicopathological parameters.

CONCLUSIONS

Polymorphisms in DNA repair genes may influence platinum-based treatment of cancer patients. Additional studies with larger population should be done. Some genes, such as ERCC1 and RRM1 should be further evaluated as potential biomarkers for the prediction of clinical outcome of NSCLC patients in the future.

Construction of a glucose responsive expression system for single-chain insulin in human mesenchymal stem cells

Katsiaryna Kastahladava

Frequency of diabetes mellitus is growing from year to year in Belarus as well as in whole world. The technology of stem cells as well as genetic engineering methods being combined open new perspectives for therapy of this disease. We have developed of the bicistronic lentiviral transfer vector pHR-Pegr1-SCI, carrying single-chain insulin gene and enhanced green fluorescent protein eGFP reporter gene under control of glucose-responsive promoter of human egr1 gene. Recombinant pHR-Pegr1-SCI based lentiviruses were used for transduction of human bone

marrow-derived mesenchymal stem cells (MSC). Under experimental conditions we observed induced 2,62-fold growth in insulin gene expression 15 minutes after glucose concentration being elevated from 3 to 20 mmol/l ($p<0,05$). Meanwhile after additional 15 minutes the expression level spontaneously decreased up to near baseline level and didn't change significantly during the subsequent 30 minutes. Therefore the functionality of this system requires additional proving on the level of transgen translation.

Developmental changes in blood indices of patients with inflammatory bowel disease through biological therapy

Kotryna Ragalevičiūtė, Rūta Saikalytė

Aim of study: Investigate and evaluate close and outermost changes of blood indices through the use of biological therapy (infliksimab or adalimumab) of patients with Crohn's disease or ulcerative colitis.

Methods: Retrospective study conducted in VUL SK HGD center. Reviewed 45 cases of patients with Crohn's disease or ulcerative colitis treated with biological therapy during 2009-2012 year. The final analysis included 24 cases. Evaluated the following data: gender, age, place of residence, distribution, form, activity of the disease, prescribed medication, laboratory tests (white blood cell and platelet count, hemoglobin and CRP levels) before and 1, 3, and 12 months after treatment. The data were processed in MS Excel 2010, according to Stjudent's test.

Results: 12 of 24 patients (50%) were men and other 12 (50%) were women. Average men age was 35,71 (±13,84) year. 20 (83,33%) patients lived in towns and 4 (16,67%) in rural areas. 4 (16,67%) patients were diagnosed with ulcerative colitis and 20 (83,33%) with Crohn's disease. No clinical remission seen in 25%

of the cases, 54,17% patients reached partial clinical remission and 20,83% reached full clinical remission. The rise of hemoglobin's concentration in blood after one year (116.61 g/l at the beginning; 132 g/l after 1 year), the decrease of platelet's count in blood after 1 month, 3 months and 1 year (385.71×10*9/l at the beginning; 326.52×10*9/l after 1 month; 305.43×10*9/l after 3 months; 299.5×10*9/l after 1 year) and the decrease of C – reactive protein's concentration in blood after 1 month (17,5 mg/l in the beginning; 6 mg/l after 1 month) were observed in blood tests.

Conclusions: Positive changes with the trend to improve further has been seen in patients with inflammatory bowel disease bloods inflammatory parameters (PLT, CRP) after first month of biological therapy.

Preparation of gold nanoparticles for bioimaging applications

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During the last few years gold nanoparticles (AuNPs) have had an important role in bioimaging because of their extensive scale of sizes and easy surface functionalization. Their advantageous properties such as localized surface plasmon resonance, fluorescence and high atomic number of gold suggest that these nanoparticles could be valuable material for X-ray and fluorescence bioimaging in order to develop accurate, high resolution imaging techniques.

Although future perspectives are promising, there are still some obstacles to overcome. Small AuNPs concentrations and lack of knowledge about stability in different conditions are the main

problems. This research investigates stability of two types of AuNPs (fluorescent and plasmonic) depending on storage temperature, reagent concentrations, centrifugation, centrifugal vacuum concentration and X-ray radiation.

Fluorescent, MES (2-(N-morpholino)ethanesulfonic acid) capped gold nanoparticles were synthesized using modified method suggested by Bao et al [1]. Spectroscopic measurements of these AuNPs (Fig. 1, A) show that they are stable over time and do not sustain any changes while exposed to radiation or stored in temperature range from 4°C to 22°C. Concentration as well as fluorescence intensity of these AuNPs can be increased more than 50 times using higher reagent concentrations. Formed precipitates could be easily eliminated by centrifugation in order to obtain more homogeneous colloid.

Plasmonic, trisodium citrate stabilized gold nanoparticles were synthesized using Turkevich et al [2] proposed method and were observed as extremely stable over time. To obtain higher concentrations of these particles two different methods were used. Higher reagent concentrations showed to be critical in formation of plasmonic AuNPs and did not provide desired results (Fig.1, B) while centrifugation and constant addition of trisodium citrate al-

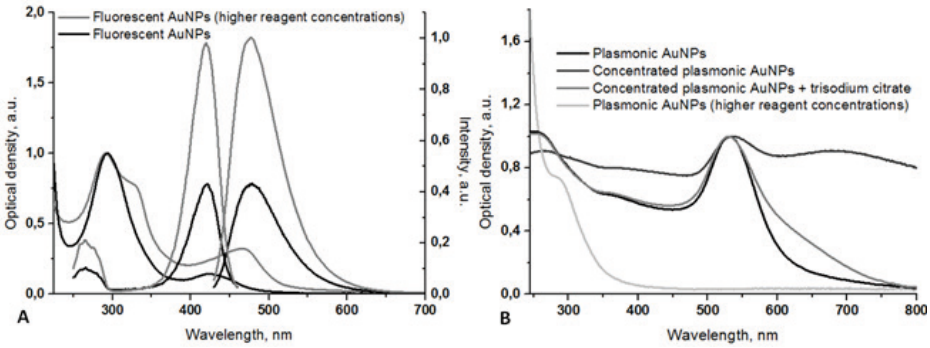

Fig.1 Fluorescence and optical density spectra of fluorescent MES capped gold nanoparticles (A). B –optical density spectra of plasmonic AuNP in different conditions.

lowed to concentrate particular particles ~150 times with no significant changes in absorption spectra.

This research evaluates pros and cons of different concentration and preparation methods of two types of promising and widely used gold nanoparticles. Experiments showed that both types do not experience any critical changes under observed environmental conditions, could be concentrated and efficiently used in bioimaging applications as contrast agents.

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[2] J. Turkevich, P. C. Stevenson, J. Hillier, A study of the nucleation ang growth prosses in the synthesis of coloidal gold, Discuss. Faraday. Soc. 1951, 11, 55-75.

Serological investigation of parvovirus B19 infection in patients with rheumatoid arthritis

Milda Naciute, Rita Nikitiene, Diana Mieliauskaite, Rita Rugiene, Mykolas Mauricas, Irute Girkontaite

Parvovirus B19 is one of smallest virus that infects humans. It is spread worldwide. Parvovirus B19 is often found in blood and tissues of rheumatoid arthritis patients. However, the infection of this virus was not investigated in Lithuania until now. The aim of this thesis was to perform serological analysis of parvovirus B19 infection in rheumatoid arthritis patients. Cytokines and parvovirus B19 specific antibodies were investigated in plasma using ELISA and Western blotting. The majority of patients had IgG class specific antibodies to parvovirus B19 and had no IgM antibodies. Such data show that the patient had parvovirus B19 infection already long time ago. The absence of IgM antibodies shows that the patients did not have acute infection. Parvovirus B19 infec-

tion modifies the cytokine levels in the plasma of patients suffering from rheumatoid arthritis. We have found reduced levels of INFγ, IL-17 and IL-10 and raised levels of IL-4 and IL-6. The levels of IL-4, IL-6 and TNFα were especially increases when the parvovirus B19 DNA was found in plasma. The levels of those cytokines were not so high when the virus DNA was obtained in the blood. Our data show that the parvovirus B19 modulates the immune response and cytokine levels in the blood of rheumatoid arthritis patients.

Studies of yeast Saccharomyces cerevisiae secreted toxin K2 effects on microbial cells survival

Neringa Venslauskaitė, Irma Orentaitė and Rimantas Daugelavičius

We studied survival of microbial cells in metal ions-enriched media containing K2 toxin. It is known that toxin K2 kills sensitive yeast cells but our results indicate that it has also a negative effect on the growth of bacteria. Results of our experiments showed that this toxin the most effectively kills M. luteus cells. The killing efficiency of toxin K2 could be explained by the permeabilizing action on bacterial membranes. The efficiency of toxin on bacteria was assayed using a system for electrochemical measurements and phenyldicarbaundecarborane (PCB–)-selective electrodes. PCB– ions were used as indicators for studies of the toxin interaction with bacterial membranes. These ions in high amount bind to membranes of inactivated cells. The highest amounts of PCB- was bound to toxin-treated grampositive S. aureus cells but gramnegative E. coli DH5α cells were also affected. However, in the case of prolonged incubation E. coli cells were able to extrude out the indicatory compound. K2 toxin is rather stabile. It was active after storage in liquid nitrogen. For K2 isolation we mechanically disrupted this toxin-producing cells after their freezing.

Gametophytes of Club Mosses in Pine Forests of South-eastern Lithuania

Radvilė Rimgailaitė

Gametophytes of Club Mosses in Pine Forests of Southeastern Lithuania 400 million years ago club mosses were the dominant group of spore-bearing vascular plants. Nowadays members of division Lycopodiophyta D. H. Scott make less then 0.4 percent of flora of the world. In the life cycle of club mosses perennial sporophytes (2n) representing asexual generation are dominant. Usually they form large overgrowths in forests. In the meanwhile gametophytes (n) – the sexual phase – are mostly subterranean and difficult to find. That is why populations of gametophytes of club mosses were examined only superficially. The gametophytes of club mosses are subterranean organisms with diameter less than 1 cm, saprotrophic, mycorrhizal and without chlorophyll. However gametophytes are responsible for renewal of populations of club mosses. They produce sexual reproduction structures: antheridia, containing male gametes and archegonia, containing female gametes. In many countries, also in Lithuania, biology of Lycopodiaceae gametophytes and mechanisms of formation of their populations were not analyzed in detail so far and knowledge about these processes is scarce. In 2012 and 2013 the research on diversity of gametophytes was carried out in forest communities (Cladonio-Pinetum, Vaccinio-Pinetum, Vaccinio-myrtillo-Pinetum, Myrtillo-Pinetum) of 60-90 years old. During the research 50x50 cm size samples of soil with forest floor were collected. In 2012, 221 gametophytes of club mosses were extracted out of them and described, and in 2013 – 253 gametophytes of club mosses. Gametophytes varied in shape and development. The structural diversity of Lycopodium L. sensu

lato gametophytes is very wide. H. Bruchmann (1898) grouped their forms that were known at that time into five morphological types, that are still being used for describing gametophytes in XXI century. All gametophytes collected are representing either type I (Lycopodium clavatum L. or L. annotinum L.) or type II (Diphasiastrum complanatum (L.) Holub or D. tristachyum (Pursh) Holub) according to H. Bruchmann's classification. Type I gametophytes have the shape of irregular bowl, type II – carrot or beetroot shape. Mature gametophytes of both types had three zones: more or less conical basal portion with rhizoids, ring meristem and gametangial cap. It was noticed that the younger gametophyte was, the less ring meristem was developed and as a result the bowl-shaped gametophytes had the edged that were not curled up, forming the shape of a disc (type I case), and carrot-shaped gametophytes had the bottom not separated with meristemic groove (type II). Initial research showed that young populations of club mosses can be discovered in pine forests of Southeastern part of Lithuania. The places where there is a big possibility to find gametophytes are usually indicated by young sporophytes. In total 474 gametophytes were found during the period of 2012–2013, located in humus soil horizon, approximately in the depth of 0,2-0,8 cm. Their size and maturity were varying. Conclusions: 1. In 2012 and 2013 research carried out in 60-90 years old pine forests in southeastern part of Lithuania showed that in these forests' soil we can find type I and type II (according to Bruchmann classification) gametophytes of club mosses. Size and shape of gametophytes vary greatly. 2. The main indicator of gametophytes population are young sporophytes, just emerged from the soil. 3 All gametophytes are usually found in humus soil horizon, less then 1cm depth.

Melatonin influences ROS production by murine macrophages and dendritic cells via MT3 receptor

Rūta Bozaitė, Vitalij Černyšiov, Irutė Girkontaitė, Mykolas Mauricas

Melatonin is known as "the hormone of the darkness". It is produced by the pineal gland mainly at night. The task of this study was to investigate the influence of melatonin to the production of reactive oxygen species (ROS) in bone marrow derived murine macrophages and dendritic cells. ROS are produced by yeasts stimulated dendritic cells and macrophages. Melatonin at concentrations 100-10mM and 100-1nM inhibits the production of ROS. Melatonin acts through MT1, MT2, MT_3 and ROR (α, β, γ) receptors. To find out which receptor is involved in the regulation of ROS production it was investigated the expression of melatonin receptors in stimulated and unstimulated cells. Only two melatonin receptors (MT_3 and ROR) were expressed in both unstimulated and LPS-stimulated cells. The amount of ROR in dendritic cells increased after LPS stimulation, while LPS stimulation did not change the expression of ROR in macrophages. The other receptors were detected under certain conditions. MT1 was expressed in LPS stimulated dendritic cells, while MT2 and RORγ were detectable in unstimulated dendritic cells only. We could not find RORβ on murine dendritic cells. However, RORβ was detected in macrophages after LPS stimulation. To found out if MT_3 receptor is involved in the regulation of ROS production selective melatonin MT_3 receptor agonist GR13531 was used instead of melatonin. It was detected that GR13531 similar as melatonin inhibits ROS production, meaning that melatonin acts via MT_3 receptor.

The Interaction of Appropriate Measures to Reduce Multidrug resistance

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Multidrug resistance (MDR) efflux pumps are transporters responsible for the extrusion of toxic substances and antibiotic from cell. Because of these systems bacteria are sensitive to increased concentrations of antibiotic, it is necessary to prescribe increased doses of these drugs. It is important to find means to overcome the resistance to antibiotic. The ways are to destroy the barrier of bacterial envelopes to antibiotics and to inhibit the efflux pumps. Polymyxin B (PMB) is a well-known and effective antibiotic. It is not a substrate of efflux pumps. However, PMB is not popular in practical medicine because of some nephrotoxicity. Bacteriophages are known as alternative means for therapy infective diseases, and Phe-Arg-β-naphthylamine (PAβN) is the best inhibitor of MDR-type efflux pumps. The combination of these means looks very attractive but it has not been studied whether is possible to combine low doses of PMB, bacteriophages and efflux pumps inhibitors.

Tetraphenylphosphonium (TPP+) is an universal substrate of MDR pumps. It is possible to study the activity of pumps using this indicatory compound. In our study the accumulation of TPP+ ions in bacteria was monitored using electrochemical system of analysis. Changes of TPP+ concentration in the medium indicated, that bacteriophage T_4 permeabilizes the bacterial envelope and causes the depolarization of cell. Our results have shown that PAβN binds with a high affinity to bacterial lipopolysaccharide (LPS). At the same time this component of the outer membrane of gram-negative bacteria is the receptor for phage T_4. Because of this phage T_4 loses the effect on the cell envelope at high concentrations of PaβN. It was also established that phage T_4 and polymyxin B compete for the binding sites in the outer membrane of the cell. So, all these antibacterial means compete for the binding to LPS and interfere with each other.

Develop procedures for purification of recombinant therapeutic antibodies against HER2, derived from a plant source

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Introduction At present monoclonal antibodies are widely used to treat various cancers. We were focused on the development and purification of antibody raised against Her2/neu oncoprotein expressed on breast cancer cells. Herceptin is used with some success in women with breast cancer. Recently, the plants were suggested to serve a platform for the production of antibodies. This approach is based on several advantages including higher biomass production and higher yield of protein. Also the assembly of protein in the plants leads to production of active antibody. The aim of this study was to develop the procedures for purification of recombinant monoclonal antibodies specific to oncoprotein HER2/neu from the plant Nicotiana benthamiana.

Materials and Methods We have designed and developed few viral vectors to transform Agrobacterium tumifaciens. We have chosen the agroinfection to follow the expression of vectors. The process of antibody extraction involved 3 steps. First, the leaves were crushed and the antibody was extracted. Then, the antibody was precipitated with ammonium sulfate at a concentration of 30-40%. Western blot was used to confirm the presence of oncoprotein. Affinity, ion exchange and gel filtration chromatography were used to purify the antibody.

Results After 5 days the leaves were collected and the protein was isolated. We have determined the optimal concentration of ammonium sulfate to precipitate protein from the extract. 30-40%

of ammonium sulfate was the optimal concentration to isolate the maximum protein. Then the protein was purified by ion exchange and gel filtration chromatography. The presence of protein at each step was confirmed by SDS-PAGE and ELISA.
Conclusion In summary, we developed the methods of purification of recombinant therapeutic antibodies. We also optimize the conditions of each stage of purification.

Metagenomics – a modern method for searching of potential biocatalysts

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The feature of enzymes (biocatalysts) to operate effectivelly not only inside the cell, but also in vitro is essential for biocatalytic application of enzymes for various scientific and industrial needs. Therefore, the demand for enzymes with new or specific characteristics is constantly increasing. Currently, in the laboratory is possible to cultivate less than 1 % of the microorganisms found in the nature, which is why only a small part of their genetic diversity can be covered by standard microbiological methods. Metagenomics is one of the modern methods for searching and investigation of new enzymes, thus, reducing the cultivation problem [1]. This method is based on the direct extraction and analysis of total DNA in the environmental samples.

The aim of our work was to detect potential biocatalysts in the constructed metagenomic DNA library. DNA extracted from sludge was fractionated, ligated with multicopy pUC19 vector and transformed into E. coli DH10B cells. Construction of metagenomic DNA library resulted in more than 3400 recombinant clones. Functional analysis of the recombinants on the selective growing

media revealed several clones with potential enzymatic activity. The partial sequence of the inserts revealed the presence of peroxidase, sulfatase, acylesterase, arylsulfatase genes. The results of functional analysis, protein electrophoresis and performed DNA sequencing will be presented in more details during poster session.

References

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Paleopathological investigation of two cemeteries from the 8th-9th century AD (Late Avar Period) in Hungary

Tamás Szeniczey, Zsolt Bernert, Tamás Czuppon, Antónia Marcsik, Géza Szabó, Attila Czövek, Katalin Bakó, Tamás Hajdu

The study of the paleopathological alterations can reveal the lifestyle and the common diseases of past populations. The purpose of our investigation was to make implications about the health status of the Avar population from two transdanubian site (Dunaszentgyörgy: 25 individuals, Dunaföldvár 61 individuals). Most of the pathological changes in the investigated anthropological materials were lifestyle-related, which are frequent in historical populations. The symptoms of a strained lifestyle e.g. enthesopathia, arthritis, degenerative lesions of the spine, was more often in both cemetery's male individuals than females. This is probably reflect a different daily task and lifestyle. The formation of larger farming settlements, which associated with an increase in the population density, is typical for the Late Avar Age. Under these factors the pathogen bacteria, like tuberculosis can spread faster. In the literature so far, one case has been described as tuberculosis in this region from the Avar Age. In two cases from Dunaföldvár we determined skeletal tuberculosis based on morphological alteration. The affected parts of the body were the sacrum and the ac-

etabulum, which are often implicated in the disease. This is an important result in paleoepidemiological point of view, because the skeletal tuberculosis occurrs in less than 5% in cases, in contrast the sample contains only 61 individuals. Therefore the tuberculosis would be more common than the previously revealed findings suggest.

The immunoregulatory action of melatonin

Vitalij Černyšiov, Dr. Mykolas Mauricas, Dr. Irutė Girkontaitė

Circadian rhythm and melatonin could influence many functions of organisms including the immune system. The absence of the main organ of melatonin synthesis (pinealectomysed animals) promotes weight loss in immune organs. These symptoms could be reversed by melatonin administration. The aim of our study was to investigate the effect of temporary suppressed melatonin synthesis (caused by disrupted circadian clock) on the quantity of leucocytes in immunized mice. BALB/c mice were immunized and kept under (1) normal lighting; (2) constant exposure to the light; exposed to the light and treated daily with melatonin. The disrupted circadian clock and melatonin synthesis did not influence the amount of cells in thymus, bone marrow, spleen, lymph nodes and Peyer's patches. However, mice kept under constant lighting (synthesis of melatonin decreased) had increased number of T cells and B2 B cells in peritoneal cavity when the immunizations were performed in the evening. Daily melatonin treatment of mice normalized the cell number. Opposite, when the immunizations with TD antigen were performed in the morning, a decreased amount of cells in peritoneum cavity of mice kept at constant lighting as compared with mice at normal lighting was detected. The number of peritoneal cells in mice kept at normal light/dark conditions depends on the time of immunization. The amount of those cells was significantly higher when the immunizations were performed in the morning versus evening. The cell

number in peritoneal cavity of mice kept at constant light conditions was not influenced by the time of immunization.

High speed biopaterning by dip-pen nanolitography with lipid inks

Vytautas Navikas, Šarūnas Vaitekonis, Martynas Gavutis, Ramūnas Valiokas

The goal of this study was to explore the possibilities of dip pen nanolithography (DPN) to fabricate lipid patterns on solid supports. DPN is scanning-probe-based lithography in which tip is used to make Nano scale chemical patterns directly transferring molecules to the surface. This method offers a number of advantages over other nanofabrication methods, but it also has some issues such an accurate control of the material amount on the tip which dramatically affects the repeatability of the structures. In this study an ink-jet deposition was used as a novel method for such a control. The lipid as an ink was chosen for a high relevance as biological sensing material. Investigation of fluorescent lipid patterns of different tips using epifluorescence microscopy showed a reliable repeatability of the material deposited on the tip. After that patterning using JPK scanning probe microscope was done on the silicon dioxide surface in order to investigate how this inking strategy affects DPN patterns. Fabricated patterns investigation using fluorescence and scanning probe microscopy showed reasonable repeatability and line widths down to 200nm. Results of this study shows that ink-jet material deposition on the tip allows reach significant improvement over conventional ink well inking.

VILNIUS UNIVERSITY

As the oldest and largest of Lithuania's higher education institutions, Vilnius University is an active participant in international scientific and academic activity and embodies the concept of a classical university – the unity of studies and research.
Vilnius university has long been an integral part of European science and culture since its establishement in 1579. As one of the oldest higher education establishments in Central and Eastern Europe, it has had a marked influence on the cultural life of Lithuania as well as her neighbouring states.

One of the main aims of the university is to position and distinguish itself in European research and education with top-level research. Vilnius university has taken upon itself the responsibility for maintaining the highest level of research and studies – fulfilling the needs of the state and society for higher education. It has recently and significantly improved the university's infrastructure through active involvement in European structural funds' projects.

Today, Vilnius university has over 22,000 students and over 1,830 teaching and research staff. The university has 12 faculties, 7 institutes, 3 university hospitals and 4 study and research centres. It has the one of the richest libraries in Europe, an astronomical observatory, a botanical garden and the cherished Church of St. Johns'. The university structure also embraces several museums, a dormitory campus, laboratories , workshops, summer resorts and student traineeship bases.

The university enjoys a unique academic atmosphere and academic freedom where priority is always attached to intellect, wisdom and tolerance.

Vilnius university remains young, dynamic, progressive and open to the world's cultural and scientific values.

FACULTY OF NATURAL SCIENCES

The Faculty of Natural Sciences is one of the 12 Vilnius university's faculties. It consists of 9 departments, 11 academic and training laboratories, the gardens of Botany, Museum and Centres of Cartography, Ecology and Environmental Sciences. The staff of faculty and students - are engaged in research and scholarships in diverse sub-diciplines within diciplines offered. The faculty offers several undergraduate programs (biology, molecular biology, genetics, microbiology, ecology and environmental sciences, biophysics, hydrology and meteorology, geology, hydrogeology and geology engineering). Graduate students can enroll in both MSc and PhD programs. Courses, including independent projects, seminars and field courses are on a focus as well.

The Faculty of Natural Sciences spans the entire spectrum educational, sports activities. Devoted and motivated students gather in student's organizations, where they have opportunities to explore, study and investigate.

The faculty is led by Dean prof. habil. Dr. Osvaldas Rukšėnas. The faculty has 164 academic and 41 research fellows.

VILNIUS UNIVERSITY LIFE SCIENCES CENTER

This unprecedented project aims to create a Life Sciences Center with particular focus on Lithuania's strategic research areas: biotechnology, structural and cell biology, genomics and their in-

dustrial applications. The project is managed by the joint effort of Vilnius University and its partners: Vilnius Gediminas Technical University and Vilnius University Institute of Oncology (project code: VP2-1.1-ŠMM-04-V-01-016). These institutions are aiming to create a competitive environment, which would attract foreign investment and world-class scientists. As one of the „Sunrise Valley" projects, it also has the goal of attracting knowledge and innovation in a form of young and active scientists.

Year 2010 marks the start of the LSC project, which is now estimated to finish in 2015. During this five year course the costs are estimated to reach nearly 142 mln. Lt, which will be primarily spent on the new research building and scientific equipment. Over 25 mln. Lt have already been invested in instrumentation, supporting research in key biotech areas: recombinant proteins; monoclonal antibodies; structural biology; computational biology; plant genomics; biocatalyst applications; novel large and small molecules therapeutics; neurophysiology; toxicology; stem cell and cancer research.

To accomplish a truly interdisciplinary research, the center will be located in a strategic location with neighboring Vilnius University Library, the faculty of Physics and emerging National Center for Physical and Technological Sciences. The new building will house an animal holding facility, a radioisotope laboratory, facilities for proteome analysis and next-generation sequencing, greenhouse,

GRID computing facility as well as lecture halls in a total area of 24 thousand square meters. A third of this area will be dedicated for educational purposes, which will be used by a nearly a thousand of current undergraduate and graduate students engaged in biological research.

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Thermo Fisher Scientific Vilniussite (Thermo Fisher Scientific Baltics, UAB) has world-class capabilities inmanufacturing products for the life science market, specifically in molecular,protein, and cellular biology, and has an outstanding research and development(R&D) center, focused on the development of new products in all aspects ofmolecular, protein, and cellular biology.

Currently there are almost 100researchers employed at the Vilnius site, making it one of the biggest privateR&D centers in the Baltic region.

In 2013 Thermo Fisher Scientific has received the Award of Socially Responsible Business in Lithuania, thecompany was selected as the Most Innovative U.S. company in Lithuania. In 2012 the company has beennamed Employer of the Year in Lithuania.

EUROMEDIC Lietuva *Sveikata - svarbiausia*

Medea Diagnostika Group (MDG)

In October 2011, Euromedic began operations in Lithuania after its acquisition of Medea Diagnostika Group (MDG), the leading diagnostics imaging services company in the Baltic States. The acquisition included seven diagnostics services centers located in four major cities -- the capital city of Vilnius, Kaunas and Panevezys with two centers each, and a center in both Siauliai and Druskininkai.

Now known as Medea Diagnostics, the centers provide a variety of services including Magnetic Resonance Imaging (MRI), Computed Tomography (CT), X-Rays, Mammography, Ultrasound examinations and medical specialist consultations.

Today, Medea Diagnostics is one of the most successful medical diagnostic services institutions in Lithuania. It holds one third of the MRI market in Lithuania in terms of installed number of MRI scanners and more than 60 percent of the market in terms of accomplished MRI exams. The success of the company is determined by more than 100 employees, approximately a quarter

of whom are highly-skilled and nationally-respected radiologists and another quarter who are highly-qualified radiology technicians.

Medea Diagnostics is dedicated to providing informative and timely medical care to all citizens of the Republic of Lithuania. Through its network of centers, Medea Diagnostics aims to be the premiere provider of the most technologically-advanced medical diagnostic services in Lithuania, where high-quality healthcare and accessibility are always ensured.

UAB "Grida" – takes care of all laboratories situated in Lithuania for more than 20 years

Currently Grida renders services for more than 200 Lithuanian laboratories.

The company cooperates with more than 30 leading global manufacturers, including such well-known trademarks as „Eppendorf", „Tecan", "GE life sciences", „Hitachi Chemical Diagnostics" „Alfa Aesar", „Biochrom", etc.

The product range of UAB „Grida" consists of more than 400,000 products.

The company has a team of high-qualified personnel consisting of biologists, biochemists, biophysicists, chemists, medics, pharmacists.

Following the highest management standards (ISO 9001:2008/ LST EN ISO 9001/:2008, GDP) and long-term experience in the field of international work UAB "Grida" renders the following services:

- Consulting on equipment of laboratories;
- Designing of laboratories;
- Selection of complete equipment with components and ma-

terials;
- Delivery and installation of equipment and training of laboratory personnel;
- Securing of constant supply of required materials and single-use facilities;
- In collaboration with the most famous scientific institutes it organizes various trainings at the "Training Centre for Biotechnologies and Biomedicine";
- Organization of international seminars – conferences.

The company clearly understands the importance of science and technologies for future generations, and constantly supports Lithuanian training and scientific institutions.

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