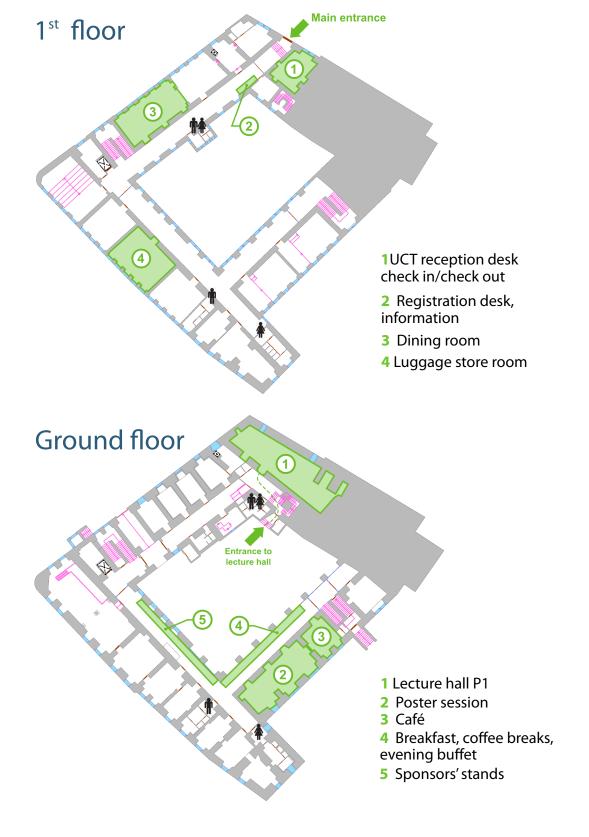
CEITEC PHD RETREAT II

20 - 21 April 2017 University Centre Telč Czech Republic



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Book of Abstracts CEITEC PhD Retreat II

20-21 April 2017

Telč, Czech Republic

Editors:

Jan Poduška Kateřina Černá Jelena Pejović-Simeunović Michaela Fojtů Adam Obrusník Zdeňka Lipovská Monika Sieberová

Masaryk University

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Welcome address

Dear students, dear invited speakers, dear friends of the CEITEC PhD Retreat

We are delighted to welcome you to the second issue of the CEITEC PhD Retreat, a two-day conference organized exclusively by students for students under the patronage and finacial support of the Central European Institute of Technology (CEITEC). Since CEITEC itself was built on the idea of creating an environment for high-level research and interdisciplinary scientific collaboration, the conference covers a relatively broad range of topics. In its three panels, Genomics and biomedicine, Material science and biosensing, Agriculture and food science, you will have a chance to see 90 contributions by PhD students as well as 7 invited talks by established experts.

The PhD Retreat brings together students from diverse fields of science to give them the possibility to gather inspiration, make new friends and present their own research in an informal and friendly environment. Though there might be a barrier to communication between researchers from different fields of science, we strongly encourage everyone to engage in discussions with others, to try to understand each other's language and to learn from each other.

We wish you a pleasant and inspiring retreat in Telč and would like to thank you for helping us to establish this event.

Your organizing committee

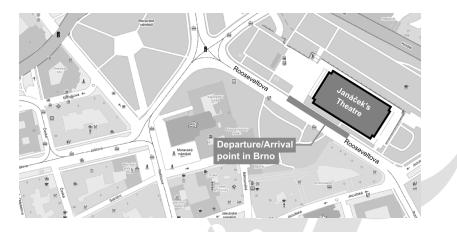
Kateřina Černá Michaela Fojtů Zdeňka Lipovská Adam Obrusník Jelena Pejović-Simeunović Jan Poduška Monika Sieberová

Practical information

» Transportation

A direct bus connection from Brno to Telč is provided by the organizers of the conference for all participants. **It is free of charge**. The time schedule of the transportation is the following:



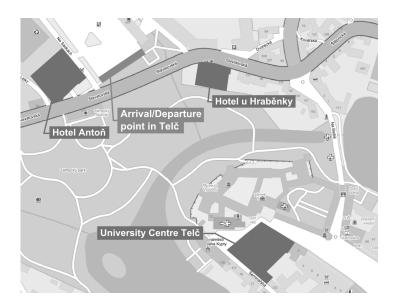


» Accommodation

Accommodation for most of the participants will be provided in the upper floors of the **University Centre Telč** (www.uct.muni.cz), which is also the conference venue. The invited speakers and the rest of the participants will be accommodated in the **Hotel U Hraběnky** (www.hotel-uhrabenky.cz) and at **Hotel Antoň** (en.hotel-anton.cz). Both hotels are just a five-minute walk from the conference venue (see the map below). We tried our best to follow the accommodation preference specified at the registration.

The participants are kindly requested to **check in at the registration desk of UCT or their hotel on Thursday**, 20 April during the extended coffee break (16.00-17.30). A storage room to store the luggage until then will be provided.

The **latest checkout time** from the UCT is 9.00. The latest checkout time for Hotel u Hraběnky and Hotel Antoň is 11.00. It will be possible to leave the luggage in the UCT luggage room after the checkout.



» Venue

The University Centre Telč (UCT, www.uct.muni.cz), owned and run by Masaryk University Brno, is a modern venue for education purposes – seminars, conferences, summer courses, teambuilding etc. It is placed in the historical heart of Telč, which is famous for its unique renaissance and baroque architecture. Since 1992, Telč has been a part of **UNESCO World Heritage**. The talks, coffee breaks and the poster session with the evening buffet will take place on the ground floor of the UCT. The registration and lunch will take place on the 1st floor. See the enclosed plan of the UCT.

» Meals

UCT, Hotel u Hraběnky and hotel Antoň will all provide breakfast on Friday morning. Lunches will be served in the dining room of the UCT. The coffee breaks and the evening buffet will be provided in the UCT's hallway on the ground floor.

» Poster session

The student poster session will take place on **Thursday, 20 April from 19.00 until 21.00**, social evening will continue after. The participants are strongly encouraged to place their posters as early in the morning as possible (right after the registration or during one of the coffee breaks). The recommended format of the posters is Ao, but it is also possible to mount smaller posters to the poster stands.

The official poster session will take 2 hours. We advise students who will be assigned odd poster numbers (1,3,5,...), to be present at your poster during the first hour and the students with even poster numbers (2,4,6,...) to be present at your posters in the second hour so that everyone has time to both present and have a look at other posters. The poster session will subsequently transfer to the whole-evening social event, where you can continue to discuss research and other topics.

By being selected for a poster, students are automatically enrolled in the **TESCAN Best Poster Award**, in which other students and our invited speakers will vote for the best poster. The winners are in for a valuable prize. The voting will be done via voting tickets. Every student will get 2 poster votes, which can be used to support his/her two favorite posters (or just one poster). By the end of the poster session, the tickets will be thrown into a ballot box located at the door to the poster room. The results of the poster session will be announced just before the closing of the conference. In case of a draw, the better poster will be chosen by the speakers.

» Talks

The talks will take place in the lecture hall on the ground floor of UCT. The **allocated time for a student talk is 10 minutes with 2 minutes of discussion**. Invited talks should not exceed 30 minutes. The equipment for the presentation (a PC, microphones and a laser pointer) will be provided.

A contest for the best student talk, similar to the best poster contest, will take place. Every participant will get 1 vote to support his/her favorite talk of the whole event. The author of the best talk will be also awarded a valuable prize.

» Internet Connection

The UCT offers wi-fi connection via **Eduroam** and also a free wi-fi during the conference. ID and password of the free wi-fi network are the following:

ID: UCTelc password: UCTkonference

Both Hotel u Hraběnky and Hotel Antoň offer their own free wi-fi connection.

» Emergency Phone Numbers

The emergency phone number is 112.

» Insurance

The organizers of the event do not accept liability for any injury, loss or damage, arising from accidents or other situations during the event. Participants are, therefore, advised to arrange accident and health insurance.

» Programme changes

The organizers cannot assume liability for any changes in the programme due to external or unforeseen circumstances.

» Contact information

Michaela Fojtů, email: michaelafojtu@gmail.com, phone: +420 737 379 079 Jan Poduška, email: poduska@ipm.cz, phone: +420 723 675 741 Adam Obrusník, email: adam.obrusnik@gmail.com, phone: +420 776 185 170

Invited talks

Abstracts and speaker biographies

Prof. Jan Černý, PhD

Group Leader, Head of Department of Cellular Biology Laboratory of Cellular Immunology Charles University Prague E-mail: cerny2@natur.cuni.cz www.natur.cuni.cz/biologie/bunecna-biologie/ osobni-stranky/jan-cerny



Biography

Jan Černý gained his PhD in Immunology at Charles University, Prague in the lab of Professor Václav Hořejší. His nowadays research interests consist not only of studying the secondary metabolites of microbes and fungi, their characterisation and biomedical application but include also using knock-in mice models for studying dynamics of endosomal system and localization of antigen-presenting cells in situ and characterising the dual probes (MRI/fluorescence) suitable for studying the pathology and physiology of tissues.

During his postdoctoral stay at Harvard Medical School, Harvard University he also dealt with other immunological and *in vivo* imaging techniques. As a highly appreciated lecturer he was awarded a lot of prizes not only for Scientific Research (Academy of Science, 2005; NATO Science Fellowship, 2000) but also as an excellent pedagogue (Siemens Prize for the best University Pedagogue, 2014; Silver Medal of Charles University, 2016).

Panel: Innovations in Genomics and Biomedicine

Chemical wars

Jan Černý

Laboratory of Cellular Immunology, Faculty of Science, Charles University, Viničná 7, Prague, Czech Republic

Many microorganisms live in multispecies communities under extreme competition. During the long-term optimization of chemical interactions in complex ecosystems, mixtures of synergistic biologically active chemicals evolved, with some of them being used as traditional medicines in many cultures. It is therefore not surprising that large numbers of potential drugs in clinical trials are either natural products or compounds derived from natural products. Given that, since the 1940s, 175 small molecules have been approved worldwide for the treatment of cancer, research on natural product-based therapeutics is obviously one of the most promising approaches. Lecture will be focused on the molecular mechanisms of action of various natural compounds as promising tools for cell biology or therapeutics, including anticancer ones.

Cristina R. Reschke, PhD

Senior postdoctoral Researcher Royal College of Surgeons in Ireland Department of Physiology & Medical Physics 123 St Stephen's Green Dublin 2, Ireland E-mail: cristinarreschke@rcsi.ie www.researchgate.net/profile/Cristina_Reschke



Biography

Cristina is a highly valued researcher in the field of epilepsy and neurosciences (Awards: Neuroscience Ireland Travel Award winner 2016; Harinarayan Young Neuroscientist Award – ILAE 2015) and one of the collaborators of the EU-funded project EpimiRNA with 16 partners from eight European countries, the USA and Brazil dealing with microRNAs (miRNAs) in epilepsy.

Cristina's education and training was as a Pharmacist and Biochemist, with a Master's Degree in Pharmacology and PhD in Pharmacology (Federal University of Santa Maria, Brazil). After her MSc training with in vivo model of methylmalonic academia, an inherited metabolic disorder characterized by neurological dysfunction and seizures, her PhD research focused on studying effects of neuroinflammation in epilepsy and effects of early-life exposure to toxins. As a part of her PhD studies, she was awarded a Visiting Scientist Fellowship and joined Dr Annamaria Vezzani's team, a world authority on neuroinflammation and treatment of epilepsy (Mario Negri Institute for Pharmacological Research, Milan, Italy).

Nowadays she is a part of the research team of Professor David C. Henshall in Ireland, who has pioneered a series of discoveries on the role of miRNAs in experimental and human epilepsy. Major Research of the group focuses on non-coding RNA (including miRNAs) in the development and treatment of epilepsy, the modelling and treatment of neonatal seizures, ATP-gated ion channels as targets for seizure control, and molecular biomarkers of epilepsy.

Panel: Innovations in Genomics and Biomedicine

Locked nucleic acid antagomirs as therapy for epilepsy

Cristina Reschke

Department of Physiology & Medical Physics, Royal College of Surgeons in Ireland, Dublin, Ireland

Epilepsy is a common, serious neurological disease characterized by recurrent seizures. Current anti-epileptic drugs (AEDs) act on a limited set of neuronal targets, are ineffective in a third of patients with epilepsy, and do not show disease-modifying properties. Evidence has emerged that a class of small, non-coding RNA called microRNA (miRNA) is important in the pathogenesis and potential treatment of epilepsy. MicroRNA-134 is involved in controlling neuronal microstructure and brain excitability and previous studies showed that intracerebroventricular injections of locked nucleic acid (LNA), cholesterol-tagged antagomirs targeting microRNA-134 (Ant-134) reduced evoked and spontaneous seizures in mouse models of status epilepticus. Translation of these findings would benefit from evidence of efficacy in non-status epilepticus models and validation in another species. Recently, we reported that electrographic seizures and convulsive behavior are strongly reduced in adult mice pre-treated with Ant-134 in the pentylenetetrazol model. Pre-treatment with Ant-134 did not affect the severity of status epilepticus induced by perforant pathway stimulation in adult rats, a toxin-free model of acquired epilepsy. Nevertheless, Ant-134 post-treatment reduced the number of rats developing spontaneous seizures by 86% in the perforant pathway stimulation model and Ant-134 delayed epileptiform activity in a rat ex vivo hippocampal slice model. The potent anticonvulsant effects of Ant-134 in multiple models may encourage pre-clinical development of this approach to epilepsy therapy.

\$ CEITEC

Stefan Baudis, PhD

Senior Scientist Technische Universität Wien Getreidemarkt 9/163, 1060 Wien E-mail: stefan.baudis@tuwien.ac.at www.researchgate.net/profile/Stefan_Baudis



Biography

Stefan Baudis is since 2015 senior scientist at the Institute of Applied Synthetic Chemistry (TU Vienna) in the research group of Robert Liska. He studied technical chemistry at the TU Vienna and specialized in synthesis and processing of polymeric biomaterials with a special focus on lithography-based additive manufacturing technologies during his doctorate. In 2011 followed a postdoc stay at Andreas Lendlein's Institute for Biomaterial Science of the Helmholtz-Zentrum Geesthacht in Teltow near Berlin. His main interest is in the development of functional, additive manufactured hydrogel system for applications in the field of biomedicine.

Panel: Nature inspired materials and biosensing

Lithography-based 3D printing of biocompatible polymers

Stefan Baudis

Technische Universität Wien, Getreidemarkt 9/163, 1060 Wien, Austria

Lithography-based additive manufacturing technologies ("3D printing") enable the generation of arbitrary, highly complex 3D structures with very high resolution. This was one of the main aspects, which attracted the interest of modern medicine. The vision is to be able to fill defects in tissues or whole organs with tailor-made, patient-specific, biocompatible polymeric constructs, which promote the regeneration of the tissue or the organ.

Lithography-based additive manufacturing technologies (L-AMTs) base on the curing of liquid formulations in layer-by-layer fashion by light. Crucial components of such formulations are the photo-curable monomer that bears polymerizable groups, and the photoinitiator that initiates the polymerization by the generation of radicals when exposed to light.

For the design of photopolymers as tissue regeneration scaffolds it is important to consider the mechanical properties of the material (which ideally matches with the properties of the tissue), the characteristics of biodegradation (which should be synchronized with the regeneration of new tissue), and – of course – the biocompatibility, i.e. the material has to promote the attachment of cells and all components, incl. degradation products, should have a very low toxicity.

Here, different approaches to 3D print scaffolds will be presented, introducing the L-AMTs stereolithography (laser- and DLP-based, for resolutions in the range of 20 μ m) and two-photon-lithography (for resolutions > 1 μ m).

Assoc. Prof. Roman Gröger, PhD

Multiscale Modelling and Measurements of Physical Properties research group leader CEITEC, Brno, Czech Republic Institute of Physics of Materials ASCR, Brno E-mail: groger@ipm.cz www.researchgate.net/profile/Roman_Groeger www.ceitec.eu/ceitec-ipm/



multiscale-modelling-and-measurements-of-physical-properties/rg11

Biography

R. Gröger received his PhD from Engineering Mechanics at the Brno University of Technology and his PhD from Materials Science and Engineering at the University of Pennsylvania in USA. He subsequently spent three years as the Seaborg postdoctoral fellow at the Theoretical Division and the Center for Nonlinear Studies of the Los Alamos National Laboratory, USA, where he focused on understanding the phase transitions in plutonium and developing phase field models that combine plasticity and phase transitions. On receiving the Marie-Curie fellowship, he returned to the Institute of Physics of Materials of the Academy of Sciences of the Czech Republic, where he currently holds the position of senior research scientist and leads the CEITEC research group 1-11 "Multiscale modelling and measurements of physical properties". R. Gröger is an associate professor at the Department of Materials Science and Engineering of the Brno University of Technology, where he teaches the advanced course "Modelling of materials II". His research focuses primarily on developing scale-bridging concepts in physics and materials science, coupling molecular simulations with electron microscopy and understanding the mechanism of nucleation of threading dislocations in III-nitride semiconductors

Panel: Nature inspired materials and biosensing

Understanding plasticity of bcc metals via computer simulations and experiments

Roman Gröger

CEITEC and Institute of Physics of Materials, Academy of Sciences of the Czech Republic

Body-centered cubic (bcc) metals (V, Nb, Ta, Mo, W, Cr and alpha-Fe) are crystallographically simple materials whose plastic deformation is nevertheless governed by processes that are not common in face-centered cubic (fcc) metals. These differences are rooted at the atomic level, where the cores of 1/2<111> screw dislocations in bcc metals are inherently nonplanar, which contrasts the plane cores of dissociated 1/2<110> dislocations in fcc crystals. Due to the nonplanar spreading of the former dislocations, their motion is not affected only by the shear stress in the 110 slip plane acting parallel to the slip direction (the Schmid stress) but, in principle, by all components of the stress tensor. These highly nontrivial dependencies were obtained by molecular statics simulations of isolated screw dislocations using accurate semi-empirical potentials. The link between the atomistic studies of isolated screw dislocations and macroscopic response of the material is established by formulating a model of thermally activated slip that predicts correct temperature dependence of the flow stress. Both models serve to develop a mesoscopic model that allows to study the evolution of texture in evolving dislocation networks. The results of molecular statics simulations have been used to observe for the first time the structure of screw dislocations by optical sectioning using high-angle annular dark field (HAADF) in STEM conditions. Direct verification of the theory is now under way using the slip trace analysis on samples of different orientations deformed at 77 K.

Prof. Petr Hořín, CSc.

Animal Immunogenomics research group leader CEITEC, Brno, Czech Republic Dept. of Animal Genetics Faculty of Veterinary Medicine Palackého 1/3, 612 42 Brno, Czech Republic E-mail: horinp@vfu.cz www.ceitec.cz/ceitec-vfu/imunogenomika-zvirat/rg26



Biography

Full professor in genetics at the Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno since 1999. Ex-dean of the faculty (1993-2000), Head of the Department of Pathobiology and of its the Institute for Animal Genetics. Research programme coordinator for Molecular Veterinary Medicine (RP7) of the Central European Institute of Technology (CEITEC), research group leader of the RG Animal Immunogenomics.

Teaches undergraduate and/or graduate students at the Faculty of Veterinary Medicine (Clinical Genetics), Faculty of Veterinary Hygiene and Ecology (Special Genetics), Faculty of Pharmacy (Applied Genetics), Faculty of Science (Animal Genetics, Immunogenetics and Immunogenomics). Supervisor of PhD and undergraduate students of three faculties.

The main research topic is genetics of animal health, especially immunogenetics, genetics of disease susceptibility. The CEITEC research group focused on analyses of genetic diversity, evolution and selection of complex genomic regions, like the major histocompatibility complex and natural killer cell receptor genes, and their associations with model diseases in equids, camelids and dogs.

Panel: Sustainable agriculture and healthy food

Host genetic susceptibility to infectious diseases

Petr Hořín

CEITEC VFU, RG Animal Immunogenomics, University of Veterinary and Pharmaceutical Sciences Brno

Although pathogens are the primary cause of infectious diseases, they are not the only factor involved in their pathogenesis. Host's defense reactions contribute to the risk, clinical symptomatology and the final outcome of infections. High levels of individual genetic variation in susceptibility to certain infections can be observed in humans as well as in animals. With the advent of genomic techniques, not only individual genes but also complex genetic mechanisms underlying host susceptibility to infections could be studied. Identification of specific mutations with strong effect allowed definition of genes and alleles responsible for individual susceptibility to infection resulting from primary immune deficiencies. Genome-wide association studies revealed genes and alleles contributing to complex mechanisms of host genetic susceptibility. In broader terms, evolutionary aspects of the host genetic susceptibility to infections can be investigated. Pathogens are considered to be one of the major driving forces of evolution. The knowledge of genes selected by previous infections, of signatures of host and pathogen interactions in the genomes of both types of these organisms as well as studies of host population diversity driven by pathogens can contribute to our understanding of the emergence of new pathogens and of their spread. A large proportion of human infections originated from wild and/or domestic animals. In this context, animal models represent a well suited tool for studying genetic mechanisms of infectious diseases. In our group, we study complex genomic regions involved in host and pathogen interactions in model domestic animal species. Selected examples documenting such interactions at the molecular and population level will be presented.

Hélène Robert Boisivon, PhD

Hormonal Crosstalk in Plant Development research group leader CEITEC, Brno, Czech Republic E-mail: helene.robert.boisivon@ceitec.muni.cz www.ceitec.cz/ceitec-mu/ interakce-hormonalnich-drah-ve-vyvoji-rostlin/rg47



Biography

Hélène Robert-Boisivon studied Medicine and Animal and Plant Biology and Geology at the University of Rouen in France. In 2000, she started as a research trainee under supervision of Dr. Frédéric Berger in the laboratory of Reproduction and Development of Plants at the Ecole Normale of Lyon where she studied Differentiation, Genetics and Immunology as MSc. student. In 2002, she worked one semester as lab technician in the lab of Dr. J. Haseloff at the Plant Sciences Department at Cambridge University in United Kingdom and started her PhD studies at the Cellular Development Laboratory at Leiden University in Netherlands under the supervision of Dr. R. Offringa. In 2007, she accepted a postdoc position in the Auxin group at the Plant System Biology Department of the Flemish Institute of Biotechnology in Ghent, Belgium, where she worked in Prof. Jiří Friml team. She moved to Brno to work at CEITEC-MU in 2012, in the Developmental and Cell Biology group (at the time headed by Prof. J. Friml). In 2014, she obtained a SOMOPROII fellowship (the South Moravian Programme for Distinguished Researchers) in the group Hormonal crosstalk in plant development at CEITEC-MU in Brno where she worked on regulation of the local auxin production in Arabidopsis thaliana in order to uncover the mechanisms of polarity axes establishment mediated by auxin during embryo development. She becomes junior group leader of the same group in 2014.

Panel: Sustainable agriculture and healthy food

The plant hormone auxin and its importance for seed and embryo development in Arabidopsis

Helene Robert Boisivon, PhD CEITEC MU, Brno, Czech Republic

Plant reproduction relies on well-defined and coordinated series of cell division and cell differentiation of the zygote. The zygote, and later the embryo, are embedded into maternal-derived integuments, whose influence on embryo development remains elusive. The plant hormone auxin is known to play a crucial role in defining the embryonic body axis. And dynamic transport of the hormone by auxin efflux carriers, PIN proteins, is involved in specifying embryonic shoot and root poles. Moreover TAA1/YUCCA-dependent auxin biosynthesis pathway has also an essential function during embryo development, as assayed by the embryonic phenotypes of certain combinations of loss-of-function mutations in these genes. Local auxin production, with feedback on auxin transport, influences different steps of embryo development. An increase of auxin production in the maternal integuments followed by auxin transport from those integuments to the zygote is important for the first asymmetric division and specification of the apical embryonic pole. At later globular stages, a new auxin source in apical cells of the embryo triggers polarization of the auxin transport to the basal pole for a proper formation of a root. Altogether these data propose a model integrating the dynamic behavior of auxin production, and its influence on the hormone transport, for the proper development of the embryo.

Acknowledgement

This work is supported by project SoMoPro II 3SGA5602 and by the Ministry of Education, Youth and Sports of the Czech Republic under the project CEITEC 2020 (LQ1601).

Prof. Renata Veselská, PhD

Professor Department of Experimental Biology, Faculty of Science Masaryk University, Brno, Czech Republic E-mail: veselska@sci.muni.cz www.veselska.me



Biography

Renata Veselská gained her PhD in Genetics at Faculty of Sciences at Masaryk University (MU). She gained postdoctoral experience as a Visiting Fellow at Georgetown University Medical Center in Washington DC in 2002. Later, she completed the Bioethics M.Sc. Program with specialization in Research Ethics at Clarkson University (formerly Union Graduate College) and Mount Sinai School of Medicine (NY, USA). She is also alumna of the Advanced Certificate Program -Research Ethics in Central and Eastern Europe (Fogarty International Center NIH, USA) and of the Intensive Bioethics Course (Georgetown University, Washington DC, USA). Currently, she is the chairperson of the Research Ethics Committee at MU and member of Society of Medical Ethics. Her research interests are focused on tumor biology, especially on solid tumors in children. She is head of the Laboratory of Tumor Biology, which is a joint research unit of the Department of Experimental Biology and Department of Pediatric Oncology. In 2015, her research group also joined the International Clinical Research Center, St. Anne's University Hospital (FNUSA-ICRC). In the field of bioethics, she is particularly interested in ethical aspects of biomedical research, especially of human genetics and cell technologies.

Panel: Extra talk

Don't Panic: The Hitchhiker's Guide to the Research Ethics

Renata Veselská

Department of Experimental Biology, Faculty of Science, Masaryk University, Brno, Czech Republic

Main aspects of the responsible conduct of research will be presented and discussed in the first part of this lecture: scientific misconduct, authorship and peer review, publication ethics, etc. The second part will be focused on the most important ethical issues in biomedical research including human subjects, human biological samples, laboratory animals and GMOs. The last part will be a brief practical overview how to survive the first contact with the institutional Research Ethics Committee.

Genomics and biomedicine

Students' abstracts



Po1: Molecular mechanisms of cross-talk between phytochrome A-mediated light signaling and cytokinin transduction pathway in Arabidopsis

<u>Veronika Balakhonova</u>¹⁺, Tereza Dobisová^{1,2+}, Klára Panzarová², Radka Kočí², Martin Trtílek² and Jan Hejátko¹

¹Functional Genomics and Proteomics of Plants, CEITEC - Central European Institute of Technology and National Centre for Biomolecular Research, Masaryk University, 62500 Brno, Czech Republic ²Photon Systems Instruments, 664 24 Drasov, Czech Republic ⁺contributed equally

Plant hormones cytokinins control plant growth and development from seed germination to leaf senescence. Cytokinin-mediated control of plant development on is largely affected by light, however, the mechanism of cytokinin/light crosstalk is mostly unknown. Cytokinin signaling is mediated via multistep phosphorelay (MSP) pathway. In Arabidopsis MSP, the sensor histidine kinase recognize cytokinins and initiate the downstream phosphorelay. Upon addition of cytokinins, the expression of A-type ARRs, the cytokinin primary response genes is initiated very promptly, providing thus a readout of MSP activity.

Here we show that one of the A-type ARR genes, ARR16, was upregulated in etiolated seedlings by far-red (FR) light irradiation. The impact of FR on ARR16 was confirmed via imaging of ARR16-mTQ reporter line. We show that the effect is mediated by the action of photoreceptor phytochrome A, suggesting a link between light and cytokinin signaling. To show the developmental importance of the regulation, we apply a novel method for the non-invasive real-time measurements of the chlorophyll content to show the interaction between cytokinin and light signaling during early stages of plant deetiolation.

Acknowledgement

Supported by Czech Science Foundation (15-22000S), Czech Biolmaging and CEITEC 2020 (LQ1601).

\$\$ CEITEC

To1: miRNA profile of mesial temporal lobe epilepsy

<u>Petra Bencurova</u>^{1,2,3}, Jiri Baloun¹, Katerina Musilova^{1,3,4}, Lenka Radova¹, Martin Pail^{2,3}, Marketa Hermanova^{2,3}, Sarka Pospisilova^{1,3,4}, Marek Mraz^{1,3,4}, Milan Brazdil^{1,2,3}

¹CEITEC, Masaryk University, Brno, Czech Republic
 ²St. Anne's University Hospital, Brno, Czech Republic
 ³Medical Faculty, Masaryk University, Brno, Czech Republic
 ⁴University Hospital Brno, Masaryk University, Brno, Czech Republic

microRNA (miRNA/miR) is a master regulator of gene expression. It is a short noncoding RNA that mediates protein production on posttranscriptional level. The expression of miRNAs is a dynamic response of a cell to the changes in the environment and its inner needs. Fine balance in miRNA production is essential for proper function of the cell and the aberrations in this control machinery might lead to various pathologies. For this reason, miRNAs are widely studied as potential biomarkers or targets of treatment in numerous diseases (cancer, cardiovascular diseases, neurological disorders, etc.). Similarly to other neurological disorders is the origin and progression of epilepsy not completely understood. miRNAs might prove as a useful tool to address this problem. In our project, we have focused on the analysis of miRNA profile in hippocampal tissue of patients with mesial temporal lobe epilepsy, the most common type of epilepsy. We have identified 20 miRNAs with altered expression in epileptic brain tissue. Putative targets those miRNAs participate in the pathways associated with epilepsy which suggests possibility of future miRNA based therapeutics.

Acknowledgement

This work was supported by GACR (GAP16-04726S), CEITEC 2020 (LQ1601) with financial support from the Ministry of Education, Youth and Sports of the Czech Republic under the National Sustainability Programme II, European Union's Horizon 2020 grant progremme (grant agreement No 692298) and by MUNI/A/1044/2015.

Po2: ATR-Chk1 pathway as a therapeutic target for leukemia and lymphoma

Miroslav Boudný¹, Alexandra Oltová¹, Kamil Paruch², Martin Trbušek¹ ¹Department of Internal Medicine – Hematology and Oncology, University Hospital Brno and Faculty of Medicine, Masaryk University, Brno, Czech Republic ²Department of Chemistry, Groeperson, Faculty of Science, Masaryk University, Brno, Czech Papublic

²Department of Chemistry, CZ Openscreen, Faculty of Science, Masaryk University, Brno, Czech Republic

During the last decade, discovery of innovative therapeutics targeting components of the DNA damage signaling and repair machinery offered a possibility for significant improvements in cancer patients' therapy. The ATR-Chk1 pathway, a critical supervisor of replication, seems to be one of such appropriate targets due to intact respective genes (ATR and CHEK1) indicating their essential role in tumor cell survival (1). We tested the potential of this pathway inhibition in leukemia and lymphoma cell lines using specific inhibitors for the Chk1 and ATR proteins (MU380 and VE-821, respectively). All tested leukemia and lymphoma cell lines (n=16) responded by a clear viability decrease after the Chk1 inhibition, which was in contrast to healthy cells derived from blood and supportive tissues (n=6). The Chk1 inhibition showed effectiveness irrespective of the TP53 defects presence. After the inhibition, we noted an increase of Chk1 phosphorylations on Ser317 and Ser345 residues presumably performed by ATR as well as an effective elimination of the pSer296 autophosphorylation, a marker of Chk1 downstream activity. Importantly, the effects of Chk1 inhibition on cell viability and genome integrity were enhanced by simultaneous ATR inhibition. This observation was in line with apparent ATR activity elicited by Chk1 inhibition during a cell attempt to cope with impaired replication control. Our results support a potential of the ATR-Chk1 pathway inhibition in treatment of leukemia and lymphoma.

Acknowledgement

Supported by MUNI/A/1106/2016.

References

[1] Manic, G., Obrist, F., Sistigu, A. & Vitale, I. Trial Watch: Targeting ATM-CHK2 and ATR-CHK1 pathways for anticancer therapy. Mol. Cell. Oncol. 2, e1012976 (2015).

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Po3: Definition of the driver reaction time

Olga Vallová¹, Veronika Svozilová², Kateřina Bucsuházy³, Ivo Stáňa³

¹CEITEC - Central European Institute of Technology, Brno University of Technology, Purkyňova 123, 612 oo Brno, Czech Republic.

²Brno University of Technology, Department of Biomedical Engineering, Technická 12, Brno 61200, Czech Republic

³Brno University of Technology, Institute of Forensic Engineering, Purkyňova 464-118, Brno 61200, Czech Republic

Nowadays, driver reaction time is a main characteristic describing a driver behavior, therefore driver reaction time is the most important driver characteristic in the field of the traffic accident. Driver reaction time is divided into three main parts (visual, psychical and physical reaction time) and their duration is a discussed topic among experts of the traffic accident field. Mostly, the duration of the driver reaction time (or its visual part) was investigated and evaluated from eyetracking measurements which can define the visual part of the reaction time accurately. But not all parts of the driver reaction time can be defined via eyetracking method. The aim of this article is to introduce the new approach to defining the physical part of the driver reaction time by combination of eyetracking and electromyography methods. Drivers are monitored during their driving in the real traffic. The eyetracker was mounted on the driver head and signals of seven muscles of the right lower limb involved in the movement of braking were acquired to determine the physical part of the driver reaction time. The results should offer a more accurate definition of the physical part of driver reaction time and its dependence on the type of stimuli dealt with by driver while driving.

Acknowledgement

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To2: Transcriptome-wide approaches revealed m6A demethylase FTO as a regulator of nuclear mRNA processing events

<u>Helena Covelo-Molares</u>¹, Marek Bartosovic¹, Pavlina Gregorova¹, Grzegorz Kudla², Stepanka Vanacova¹

¹CEITEC – Central European Institute of Technology, Brno, Czech Republic ²MRC Human Genetics Unit, Edinburgh, UK

N6-methyladenosine (m6A) is the most prevalent internal messenger RNA (mRNA) modification and, since the discovery of the m6A demethylase FTO (so called 'eraser'), the first example of reversible RNA methylation. On the functional level, m6A regulates mRNA fate -stability, translation or splicing- and it plays a role in mammalian cell differentiation. Whereas the m6A methylase complex has been extensively studied, the m6A erasers remain largely unexplored. In my laboratory, we performed FTO cross-linking and immunoprecipitation coupled to high-throughput sequencing (CLIP-Seq) to uncover the RNA targets of FTO. Additionally, we analyzed by RNA-Seq the gene expression changes after depletion of FTO (FTO *knockout*) on a mammalian cell line. By combining these two approaches, we discovered that FTO regulates nuclear mRNA processing rather than gene expression and that preferentially binds to intronic regions of pre-mRNAs, around alternative spliced (AS) exons and in the proximity of poly(A) sites. Our study contributes to the understanding of m6A-regulated processes pointing to splicing and 3' end processing as the major nuclear functions of m6A demethylase FTO.

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Po4: Effects of ethylene and its inhibitor 1-methylcyclopropane on *Arabidopsis* roots under defined atmosphere

Abigail Rubiato Cuyacot¹, Marketa Zdarska¹, Martin Trtilek² and Jan Hejatko¹ ¹Functional Genomics and Proteomics of Plants, CEITEC - Central European Institute of Technology and National Centre for Biomolecular Research, Masaryk University, Kamenice 5, Brno, Czech Republic ²Photon Systems Instruments, 664 24 Drasov, Czech Republic

Ethylene (C_2H_4) is a simple gaseous phytohormone that is involved in many aspects of the plant life cycle such as seedling growth, leaf, root, stem and flower development, fruit ripening and organ senescence. 1-Methylcyclopropene (1-MCP) on the other hand is a synthetic plant growth regulator, a volatile gas at standard temperature and pressure. It has a similar structure with ethylene and is known to inhibit ethylene perception based on its stable binding to the ethylene receptor in plants, thus blocking the effects of ethylene. In this study, we employed 1-MCP to examine the role of ethylene inhibitor in the development of *Arabidopsis* roots.

We have established a cultivation system operated by gas mixing system (GMS) allowing plant growth under defined atmosphere via controlled air/N2 flow, concentration and pressure inside sealed chambers. Ethylene gas is incorporated under GMS 150 ($N_2 + C_2H_4$) and on the contrary, 1-MCP is slightly handled in a different manner since it is a powder that releases its gas when dissolved in water. Dissolved 1-MCP powder is directly added inside the sealed chamber with non-stop ventilation (air/N2 flow) administered by GMS.

We evaluated C_2H_4 and 1-MCP responses in the roots of *Arabidopsis thaliana* via measurements of the root apical meristem (RAM) size of young seedlings and via various ethylene-responsive fluorescent reporter lines. Effects of ethylene and 1-MCP at given concentration and pressure can be observed directly followed 24hr treatment. Our results implied these gases to have opposing effects in terms of RAM sizes on *A. thaliana* lines tested. Mutant lines that are unable to bind C_2H_4 were found to be insensitive with 1-MCP as well. In addition, 1-MCP is observed to diminish the ethylene-responsive reporter lines expression when compared to the C_2H_4 per se. Details on the methodology will be presented and further information on the ethylene-mediated responses and their role in the root development will be shown.

Acknowledgement

Supported by Czech Science Foundation (13-25280S), RIAT-CZ, Czech Biolmaging and CEITEC 2020 (LQ1601).

Po5: BreakingPoint: an effective purpose-build tool for fusion genes identification from Target Capture NGS datasets in pediatric acute lymphoblastic leukemia

<u>Andrea Grioni</u>^{1,2}, Nikos Darzentas², Giovanni Cazzaniga¹ and Vojtech Bystry² ¹CEITEC - Central European Institute of Technology, Masaryk University, Brno, Czech Republic ²Tettamanti Research Center, San Gerardo Hospital, Monza, Italy

The most common childhood cancer is acute lymphoblastic leukemia (ALL), which is mainly characterized by chromosomal translocations leading to fusion genes. ALL fusion genes typically affect a number of recurrent genes (target) rearranged with several variable partners and specific chemotherapeutic agents act against their molecular products. Therefore, a sensitive identification of fusion genes carried by patients is crucial to design the best therapy. Target Capture Next Generation Sequencing (TC-NGS) allows a focused sequencing of genes involved in ALL; thus, this technology can be used for a fast identification of both known and new fusion genes in ALL patients. TC-NGS datasets have features that require an increased sensitivity of bioinformatics tools for fusion genes detection. We developed BreakingPoint, a purpose-build tool for fusion gene identification from TC-NGS datasets of any biological material. BreakingPoint was tested on real data of patients resulted negative for fusion genes through standard PCR-based methods at the diagnosis. Moreover, we compared BreakingPoint with TopHat[1] and Delly[2], other well-known tools for structural variant identification. BreakingPoint detected both known and new fusion genes in samples resulted negative at diagnosis and showed an increased sensitivity for fusion gene detection in comparison with TopHat and Delly. Our results indicate that TC-NGS assay in association with BreakingPoint analysis can be usefully applied for routine diagnosis in ALL.

References

[1] C. Trapnell, TopHat: discovering splice junctions with RNA-Seq, Bioinformatics, 2009;25(9):1105-11.

[2] Tobias Rausch, Thomas Zichner, Andreas Schlattl, Adrian M. Stuetz, Vladimir Benes, Jan O. Korbel, Bioinformatics 2012 28: i333-i339.

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To3: Biomacromolecular structure quality – improvement or stagnation?

Vladimír Horský¹, Veronika Bendová^{1,2}, Radka Svobodová Vařeková¹, Jaroslav Koča¹

¹National Centre for Biomolecular Research, Faculty of Science, and CEITEC - Central European Institute of Technology, Masaryk University Brno, Kamenice 5, 625 oo Brno-Bohunice, Czech Republic

²Department of Mathematics and Statistics, Faculty of Science, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

The general availability of biomacromolecular structural data is one of the most important results of modern life sciences. 13 Nobel prizes were awarded for research based on this data [1]. However, quality issues of structures has come into consideration when erroneous data caused retraction of articles from several respected journals [2]. Scientific community reacted by developing tools for validating biomacromolecular complexes. Protein Data Bank, major structural database, offers validation reports which enable users to assess several facets of database entry quality. Therefore, we became curious whether these new validation resources have any impact on quality of newly published biomacromolecules. We are also interested in ligand quality, which is more critical due to the fact that ligands do not enjoy as much care.

We have performed wide scale analysis of trends in quality and size of biomacromolecules and their ligands. 70 diverse factors have been considered. Information about structures and their quality have been obtained from the Protein Data Bank, while the state of ligand quality has been sourced by our database ValidatorDB.

Existence of some discovered trends was expected (e.g., newer structures have better quality). Other trends surprised us (e.g., ligand quality is stagnant at best, currently utilized structure validation methods do not validate ligands well). Discovered trends are openly available in the ValTrendsDB database (ncbr.muni.cz/ValTrendsDB).

References

[1] Protein Data Bank Europe web pages: Structural biology related Nobel Prizes: http://www.ebi.ac.uk/pdbe/docs/nobel/nobels.html

[2] Matthews, B.W. (2007) Five retracted structure reports: inverted or incorrect? Protein Sci., 16, 1013–1016.

To4: Reaction mechanism of mycobacterial glycosyltransferase GlfT2: computational study

Pavel Janoš^{1,2}, Stanislav Kozmon^{2,3}, Igor Tvaroška^{2,3}, Jaroslav Koča^{1,2}

¹ Faculty of Science-National Centre for Biomolecular Research, Masaryk University, Brno, Czech Republic
 ² Central European Institute of Technology (CEITEC), Masaryk University, Brno, Czech Republic
 ³ Institute of Chemistry, Slovak Academy of Sciences, Bratislava, Slovak Republic

Galactofuranosyltransferase 2 (GlfT2) is one of key enzymes involved in biosynthesis of *Mycobacterium tuberculosis* cell wall. It catalyzes the transfer of galactofuranosyl (Galf) unit from donor substrate UDP-Galf onto a growing polysaccharide chain in alternating -1-5 or -1-6 linkages. The resulting galactan is part of mycolyl-arabinogalactan complex, the largest component of the mycobacterial cell wall. GlfT2 is interesting from a mechanistic point of view as well. It has a dual activity, works in processive manner and uses galactose in a furanose form.

We use hybrid QM/MM Car–Parrinello molecular dynamics simulations in order to study the reaction mechanism of GlfT2. In this approach the key region where the enzymatic reaction is occurring is treated at a DFT level of theory (QM), while the rest of the system is described by computationally less demanding force field (MM). When coupled with string method we can explore the reaction mechanisms and obtain the free energy profile of the reaction as well as estimation of the transition state structure.

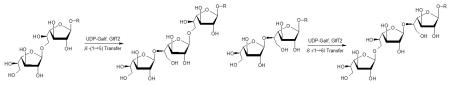


Fig. 1: Reaction catalyzed by GlfT2.

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Po6: Properties of human embryonic stem cells and their differentiated derivatives depend on non-histone DNA-binding HMGB1 and HMGB2 proteins

Alireza Jian Bagherpoor¹, Dasa Dolezalova², Tomas Barta², Martin Kučírek¹, Soodabeh Abbasi Sani¹, Milan Ešner², Michaela Kunova Bosakova³, Vladimír Vinarský², Lucie Peskova², Aleš Hampl², Michal Štros¹

¹Laboratory of Analysis of Chromosomal Proteins, Institute of Biophysics, Academy of Sciences of the Czech Republic, Brno, Czech Republic

² Department of Histology and Embryology, Faculty of Medicine, Masaryk University, Brno, Czech Republic ³ Department of Biology, Faculty of Medicine, Masaryk University, Brno, Czech Republic

HMGB1 and HMGB2 proteins have been implicated in numerous cellular processes, including proliferation, differentiation, apoptosis, and tumor growth. It is unknown whether they are involved in regulating the typical functions of pluripotent human embryonic stem cells (hESCs) and/or those of the differentiated derivatives of hESCs. Using inducible, stably transfected hESCs capable of the shRNA-mediated knockdown of HMGB1 and HMGB2, we provide evidence that the downregulation of HMGB1 and/or HMGB2 in undifferentiated hESCs does not affect the stemness of the cells and induces only minor changes to the proliferation rate, cell-cycle profile, and apoptosis. After differentiation is induced, however, the downregulation of those proteins has important effects on proliferation, apoptosis, telomerase activity, and the efficiency of differentiation towards the neuroectodermal lineage. Furthermore, those processes are affected only when one, but not both, of the two proteins is downregulated; the knockdown of both HMGB1 and HMGB2 results in a normal phenotype. Those results advance our knowledge of the regulation of hESC and human neuroectodermal cell differentiation and illustrate the distinct roles of HMGB1 and HMGB2 during early human development.

To5: Nucleoside inhibitors of tick-borne encephalitis virus: structure-activity relationships and viral resistance study

Luděk Eyer^{1,2}, Tomáš Kastl¹, Darina Zaouharová¹ and Daniel Rúžek^{1,2} ¹Department of Virology, Veterinary Research Institute, Hudcova 70, CZ-62100 Brno, Czechia ²Institute of Parasitology, Biology Centre of the Czech Academy of Sciences, Branišovská 31, CZ-37005 České Budějovice, Czechia

Tick-borne encephalitis virus (TBEV), causative agent of tick-borne encephalitis (TBE), is commonly spread across Central and Eastern Europe and is mostly transmitted to human via bite of ticks from the genus lxodes. TBEV infection manifests usually as severe meningitis, encephalitis or meningoencephalitis and may result in permanent brain damage.

At present there is no direct drug therapy against this disease. We identified 2'-C-methyl- and 4'-C-azido-substituted nucleoside analogues, which have a significant antiviral effect with only negligible cytotoxicity for cell culture, what makes them promising candidates for further therapeutic research. Additionally, a drug resistant mutant of TBEV was isolated under the selection pressure of 7-deaza-2'-C-methyadenosine. Its single mutation S603T within the active site of NS5 RNA-dependent RNA-polymerase is responsible for the high-level resistance to 2'-C-methylated nucleosides analogues. However, this mutation also caused a loss of viral fitness in cell culture and notably compromised virulence potency. Following investigation revealed the ability of the mutant to flexibly revert this mutation back to its wild-type genotype, when cultivated without the presence of the compound.

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Po7: Evolutionarily conserved biological roles of ADAR RNA editing enzymes

<u>Anzer Khan</u>¹, Dragana Vukic¹, Nagraj Sambrani¹, Simona Paro¹, Leeanne McGurk¹, Xianghua Li¹, Mary A. O'Connell¹, and Liam P. Keegan¹ ¹*CEITEC at Masaryk University, Kamenice 753/5, A35/143, 625 oo Brno, Czech Republic*

ADAR RNA editing enzymes deaminate adenosine bases to inosines in RNA. During translation inosine is read as guanosine and this editing diversifies the proteins present in the organism. In vertebrates, the editing by ADAR1 protein also helps to differentiate between self and non-self dsRNA. Recent published work from our lab on the mouse *Adar1* mutant demonstrated that ADAR1 prevents cellular dsRNA from aberrantly activating cellular innate immune responses. A mutation in human *Adar1* leads to an extreme condition known as Aicardi-Goutieres Syndrome in which children with a defective ADAR1 aberrantly express antiviral interferon and die with encephalitis.

Drosophila has one *Adar* gene which is an orthologue of vertebrate Adar2. *Adar*^{5G1} null mutant flies show locomotion defects, are male sterile and develop age-dependent neurodegeneration. There is also aberrant upregulation of transcripts encoding Anti-Microbial Peptides (AMPs), in *Adar*^{5G1} flies. *Drosophila Adar* mutant phenotypes are rescued by human Adar2 expression. Deciphering the role of Adar in Drosophila will help us to understand the evolutionarily conserved roles of ADAR2-type RNA editing enzymes.vertebrate innate immune signalling are at least partially conserved in *Drosophila melanogaster* and preliminary results indicate a potential role of CNS-associated Toll signalling in rescuing *Adar*^{5G1} mutant phenotypes.

We previously performed a genetic screen for suppressors of the reduced viability associated with the $Adar^{5G_1}$ null mutant. This screen identified a strong rescue by reduced *Tor* gene dosage of all tested *Adar* mutant phenotypes, except male infertility. We propose that $Adar^{5G_1}$ mutant cells aberrantly activate autophagy to clear virus RNA or aberrant intracellular dsRNA.

Po8: B cell receptor signaling activity is associated with genomic defects in chronic lymphocytic leukemia

<u>Helena Kočková</u>^{1,2}, Karla Plevová^{1,2}, Jitka Malčíková^{1,2}, Jana Kotašková^{1,2}, Vojtěch Bystrý¹, Veronika Mančíková¹, Martin Trbušek², Michaela Hložková², Yvona Brychtová², Michael Doubek^{1,2}, Šárka Pospíšilová^{1,2} ¹*CEITEC, Masaryk University, Brno, Czech Republic* ²*IHOK, University Hospital Brno and Faculty of Medicine, Brno, Czech Republic*

Objectives: Chronic lymphocytic leukemia (CLL) is the most common leukemia of elderly people characterized by expansion of pathological B-lymphocytes. Survival and proliferation of these leukemic cells are crucially driven by B cell receptor (BCR) signaling pathway. CLL has heterogeneous genetic background with recurrence of certain chromosomal aberrations and gene mutations. Such heterogeneity is also reflected in highly variable disease course.

Aim: We aimed to understand if and how BCR signaling is involved in accumulation of genomic defects in CLL.

Methods: Fresh frozen CLL samples, separated from peripheral blood of 78 patients, were used for BCR signaling stimulation using specific antibody. Samples were fixed, permeabilized, labeled against phosphorylated components of BCR signaling and measured by flow cytometry. BCR signaling activity was compared to the basal phosphorylation in unstimulated cells. The obtained data were correlated with genetic findings and clinical parameters.

Results: We identified a number of associations of BCR signaling activity with patients' clinical and laboratory characteristics on the level of basal and also activated phosphorylation. Generally, genomic defects are predominantly associated with lower phosphorylation of several BCR signaling components. In particular, phosphorylation of upstream kinases ZAP and SYK was most prominently associated with del11q, and mutations in *NOTCH1* and *TP53*.

Conclusion: Our findings provide further evidence for functional crosslink between BCR signaling activity and genomic defects in CLL.

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Pog: Simulation of AML clonality in NSG mouse

Zdenka Kosarova¹, Martin Culen^{1,2}, Ivana Jeziskova², Adam Folta², Jana Chovancova^{1,2}, Tomas Loja³, Nikola Tom^{1,3}, Dana Dvorakova^{1,2}, Zuzana Sustkova², Lukas Semerad² and Zdenek Racil^{1,2,3}

¹ Faculty of Medicine, Masaryk University, Brno, Czech Republic

² University Hospital Brno, Czech Republic

³CEITEC, Masaryk University, Brno, Czech Republic

Acute myeloid leukemia (AML) is often defined by distinct clonal hierarchy. Mutational screening in patient-derived xenografts (PDX) allows the characterization of subclones with leukemia-initiating capacity [1]. To compare the AML clonality pre- and post- transplant in immunodeficient mice, we analyzed mutations in 20 genes associated with myeloid leukemia using next-generation sequencing (NGS).

AML bone marrow (BM) leukocytes (9 clinical samples) were injected into NOD scid gamma (NSG) mice (1-4 mice per sample, 26 mice in total). Human leukocytes (hCD45+) harvested from murine BM were analyzed and sorted on FACSAria II, (BD Biosciences). Targeted amplicon sequencing using ClearSeq AML Haloplex (Agilent) was performed on MiSeq and NextSeq instruments (Illumina). NGS data were processed using VarDict, VarScan and Pindel algorhytms.

The primary samples carried 1-3 mutations in the genes: *FLT*₃ (*FLT*₃-*ITD*, n=4), *NPM*₁ (n=4), *DNMT*₃A (n=3), *NRAS* (n=1), *ASXL*₁ (n=1), *TP*₅₃ (n=1). In 7/9 sample pairs, all engrafted mice carried the same mutations as the corresponding primary sample. In 2 patients, the detected differences showed outgrowth/decline of distinct AML subclones. Data indicate the general capability of the PDX model to simulate AML clonality, where minor deviations reflect the growth capacity of individual subclones.

Acknowledgement

Supported by the project from Ministry of Health of the Czech Republic with reg. no. 15-25809A and project MUNI/A/1106/2016.

References

[1] J. M. Klco *et al.*, Functional Heterogeneity of Genetically Defined Subclones in Acute Myeloid Leukemia, Cancer Cell, 2014, 25(3): 379-392.

P10: CD20 knockout cell lines obtained using CRISPR/Cas9 have no significant defect in BCR signaling in B-lymphoid malignancies

<u>Veronika Kozlová</u>¹, Aneta Ledererová¹, Viera Vakulová¹, Michael Doubek^{1,2}, Jiří Mayer^{1,2}, Šárka Pospíšilová^{1,2}, Michal Šmída^{1,2}

¹Central European Institute of Technology (CEITEC), Brno, Czech Republic

²Department of Internal Medicine - Hematology and Oncology, Medical Faculty of Masaryk University and University Hospital Brno, Czech Republic

Standard of care for B-lymphoid malignancies still relies on the administration of monoclonal antibodies, with CD20 antigen being the prime target. Although effective at first, repeated cycles of anti-CD20 monoclonal antibody therapy often result in the loss of CD20 on the surface of malignant B-cells and consequently in the therapy resistance and therapy failure. In spite of the widespread use of CD20 monoclonal antibodies, the exact mechanisms regulating CD20 expression stay largely unrevealed and it mainly remains unclear whether they can be exploited pharmacologically to modulate expression of CD20 in the clinic.

Therefore we have utilized the state-of-the-art CRISPR/Cas9 system to fully knockout CD20 gene in B-cell lymphoma (Ramos) and chronic lymphocytic leukemia (MEC1) cell lines. As expected, these cells are totally resistant to CD20 monoclonal antibodies. CD20 was originally proposed to function as a calcium channel and to contribute to B-cell receptor signaling, however the exact function of CD20 remains largely elusive yet. We demonstrate that B-cells with the loss of CD20 have fairly normal B-cell receptor signaling with no signs of any large defect. Also calcium flux in response to BCR triggering seems to be normal in CD20-deficient cells, thus suggesting that CD20 is dispensable for proper B-cell receptor signaling.

In conclusion we can say that the expression of CD20 molecules is not critical for the B-cell receptor function. Further analysis of mechanisms regulating CD20 expression and function is critically needed.

Acknowledgement

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To6: Study of Fox-1 RRM – the molecular recognition and hydration

Miroslav Krepl^{1,2}, Jiri Sponer^{1,2}

¹Institute of Biophysics, Academy of Sciences of the Czech Republic, Kralovopolska 135, 612 65 Brno, Czech Republic

² Regional Centre of Advanced Technologies and Materials, Department of Physical Chemistry, Faculty of Science, Palacky University Olomouc, 17. listopadu 12, 771 46 Olomouc, Czech Republic

RNA Recognition Motif (RRM) proteins represent an abundant class of proteins which are widely conserved in eukaryotic genome. We use molecular dynamics (MD) simulations in conjunction with NMR spectroscopy to interpret and expand the available data on the Fox-1 RRM which is implicated in several diseases affecting cells' development. We show that several segments of the protein-RNA interface may involve competition between dynamical local substates rather than firmly formed interactions, which is indirectly consistent with the primary NMR data. We also propose a protocol for "MD-adapted structure ensemble" as a way to integrate the simulation predictions and expand upon the deposited NMR structures [1].

In the second part of our work, we utilize X-ray and NMR structures of the Fox-1 RRM to study structured hydration. The MD excellently reproduces the most occupied hydration sites. Simulations of the protein/RNA complex show hydration consistent with the isolated protein complemented by the hydration sites specific to the protein/RNA interface. We then characterize two of them using NMR spectroscopy and RNA binding with switchSENSE. Both hydration sites are experimentally confirmed and their abolishment reduces the binding free energy. Significantly, one of the hydration sites is evolutionarily conserved within the RRM domains genome. The MD is an effective tool for predicting and interpreting the hydration patterns of protein/RNA complexes, which are not easily detectable in NMR experiments but affect stability of protein/RNA complexes.

References

[1] Krepl, M.; Cléry, A.; Blatter, M.; Allain, F. H. T.; Sponer, J., *Nucleic Acids Res.* 2016, 44, 6452-6470.

P11: Role of upstream open reading frames in auxin dependent processes

Timofeyenko Kseniya¹, Dmitry Konovalov², Kamil Růžička¹, Jan Hejátko¹ ⁷Functional Genomics and Proteomics of Plants – Central European Institute of Technology, Masaryk University, Brno, Czech Republic ²KinrossResearch, Minsk, Belarus

Upstream open reading frames (uORFs) are important post transcriptional regulatory elements situated in the gene 5'-untranslated regions. They are proposed to regulate (repress) expression of their downstream main ORFs. However, despite their large occurrence in significant part of eukaryotic (and plant) genes, their functional relevance has been poorly studied up to now. In addition, the role of auxin in uORF mediate signaling has been also suggested. To resolve this, we set up a bioinformatics pipeline to find uORFs in auxin-related genes. We took advantage of numerous sequenced plant genomes and determined conservation as a measure of expected functional relevance of a putative uORF found.

Using sequence conservation as a hint again, we further aim to expand our bioinformatics pipeline to identify also conserved noncanonical (non-AUG initiated) uORFs to get more precise list of uORFs present in genes directly connected with auxin dependent processes. In addition, we already confirmed the presence of a highly conserved uORF in the gene encoding crucial transcriptional regulator AUXIN RESPONSE FACTOR 4 (ARF4). In the experimental part of the project, we will test the functional impact of this uORF on the expression the ARF4 main ORF and the resulting functional and phenotypic consequences.

To7: Antimicrobial resistance in Enterobacteriaceae in wild birds

<u>Iva Kutilova</u>^{1,2}, Martina Masarikova^{2,3}, Ivana Jamborova^{1,2}, Nicol Janecko^{1,4}, Ivan Literak^{1,2}, Alois Cizek^{2,3}, Monika Dolejska^{1,2}

¹Department of Biology and Wildlife Diseases, Faculty of Veterinary Hygiene and Ecology, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

²CEITEC, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

³Department of Infectious Diseases and Microbiology, Faculty of Veterinary Medicine, University of Veterinary and Pharmaceutical Sciences Brno, Brno, Czech Republic

⁴Department of Population Medicine, University of Guelph, Canada

Significant increase of antibiotic resistance has been observed in gram-negative bacteria, which are becoming resistant to almost every antibiotic available. The horizontal gene transfer mediated by plasmids plays an important role in the rapid spread of resistance. Infections caused by bacteria resistant to beta-lactam antibiotics, mainly cephalosporins and carbapenems, represent one of the major issue of human and veterinary medicine. The primary mechanism of resistance to cephalosporins is the production of beta-lactamases, especially extended-spectrum beta-lactamase (ESBL) and AmpC beta-lactamases. Recently, the antibiotic resistant bacteria have been documented in the wildlife. Synanthropic birds which live in close proximity to urban and agriculture areas are in higher risk of colonization by resistant bacteria.

We investigated 1073 and 1039 faecal samples from wintering rooks [1] and American crows in Europe and North America, respectively, for resistance to beta-lactams. Moreover, 504 cloacal samples from gull chicks were studied for carbapenemase-producing Enterobacteriaceae in Australia [2].

A total of 152 (14%, n=1073) and 174 (17%, n=1039) cefotaxime-resistant *Escherichia coli* in Europe and North America, respectively, were obtained. The major mechanisms of resistance were production of ESBLs and AmpC beta-lactamases associated with transferable plasmids. High prevalence of isolates carrying carbapenemase gene *bla*_{IMP} (40%, 80/200) was found in gulls in Five Islands, Australia.

Our data pointed out that wild birds are important reservoirs of bacteria resistant to critically important antibiotics. We documented widespread occurrence of Enterobacteriaceae with ESBL, AmpC and carbapenemase genes associated with high-risk *E. coli* clones such as ST95, ST131, ST405 and ST648 in wild birds worldwide.

Acknowledgement

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References

[1] Jamborova I. et al. Appl Environ Microbiol. 2015, 81:648 –657.

[2] Dolejska, M. et al. J Antimicrob Chemother. 2016, 71: 63–70.

P12: p53 and DNA damage response pathway in anaplastic large cell lymphoma

<u>Cosimo Lobello</u>¹, Jakub Hynšt¹, Sarka Pospisilova¹ ¹CEITEC – Central European Institute of Technology, Brno, Czech Republic

Anaplastic large cell lymphoma (ALCL) is a rare T cell non-Hodking's lymphoma. The World Health Organization distinguishes ALCL in two different entities: anaplastic lymphoma kinase (ALK)-positive and ALK-negative, according to the presence of ALK translocation [1]. Although ALK translocation plays a main role in the cell transformation in ALK-positive ALCL, several evidences [2] suggest that per se this translocation is not sufficient and additional cooperative mutations are likely required for the lymphomagenesis. Several works have tried to characterize the differences between the entities, but the issue is still open. The aim of this project is to investigate the differences based on the next generation sequencing (NGS), focusing, in particular, on ALK-positive lymphoma and to explore, mainly p53 and DNA damage response pathways. An initial sequencing focused on TP53 has confirmed that TP53 is uncommonly mutated in both, ALK-positive and negative ALCL [3]. For this reason, we have decided to extend the panel of genes and we want to investigate the 275 genes most commonly mutated in cancer and/or deregulated in ALCL. Moreover, our preliminary works on microRNA (miRNA) have shed light on further divergences between ALK-positive and negative ALCL. We found upregulated miRNAs miR-214-3p, 222-3p and 342-3p in ALK-positive ALCL. These miRNAs could play a role in the regulation of PI3K-PTEN-AKT pathway, known to be constitutionally activated by ALK-translocation[4]. Further work will be necessary to determine the possible role of these miRNAs and gene mutations in ALCL and to increase the knowledge concerning these mysterious entities.

References

[1] Delsol G, Falini B, Muller-Hermelink HK, et al. In: Swerdlow S, Campo E, Harris N, et al, eds. WHO Classification of Tumours of Haematopoietic and Lymphoid Tissues. Lyon: International Agency for Research on Cancer; 2008:312–316.

[2] McDuff F.K.E. and Turner S.D. PLoS One. 2011; 6(3): e17854.

[3] Rassidakis GZ, Thomaides A, Wang S, Jiang Y, Fourtouna A, Lai R, Medeiros LJ. Leukemia. 2005 Sep;19(9):1663-9.

[4] Chiarle R, Voena C, Ambrogio C, Piva R, Inghirami G. Nature Reviews Cancer 8, 11-23 (January 2008) | doi:10.1038/nrc2291

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P13: MALDI TOF MS imaging of 3D neuroblastoma cell cultures

<u>Markéta Machálková</u>¹, Adam Pruška¹, Jarmila Navrátilová², Jan Šmarda², Jan Preisler¹

¹Laboratory of Bioanalytical Instrumentation, Brno, Czech Republic ²Laboratory of Cell Differentiation, Brno, Czech Republic

Matrix-assisted laser desorption ionization mass spectrometry imaging (MALDI MSI) has become a routine technique for analyte visualization across biological samples. In a single experiment, it enables imaging both exogenous and endogenous compounds, such as drugs, proteins and lipids. Additional great benefit of this approach is no need for molecule labelling, in comparison with the methods such as fluorescent microscopy, immunohistochemistry etc.

In our study, 3D cell cultures SK-N-Be(2) and SH-SY5Y were analyzed [1]. The cell formations (spheroids) were embedded in gelatine, frozen, cryo-sectioned into thin slices and thawed to conductive glass slides. For uniform matrix coating, commercial iMatrixSpray sprayer [2] was employed, its parameters were optimized and the results were compared with sublimation method. Developed protocols were applied to the analysis of spheroids treated by potential cancerostatics metaiodobenzylguanidine and perifosine. Initial images showing distribution of the drugs and selected lipids with CHCA and DHB matrices were acquired.

References

[1] Liu X. et al.: Anal. Chem. 85(13), 6295-6302 (2013).

[2] Stoeckli M et al.: CHIMIA 68(3), 146-149 (2014).

P14: Acoustic analysis of poem recitation for identification of hypokinetic dysarthria in Parkinson's disease patients

<u>Ján Mucha</u>¹, Zoltán Galáž¹, Tomáš Kiska¹, Jiří Mekyska¹, Vojtech Zvončák¹, Zdeňek Smékal¹

¹Department of Telecommunications and SIX Research Centre, Brno University of Technology, Technická 10, 61600 Brno, Czech Republic

Parkinson's disease (PD) is the second most frequent neurodegenerative disorder. Up to 90 % of PD patients suffer from speech disorder called hypokinetic dysarthria (HD). The goal of this work was to perform quantitative acoustic analysis of poem recitation in order to identify presence of HD. We employed conventional acoustic features to quantify specific HD disorders such as articulation, prosody, speech fluency and quality. It was observed that there is only mildly strong correlation between these speech features and diagnosis of the speakers. Next, we performed an univariate classification with these results of sensitivity in specific HD domains: imprecise articulation (62.63 %), dysprosody (61.62 %), speech dysfluency (71.72 %), and speech quality deterioration (59.60 %). The classification performance was improved by a multivariate classification, where we achieved sensitivity of 83.42 % using only two features describing imprecise articulation and speech quality deterioration in HD. Promising potential of the selected speech features and especially the use of poem recitation task to quantify and identify HD in PD was demonstrated.

References

[1] Z. Galaz, J. Mekyska, Z. Mzourek, Z. Smekal, I. Rektorova, I. Eliasova, M. Kostalova, M. Mrackova, and D. Berankova, Comput. Methods. Programs. Biomed., vol. 127, pp. 301 – 317, 2016.

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To8: The impact of choice of reference gene annotation

Jan Oppelt^{1,2}, Marek Mráz¹

¹CEITEC-Central European Institute of Technology, Masaryk University, Brno, Czech Republic ²National Centre for Biomolecular Research, Faculty of Science, Masarykova univerzita, Brno, Czech Republic

Analysis of various sequencing data often relies on so called *reference gene annotation*. Reference gene annotation describes where the genes are located in a genome, how many there are and how they look like. It sounds simple, but as always there is a catch.

Thanks to a progress in massive-parallel sequencing methods we are able to analyze not only genes themselves but also their isoforms. We can also explore differences in expression between samples in different conditions. But before we are able to examine the changes we have to determine the level of expression of each gene.

Unfortunately, most of the common model organisms have multiple reference gene annotations curated by different consortia. The main difference lies in required gene evidence – some consortia strictly require experimental evidence whereas some include gene predictions. This results in difference in number of genes and associated isoforms.

We analyzed human gene expression data using 5 different reference gene annotations and explored the impact on the expression of genes and isoforms. We have observed that choice of the annotation has a considerable impact on the expression levels as well as on the evaluation of differential expression.

References

[1] A. Dobin *et al.*, STAR: ultrafast universal RNA-seq aligner, Bioinformatics. 2013. doi: 10.1093/bioinformatics/bts635.

[2] B. Li *et al.*, RSEM: accurate transcript quantification from RNA-Seq data with or without a reference genome, BMC Bioinformatics. 2011. doi: 10.1186/1471-2105-12-323.

[3] M. D. Robinson *et al.*, edgeR: a Bioconductor package for differential expression analysis of digital gene expression data, Bioinformatics. 2010. doi: 10.1093/bioinformatics/btp616.

P15: GLASS: assisted and standardized assessment of gene variations from Sanger sequence trace data

<u>Karol Pal</u>¹, Vojtech Bystry¹, Tomas Reigl¹, Martin Demko¹, Adam Krejci¹, Boris Tichy¹, Sarka Pospisilova^{1,2}, Jitka Malcikova^{1,2}, Nikos Darzentas¹ ¹CEITEC – Central European Institute of Technology, Masaryk University Brno, Czech Republic ²Hospital Brno, Brno, Czech Republic

Background: Despite great advances in Next Generation Sequencing, Sanger sequencing still represents a widely used methodology for mutation detection. The reliability and accuracy of Sanger sequencing mutation is influenced also by the software analysis of Sanger data. Moreover, the uniform designation of mutations according to the HGVS nomenclature is crucial for reporting and inter-laboratory comparison.

Aims: We have developed an easy to use helpful tool for analyzing Sanger sequencing data with advanced functionality which still follows the workflow of "manual inspection" of traces and assists the user allowing him to focus on cases where expertise is needed.

Methods: GLASS is written in the R programing language and is implemented as a web service. The program uses an interactive web page friendly visualization of the chromatogram for which a custom D₃ based JavaScript library was developed. The program performs several steps of alignment to determine the reference gene and orientation of the analyzed sample and also predicts and annotates possible indels and the frequency at which they occur.

Results: GLASS is a bioinformatic implementation of best practices of labs with published know-how in the analysis of TP₅₃ and other clinically relevant genes. GLASS is freely available online at http://bat.infspire.org/genomepd/glass/.

Acknowledgement

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\$\$CEITEC

P16: Atomic force microscope-based analysis of cancer cell stiffness: its potential as a biomarker of epithelial-mesenchymal transition in head and neck cancer-pilot study

Barbora Peltanova^{1,3}, Monika Kratochvilova³, Marketa Svobodova^{1,3}, Hana Polanska^{1,3}, Martina Raudenska^{2,3}, Jaromir Gumulec^{1,2,3}, Jan Pribyl⁴, Tomas Vicar¹, Michal Masarik^{1,2,3}

¹Department of Pathological Physiology, Faculty of Medicine, Masaryk University, Brno

²Central European Institute of Technology, Brno University of Technology

³Department of Physiology, Faculty of Medicine, Masaryk University

⁴Central European Institute of Technology, Masaryk University/Kamenice 5, CZ-625 oo Brno, Czech Republic

Background: During epithelial-esenchymal transition (EMT), cells lose epithelial features (low motility and strong cell–cell contacts) while gaining mesenchymal traits associated with metastasis (increased motility, weak cell–cell contacts, CD90 expression). Recently, it was demonstrated that cell stiffness could serve as a biomarker of metastatic potential.

Methods: Starting Kit (CD90 MicroBeads- human, Miltenyi Biotec) were used for separation of CD90-positive subpopulations. AFM measurements were obtained in a humidified incubator (37°C; 5% CO2) with force measurements recorded at a pulling rate of 1 Hz. Young's modulus was calculated by Hertzian-Sneddon model.

Results: Cell stiffness of CD90-positive tumour cells derived from patients with stage of lymph node metastasis No-1 was significantly higher than cell stiffness of CD90-positive tumour cells derived from patients with stage N2-3.

Conclusion: Our results indicate, that cell stiffness could be a promising predictor for metastatic potential of head and neck cancer.

Acknowledgement

This work was supported by the Ministry of Health of the Czech Republic (grant no. 16-29835A) and by Grant Agency of the Czech Republic (GA16-12454S).

P17: Glycobiochemistry: from bugs to cystic fibrosis

Daniel Pokorný¹, Gita Jančaříková^{1,2}, Michaela Wimmerová^{1,2,3}

¹National Centre for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 753/5, Brno 625 00, Czech Republic

²Central European Institute of Technology, Masaryk University, Kamenice 753/5, Brno 625 oo, Czech Republic

³Department of Biochemistry, Faculty of Science, Masaryk University, Kamenice 753/5, Brno 625 00, Czech Republic

Glycobiochemistry group is focused on structure and function of two groups of proteins: lectins and glycosyltransferases. Glycosyltransferases are enzymes catalyzing transfer of activated nucleotide sugars to an acceptor molecule yielding a proper glycoconjugate. Lectins (sugar-binding proteins) are devoid of catalytic activity, but play equally critical role in wide range of both physiological and pathological processes, such as cell-cell recognition, subcellular localization of (glyco)proteins, pathogen adhesion to host cells, etc. The latter role is of special importance for us, because inhibition of such lectins via suitable antagonist could prevent the pathogen from adhesion to host cells. In some cases, this could lead to abolishing the infection completely.

Among the sugar-binding proteins we study are lectins from pathogens, e.g AFL from *A. fumigatus*, aspergillosis-causing fungus [1], PA-IL and PA-IIL [2] from *P. aeruginosa*, a bacterium causing severe lung infections in patients with cystic fibrosis, PLL lectins [3] from *P. luminescens*, a bacterium that associates with nematodes and attacks insects, PHL lectins from *P. asymbiotica* which infects insects and human, and others. We use a broad range of molecular biology and biophysics methods to study their structure (CD spectroscopy, analytical ultracentrifugation, X-ray crystallography), stability (differential scanning fluorimetry/calorimetry), interaction with ligands (isothermal titration calorimetry, surface plasmon resonance, micro-scale thermophoresis, glycan array) and their biological activity to assess the function of the protein and its involvement in particular process.

References

- [1] Houser, J. et al. PLoS ONE 8, 2013
- [2] Chemani, C. et al. Infect. Immun. 77, 2009
- [3] Kumar, A. et al. J. Biol. Chem. 291, 2016

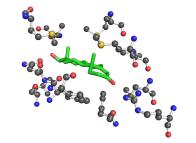
\$\$ CEITEC

P18: Effective on-demand mining of structural databases

Lukáš Pravda¹, Sehnal David¹, Radka Svobodová Vařeková¹, Jaroslav Koča¹ ¹Central European Institute of Technology, Masaryk University, Brno, Czech Republic.

The majority of *in silico* experiments often heavily relies on a data collection. Indeed, identification of biomolecular substructures (patterns) within biomolecular databases, such as Protein Data Bank is a common procedure in structural bioinformatics and related fields. We are seeking for well-defined molecular patterns such as binding or catalytic sites, transcription factors, protein structural, or sequence motifs, etc. These are in turn used to aid structural and functional characterization and comparison of proteins, analysis of newly determined protein structures, identification of similar binding sites in off-target proteins, discovery of new inhibitors, facilitation of protein-protein interaction and more. This is usually done using a plethora of one-time-only use in-house programs often in combination with dedicated software tools. Development of such solutions is generally error-prone and time-consuming. Hence the question is, can we do any better? Do we really need all these single purpose programs? Or can we extract biologically important sites in an easy user-defined and customizable way?

In this talk we will present our unique online solution PatternQuery (PQ - http://ncbr.muni.cz/PatternQuery) – for mining structural databases such as Protein Data Bank.



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To9: Telomere- and telomerase-associated proteins in flowering plants

<u>Šárka Schořová</u>¹, Petra Procházková Schrumpfová^{1,2}, Jiří Fajkus^{1,2} ¹Laboratory of Functional Genomics and Proteomics, NCBR, Faculty of Science, Masaryk University, Kotlářská 2, CZ-611 37 Brno, Czech Republic

²Mendel Centre for Plant Genomics and Proteomics, CEITEC – Central European Institute of Technology, Masaryk University, Kamenice 5, CZ-625 oo Brno, Czech Republic

The interactome of mammalian telomeric protein complex has been described in detail, but data on functions and interactions of the plant telomere-associated proteins arised only recently. Recently we characterized plant specific group of proteins called Telomere Repeat Binding (TRB), homologues of mammalian Shelterin proteins TRF1 and TRF2 (Telomere Repeat Factor 1 and 2), as interactors of telomeric DNA and telomerase [1,2,3,4].

Our recent results demonstrate newly found interaction partners of TRB proteins and their effect in telomere maintenance or telomerase activity in Arabidopsis. Mammalian homologues of the newly found TRB interactors play important roles in telomerase complex assembly and in multiple other cellular pathways. The newly identified TRB interactors may thus be involved not only in telomere biology but also in other (non-telomeric) functions. This way, our research on telomere interactome will contribute to elucidation of the respective cellular mechanisms.

Acknowledgement

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References

- [1] Schrumpfová, PP., et al. (2014). Plant J. Mar;77(5):770-81.
- [2] Schrumpfová, PP., et al. (2016). Plant Mol Biol. Jan;90(1-2):189-206.
- [3] Schrumpfová, PP., Schořová, Š., Fajkus, J. (2016). Front Plant Sci. Jun 28; 7:851.
- [4] Majerská J and Schrumpfová PP, et al. (2016). Protoplasma. Nov 16. In press

T10/P68: Regulation of B-cell receptor signalling in chronic lymphocytic leukaemia (CLL) microenvironment via modulation of GAB1 protein levels

<u>Václav Šeda</u>^{1,2+}, <u>Eva Vojáčková</u>¹⁺, Tomáš Loja¹, Gabriela Pavlasová^{1,2}, Kateřina Černá^{1,2}, Kateřina Musilová^{1,2}, Veronika Svobodová^{1,2}, Sonali Sharma¹, Marek Mráz^{1,2} ¹CEITEC MU, Masaryk University, Brno ²University Hospital Brno, Brno contributed equally

Abstract removed upon authors' request.

Acknowledgement

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T11: Identification of genes regulating plant meiosis by supresor screening in *Arabidopsis thaliana*

Sorin Tanasa

¹CEITEC, Brno, Czech Republic

Meiosis is a specialized cell division that produces haploid cells from diploid precursors in two rounds of chromosomal divisions. While we have relatively detailed knowledge on evolutionary conserved genes governing meiotic recombination and chromosome segregation, those dictating the regulation of meiotic progression are evolutionary less conserved and studied. In our previous work we showed that the nonsense-mediated mRNA decay (NMD) factor SMG7 is required for completion of meiosis in Arabidopsis thaliana. Smg7 mutants are characterized by an irregular anaphase II, which in turn is associated with delayed chromosome decondensation and aberrant rearrangement of the spindle. The meiotic function of Smg7 protein does not seem to be related to its NMD function, but rather represents a distinct gene function. To shed a light on the meiotic role of SMG7, we have performed a forward EMS-based genetic screen to identify suppressor mutations that rescue reduced fertility of SMG7-deficient plants. 90 mutant lines with increased fertility were isolated. Candidate mutations were identified by analyzing mapping populations by whole genome sequencing. Currently we are performing an in depth functional characterization of selected gene candidates. These experiments include detailed characterization of fertility, meiosis, protein localization studies and epistatic analysis with other genes involved in meiotic progression.

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T12: ToTem – automated software solution for program selection and parameter optimization

<u>Nikola Tom</u>¹, Ondrej Tom, Jitka Malcikova^{1,2}, Sarka Pavlova^{1,2}, Blanka Kubesova^{1,2}, Sarka Pospisilova^{1,2} ¹Molecular Medicine, CEITEC – Central European Institute of Technology ²University hospital Brno

Next generation sequencing (NGS) is a high-throughput method for determination of the nucleotide sequence in given DNA or RNA molecule. The primary outcome of NGS method needs to be further analyzed e.g. to correctly detect mutations. A key part of the data analysis is the selection and adjustment of adequate analytical programs (tools) which require heavy testing and optimization.

That was the motivation for the creation of ToTem. Specifically, in the first step, the user selects bioinformatics tools and defines their parameters that are going to be tested at defined values. Subsequently, all the combinations of parameters' settings are run to generate results. The results produced by individual settings are compared to the reference data representing desired output and sorted according to selected metrics. In a pilot bioinformatics experiment, 2 tools for variant detection were tested - VarScan2 (320 combinations) and VarDict (2880 combinations). Analysis was performed on 40 samples (deep sequencing of exons 2-11 of *TP53* gene; 254 mutations).

Using ToTem, more precise results in comparison to default programs' settings were achieved: VarScan2 (default = 0.218 sensitivity, specificity = 1 vs. 0.944 and 0.994); VarDict (default = 0.278 sensitivity, specificity = 1 vs. 0.915 and 0.977).

Applying ToTem for the automated optimization of the settings for the variant detection in DNA samples resulted in the several fold higher detection accuracy.

Acknowledgement

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P2o: Involvement of TRAMP complex in transcription termination of protein-coding genes

<u>Viacheslav Zemlianski</u>¹, Jana Laláková^{1,2}, Andrea Fořtová¹, Tomasz K. Kabziński¹, Karel Kubíček¹, Richard Štefl¹, Štěpánka Vaňáčová¹ ¹CEITEC, Masaryk University; Brno, Czech Republic ²Karolinska Insitute; Stockholm, Sweden (current address)

The yeast RNA polymerase II (RNAP II) synthesizes both protein-coding and non-coding RNAs (ncRNA). Correct transcription termination is extremely important for nascent transcripts metabolism and stability. RNAP II transcription is regulated by the dynamic phosphorylation of the C-terminal domain (CTD). CTD consist of multiple hexapeptide repeats with the sequence Y1S2P3T4S5P6S7. There are two different mechanisms for mRNAs and ncRNAs, associated with different CTD phosphorylation pattern. The CTD phosphorylation at Ser2 position is recognized by the Rtt103p, which is believed to stimulate termination of coding transcripts. Rtt103p recruits Rat1p exonuclease and Rai1p pyrophosphatase, which degrade the downstream cleavage product and displaces RNAPII from template DNA. Termination of ncRNA transcription, in turn, depends on the recognition of pSer5 by Nrd1p and consequent connection with Nrd1p-Nad3p-Sen1p (NNS) complex. NNS complex recruits the TRAMP-exosome machinery which trims the 3' ends of nascent transcripts. TRAMP complex was not previously expected to participate in mRNA processing. Our data shows that Trf4p can recognize Rtt103p and bind to it. Moreover, depletion of TRF4 gene causes the accumulating of 3' extended mRNA. Presented data shows that TRAMP complex is also involved in mRNA metabolism.

References

[1] Tudek A., Porrua O., Kabzinski T., Lidschreiber M., Kubicek K., Fortova A., Lacroute F., Vanacova S., Cramer P., Stefl R., Libri D.; Molecular Cell 55 (2014): 467-481

[2] San Paolo S., Vanacova S., Schenk L., Scherrer T., Blank D., Keller W., Gerber A.P.; PLoS Genet. Jul;5(7) (2009): e1000555

[3] Vanácová S., Wolf J., Martin G., Blank D., Dettwiler S., Friedlein A., Langen H., Keith G., Keller W.; PLoS Biol. Jun;3(6) (2005): e189

[4] Buratowski S.; The CTD code; Nature structural biology 10 (2003): 679-680

P21: Modified tRNA adenines and their interaction with cytokinin homeostasis in plant development

Elena Zemlyanskaya¹, Jan Hejátko¹ and Kamil Růžička¹

¹Functional Genomics and Proteomics of Plants – Central European Institute of Technology, Masaryk University, Brno, Czech Republic

Cytokinins are a class of plant hormones involved in a wide range of biological processes affecting plant growth and development. They are adenine derivatives with aromatic or isoprene side chain. In the major biosynthetic pathway in plants, cytokinins are synthesized from precursor molecules by the enzyme isopentenyl transferase, which utilizes adenosine phosphates (either AMP, ADP or ATP) as a source of adenine. Isoprenoid cytokinins (e.g., N6-(cis-hydroxyisopentenyl) adenosine, also known as cis-zeatin) are present also in tRNAs (A37 position), and these are considered as an additional source of free cytokinins. Although this phenomenon has been known for decades, the biological role of tRNA-mediated cytokinin production still remains poorly understood. Our extensive search in public databases revealed that there are also several other modifications present at A37 position of tRNA. We hypothesize that these molecules may have cytokinin activity as well.

To this end, cytokinin activity of A37 tRNA modification t6A was tested, and the candidate mutants with abolished t6A formation in *A. thaliana* have been analyzed for cytokinin related defects. We found that mutations in different genes involved in t6A formation have different impacts on plant development. We reveal that, in addition to several defects possibly ascribed to altered cytokinin activity, several genes are essential for early morphogenesis steps, including development of the gametophyte.

P22: TDS (TUT-DIS3L2 Surveillance) targets aberrant ncRNAs and short transcripts of protein-coding genes in human cytoplasm

Dagmar Zigackova¹, Zuzana Feketova¹, Josef Pasulka¹, Dmytro Ustianenko¹, Andrea Fortova¹, Lukas Bednarik¹, Mihaela Zavolan², Stepanka Vanacova¹ ¹Central European Institute of Technology, Masaryk University, Brno, Czech Republic ²Biozentrum, University of Basel, Basel, Switzerland

The 3'-terminal RNA uridylation catalyzed by the terminal uridyltrasferases (TUTases) mediates degradation of various RNAs and processing of some ncRNAs. DIS_{3L2} is mammalian oligo(U) specific exonuclease, that is involved in decay of uridylated precursors of let-7 miRNAs, tRNAs and cleaved mRNAs. Its mutation is linked to the Perlman syndrome development and Wilms tumor progression. However, the function of uridylation and the involvement of DIS_{3L2} in these diseases remains largely unknown.

By using catalytical mutant of DIS₃L₂ as a bait, we have identified uridylated aberrant forms of multiple types of coding and noncoding RNAs. We demonstrate, that extended and aberrantly processed forms of ncRNAs, such as snRNAs, rRNA, tRNAs, YRNAs, and also transcripts originating from pseudogenes are uridylated, and then bound and degraded by DIS₃L₂. Most interestingly, we uncovered a fraction of reads mapping to 5' termini of protein coding genes. The uridylation positions overlap with the position of stalled RNA polymerase II indicating, that these fragments originate from RNA Pol II stalling. Next, we show, that uridylated 5' fragments of mRNAs are exported to cytoplasm, where they are removed by the activity of DIS₃L₂.

In summary, our results demonstrate, that TUT-DIS₃L₂ surveillance (TDS) is a general cytoplasmic RNA mechanism assuring the removal of aberrant transcripts.

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T13: Validation and analyses of biomacromolecular ligand conformations

Zuzana Žufanová¹, Radka Svobodová Vařeková², Michaela Wimmerová^{1,2} ¹Department of biochemistry, Faculty of Science, Masaryk University, Brno ²Central European Institute of Technology, Brno

With the immense increase in biomolecular structural data stored in databases in recent years, validation methods have been developed to assess the quality and accuracy of the biomolecular structures. Currently, most the structure validation programs focus on the validation of biomacromolecules as opposed to small ligands. This progression has been primarily due to complications arising from the large chemical variability of ligands.

Existing programs for ligand validation prioritize evaluation of the correctness of specific properties such as torsion angles, bond length, and atom clashes, or focus on annotation validation (validation by comparing the structure with a correct model). Examples of validation programs using annotation validation are MotiveValidator (MV) [1] and ValidatorDB (VDB) [2], both of which examine the ligand structure completeness, ligand chirality, and ligand annotation errors.

In this work, we focus on extending the functionality of MV and VDB by developing methodology to validate the ligand conformation. Our methodology is based on obtaining correct models from the literature and other existing databases, and comparing the two structures using Ertl chemical scaffolds. Ertl scaffolds will allow us to perform more accurate analysis, as they feature information about key parts of ligands such as the central part of the molecule without acyclic parts, and exocyclic double bonds.

References

[1] R. Svobodová Vařeková et al, Nucleic Acids Research, 2014.

[2] D. Sehnal, R. Svobodová Vařeková et al, Nucleic Acid Research, 2015.

P69: Enzymatic assay for evaluation of virolytic activity of antimicrobial peptides based on Ebola virus–like particles

<u>Marie Pešková</u>¹, Zbyněk Heger^{3,4}, Vojtěch Adam^{3,4}, Vladimír Pekařík² ¹Faculty of Medicine, Masaryk University, Brno, Czech Republic ²Institute of Physiology, Faculty of Medicine, Masaryk University, Czech Republic ³Department of Chemistry and Biochemistry, Mendel University, Czech Republic ⁴Central European Institute of Technology (CEITEC), University of Technology, Czech Republic

With the emergence of new antibiotic-resistant bacterial strains, new ways of their treatment must be investigated. Antimicrobial peptides (AMP) represent one of such ways, but there is a need to conclusively prove and evaluate their cytotoxic and membranolytic activities, for current assays based on liposomes or haemolysis are burdened by the artificial nature of liposomes and distinctive composition of used erythrocytes. To simulate the native membranes, we have used virus–like particles (VLPs), because they strongly resemble parental viruses by the structure but carry intact membrane of the host cell. VLPs do not carry genetic information and most often contain only one or a few viral proteins. Thus we have created an assay based on enzymatic Ebola VLPs containing nanoluciferase reporter in the virus core, which after disruption of the cell wall reacts with nanoluciferase substrate providing an unparalleled sensitivity of the assay. We have tested several AMP in the assay and compared the results with lentivirus inactivation and haemolytic assays.

The results show that the designed assay has a great sensitivity and is able to reveal the potential of the peptides to interact with the cell membrane. According to our results from the assays, we have identified the CAM-W (KWKLWKKIEKWGQGIGAVLKWLTTWL) peptide as the most potent against filoviral VLP and lentiviral vectors. The approach has a potential to identify new peptides with antimicrobial, antivirolytic, and anti-tumour activities in the future.

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P70: Acute inflammatory response of Schwannoma cells to lipopolysaccharide stimuli

<u>Marcela Kohoutková</u>¹, Andrea Korimová¹ and Petr Dubový¹ ¹Department of Anatomy, Faculty of Medicine, Masaryk University, Brno, Czech Republic

Wallerian degeneration is a process of stereotypical reactions of Schwann cells which results in the reparation of the damaged nerve. Inflammatory activation of Schwann cells can be triggered by endogenous ligands produced during Wallerian degeneration or exogenous pathological molecules via Toll-like receptors (TLRs) [1,2]. Lipopolysaccharide (LPS) was used as a prototypical ligand for activating intracellular immune response of Schwannoma cells. Acute inflammatory response of Schwannoma cells is associated with increased production of cytokines and transcription factors [3]. We studied proteins that associate with inflammation most commonly - STAT3, IL-6 and receptor TLR4, which can transduce inflammatory signals to the cell. Rat Schwannoma cells (RT4-D6P2T) were treated with LPS (c=10ng/ml) for 1 hour to induce acute inflammatory responses. TLR4, pSTAT3 and IL-6 proteins were studied by immunocytochemistry and Western blot analysis. We observed dynamic changes of TLR4 deposits in the cell cytoplasm but not in the cell surface. LPS treatment induced enlarging of early endosomes in Schwannoma cells. We also found significantly increased levels of pSTAT3 and IL-6 proteins after LPS treatment indicating their crucial role in the induction of inflammation cascade in Schwann cells.

References

[1] S. Rotshenker, Wallerian degeneration: the innate-immune response to traumatic nerve injury., J. Neuroinflammation. 8 (2011) 109.

[2] E. Ydens, G. Lornet, V. Smits, S. Goethals, V. Timmerman, S. Janssens, The neuroinflammatory role of Schwann cells in disease, Neurobiol. Dis. 55 (2013) 95–103.

[3] H.N. Hao, J.D. Peduzzi-Nelson, P.J. VandeVord, K. Barami, S.P. DeSilva, D. Pelinkovic, L.G. Morawa, Lipopolysaccharide-induced inflammatory cytokine production by Schwann's cells dependent upon TLR4 expression, J. Neuroimmunol. 212 (2009) 26–34.



Material science and biosensing

Students' abstracts



P23: The effect of biologically active substance on the structure and biocompatibility of collagenous scaffolds for tissue engineering

<u>Johana Babrnáková</u>¹, Veronika Švachová¹, Jana Brtníková¹, Petr Sedláček², Eva Prosecká³, Barbara Kubešová⁴, Lucy Vojtová^{1,5}

¹CEITEC – Central European Institute of Technology, Brno University of Technology, Brno ²Materials Research Centre, Faculty of Chemistry, Brno University of Technology, Brno ³Institute of Experimental Medicine, Academy of Science of the Czech Republic, Prague ⁴VA-BIOS s.r.o., Navrátilova 8a, 616 oo Brno; 5SCITEG, a.s., Brno

The influence of biologically active additives, crosslinking agents, and enrichment with growth factors on the morphological properties of collagen-based scaffolds and their in-vitro bioactivity with mouse fibroblasts 3T3 have been investigated. 3D porous collagen scaffolds were modified with both antibacterial natural polysaccharides (chitosan and oxidized cellulose) and growth factors delivered in the form of blood platelet lysate. Addition of blood platelet lysate decrease the pore size of pure collagen scaffold but increased its porosity as demonstrate scanning electron microscopy pictures (Fig. 1).

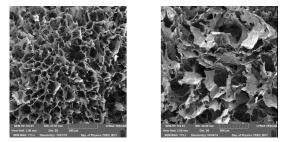


Fig. 1: SEM image of 3D collagen scaffold enriched with blood platelet lysate (left) and without platelet lysate (right).

Surprisingly, in-vitro tests of prepared scaffolds on mouse fibroblasts 3T3 showed that scaffolds enriched with platelet lysate exhibited significant synergistic effect with antibacterial additives on cells cultivation as determined by MTS assay and PicoGreen method. Therefore, these newly developed antibacterial collagen sponges involving growth factors could be used as scaffold for growing cells in systems with low mechanical loading having potential application in soft tissue engineering.

[1] J. Babrnáková, The effect of biologically active substances on the structure and properties of collagenous substrates. Brno university of Technology, Faculty of Chemistry, Brno, 2016.

¢° ⊂ EITEC

P24: Misalignment aberrations in electron beam lithography

Viktor Badin

Institute of Physical Engineering, Faculty of Mechanical Engineering, Brno University of Technology

Studying aberrations is vital in charged particle optics applications such as electron microscopy and electron beam lithography. Our studies deal with parasitic aberrations arising from the misalignment and/or imperfect manufacturing of optical components. For instance, in case of an electrostatic deflector, these imperfections include non-nominal width, radial and axial misplacement or rotation of the electrodes. The main goal of the research is to reduce the dimensionality of the perturbed system calculations — instead of relatively simple but very resource intensive 3D calculations we aim to transform the problem into 2D and use much faster tools to solve it. Our main tool to accomplish this is the multipole field expansion of the boundary condition using Fourier series.

The 3D field calculation takes many hours on an expensive computational server while the 2D problem can be solved in a much denser mesh in mere seconds to minutes on a regular PC which is what we aim to achieve. Besides deriving the theory and evaluating the errors of the used methods, the final goal of this research is to implement a robust tolerancing software package to be used for designing electron microscopes and lithography systems.

P25: Pressureless spark plasma–sintered Bioglass[®] 45S5 with enhanced mechanical properties and stress–induced new phase formation

<u>Luca Bertolla</u>¹, Ivo Dlouhý¹, Peter Tatarko^{2,3}, Alberto Viani⁴, Amit Mahajan², Zdeněk Chlup¹, Michael J. Reece¹, Aldo R. Boccaccini⁵

¹Institute of Physics of Materials ASCR, CEITEC IPM, Zizkova 22, 61662 Brno, Czechia

²Nanoforce Technology Limited, Queen Mary University of London, London E1 4NS, United Kingdom

³Institute of Inorganic Chemistry, Slovak Academy of Sciences, Dúbravská cesta 9, 84536, Bratislava 45, Slovakia

⁴Centre of Excellence, Telč Batelovská 485-6588 56 Telč, Czechia

⁵Institute of Biomaterials, Department of Materials Science and Engineering, University of Erlangen-Nuremberg, 91058 Erlangen, Germany

Commercial Bioglass[®] 4555 powder was sintered using spark plasma sintering (SPS) technique without the assistance of mechanical pressure with heating and cooling rate of 100 °C/min, dwell temperature of 1050 °C and dwell time of 30 min. Such route enabled the production of samples exhibiting superior mechanical properties in comparison with Bioglass[®] sintered in furnace. In particular, flexural strength and fracture toughness reached values close to those of apatite-wollastonite bioceramics, already widely used in clinical applications. The residual stresses implemented by indentation promoted the formation of a new phase in samples sintered by SPS. Complementary use of Raman and energy dispersive spectroscopy (EDS) indicated the phase as sodium carbide and a formation mechanism was proposed.

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P26: Supramolecular covalence in bifurcated chalcogen bonding

Pankaj Lochan Bora^{1,2}, Martin Novák^{1,3}, Jan Novotný¹, Cina Foroutan-Nejad¹, Radek Marek^{1,2,3}

¹CEITEC – Central European Institute of Technology, Masaryk University, Kamenice 5, Brno, Czech Republic ²Department of Chemistry, Faculty of Science, Masaryk University, Kamenice 5, Brno, Czech Republic ³National Center for Biomolecular Research, Faculty of Science, Masaryk University, Kamenice 5, Brno, Czech Republic

Supramolecular interactions were generally classified as non-covalent. However, recent studies have demonstrated that many of these formally non-covalent interactions are stabilized by a significant covalent component. Herein we show for systems of the general structure $[MX_6]^{2-}$:YX² (M = Se or Pt; Y = S, Se, or Te; X = F, Cl, Br, I) featuring bifurcated chalcogen bonding that, while the electrostatic parameters are useful for estimating the long-range electrostatic component of the interaction, they fail to predict the correct order of the binding energies in a series of compounds. Instead, the Lewis basicity of the individual substituents X on the chalcogen atom governs the trends in the binding energies via fine-tuning the covalent character of the chalcogen bond. The effects of substituents on the binding energy and the supramolecular electron sharing are consistently identified by an arsenal of theoretical methods ranging from approaches based on the quantum chemical topology to analytical tools based on the localized molecular orbitals. The chalcogen bonding investigated in this work is driven by orbital interactions with significant electron sharing, which can be designated as supramolecular covalence.

References

[1] Bora et al. Chem. Eur. J. 0.1002/chem.201700179(Accepted manuscript).

- [2] Jolleys et al. Dalton Trans., 42, 2963-2972 (2013).
- [3] Politzer et al. Theor. Chem. Acc., 108, 134-142 (2002).
- [4] Zhang et al., J. Phys. Chem. A, 113, 8132-8135 (2009).
- [5] Elguero et al. J. Chem. Theory Comput., 9, 5201-5210 (2013).

P27: High resolution measuring with X-ray computed tomography

<u>Adam Brinek</u>¹, Marketa Tesarova¹, Tomas Zikmund¹, Jozef Kaiser¹ ¹Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic

The ability to inspect the interior of an object in a nondestructive way is of fundamental importance in a wide range of academic and industrial applications, ranging from life science research, to medical diagnosis. X-ray tomography provides a tool to obtain 3D image of the object computed from a series of X-ray projections, acquired from a range of viewing angles. The 3D image is calculated by the reconstruction algorithm. In recent years, significant progress has been made in the field of advanced X-ray computed tomography (CT) imaging, pushing the limits of voxel resolution far beyond 1 micrometer [1].

This work shows the possibilities of the X-ray CT focused on the achieved resolution. The sample of human skull (10 centimeter in diameter) was observed with resolution of 114 μ m. Measurement provided highly visible structure of the bone in the images. Another investigated example was *Octodon Degu* jawbone with the length approximately 10 cm [2]. Micro CT successfully achieved 8 μ m voxel resolution and individual parts of each tooth were easily recognizable. The samples of mouse embryo with about 4 μ m voxel resolution were studied and various organs were observed and analyzed [3]. The last example was Proteus from Tular Cave. This measurement was made with high-ultra 0.5 μ m voxel resolution and it was possible to recognize individual cells in the images.

References

[1] Stuart R. Stock: MicroComputed Tomography: Methodology and Applications, CRC Press, 2008, ISBN-10: 1420058762.

[2] V. Jekl, T. Zikmund, K. Hauptman, Dyspnea in a Degu (Octodon degu) Associated with Maxillary Cheek Teeth Elongation, J. Exot. Pet Med., 2016, ISBN 10.1053/j.jepm.2016.03.003.

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P28: Functional interconnected magnesium-calcium phosphate composites: fabrication and characterization.

<u>Mariano Casas Luna</u>¹, Edgar B. Montufar¹, Sebastián Díaz de la Torre², Jozef Kaiser¹, Ladislav Celko¹.

¹Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic. ²Instituto Politécnico Nacional, Centro de Investigación e Innovación Tecnológica, Mexico City, Mexico.

Calcium phosphates (e. g., hydroxyapatite and tricalcium phosphate) have been widely investigated and used as bioactive materials. Nevertheless, their poor mechanical properties limit them for load bearing applications. New types of metallic and calcium phosphate composites are gaining attraction to create load-bearing implants for bone regeneration. Magnesium alloys appear as one of the best alternatives as biodegradable materials due to their bioactive and mechanical properties similar to the human bone. The present work is focused on the design of an interpenetrated magnesium – tricalcium phosphate (Mg-TCP) composite, meanwhile the development of a new advance technique for infiltration is introduced. Current assisted metal infiltration (CAMI) is a novel technique that allows molten metal infiltration of brittle ceramic preforms in less than 15 minutes. In the study, TCP porous preforms are fabricated via robocasting and later infiltrated with pure Mg through CAMI. The final specimens were analysed by X-ray microtomography (CT), scanning electron microscopy (SEM), elemental chemical analysis (EDS) and X-ray diffraction (XRD).

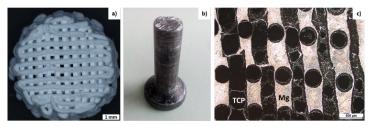


Fig. 1: a) Robocast TCP preform, b) TCP preform infiltrated with Mg and C) Optical microscopy picture of the Mg-TCP composite.

P29: Black phosphorous potentiated drug delivery of oxaliplatin for ovarian cancer treatment

Michaela Fojtů^{1,2}, Chia Xinyi³, Zdeněk Sofer⁴, Michal Masařík^{1,2}, Martin Pumera³

¹Department of Physiology, Faculty of Medicine, Masaryk University, Brno

²Centre for Structural Biology, CEITEC, Brno

³ Division of Chemistry and Biological Chemistry, School of Physical and Mathematical Sciences, Nanyang Technological University, Singapore

⁴Department of Inorganic Chemistry, University of Chemistry and Technology, Prague

Black phosphorous (BP) represents together with graphene, graphene oxide, and transition metal dichalcogenides (TMDs) group of materials called two-dimensional (2D) nanomaterials. 2D nanomaterials have a broad application potential that ranging from biosenzing, utilization in catalysis, water purification, to application optoelectronics. BP is thermodynamically the most stable phosphorous allotrope. It is composed of two-dimensional layers of phosphorous atoms that are sticked together via weak van der Waals forces, and therefore enabling preparation of few-layer BP structures and formation of ultrathin two-dimensional nanosheets by exfoliation. Besides the great application potential in material sciences, BP is recently unrevealing its auspicious potential in biomedicine. In our study, we synthesized BP nanoparticles and explored their applicability in targeted drug delivery. BP was loaded with platinum agents cisplatin and oxaliplatin and subjected in vitro evaluation of targeted drug delivery. BP was able not only to load investigated platinum derivatives on its surface by simple electrochemical interactions, but also to transfer the therapeutic cargo, target the specific tissue and combine its effect with oxaliplatin, which led not only to preserving, but to further potentiation of its anticancer effect for around 21%.

Acknowledgement

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T14: CMOS compatible piezoelectric resonator with FET structure for graphene monolayer properties modulation

Imrich Gablech^{1,2}, Jan Pekárek^{1,2}

¹Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic ²Faculty of Electrical Engineering and Communications, Department of Microelectronic, Brno University of Technology, Brno, Czech Republic

This abstract proposes a new method of graphene monolayer properties characterization under precisely specified conditions. It combines MEMS/NEMS resonator with FET structure. This approach allows to change graphene properties separately or together via two methods. The mechanical way is based on induced strain from the resonator which is graphene monolayer situated on. It brings the opportunity to measure graphene properties induced by the changes of mechanical strain and frequency of forced vibrations without the influence from external electric field. The other way uses FET structure to influence graphene monolayer using an electric field from bottom gate. This technique exploits graphene monolayer as a tunable sensor for molecule detection. There is no limit to measure concentration in units of ppb in terms of structure design. This approach of fabrication CMOS-compatible and biocompatible tunable frequency-modulated piezoelectric MEMS/NEMS resonators with graphene monolayer should be very useful in many fields for molecule level detection.

References

[1] SCHEDIN, F. et al., Detection of individual gas molecules adsorbed on graphene. Nature materials, 2007, vol. 6, no. 9, pp. 652-655. ISSN 1476-1122.

[2] NOVOSELOV, K. S. et al., Electric field effect in atomically thin carbon films. science, 2004, vol. 306, no. 5696, pp. 666-669. ISSN 0036-8075.

[3] BAE, S.-H. et al., Graphene-based transparent strain sensor. Carbon, 2013, vol. 51, pp. 236-242. ISSN 0008-6223.

P30: Membrane interaction of pegylated superparamagnetic nanoparticles

Noga Gal, Andrea Scheberl, Andrea Lassenberger, Laia Herrero Nogareda, and Erik Reimhult

Department of Nanobiotechnology, Institute for biologically inspired materials, University of Natural Resources and Life Sciences Vienna, Muthgasse 11-II, A-1190 Vienna, Austria

Iron oxide core-shell nanoparticles are gaining ever increasing interest for separation and imaging in biotechnology and biomedicine1,2, due to supposed low cytotoxicity and their superparamagnetic properties. Hydrophilic polymer-coated nanoparticles are believed to have low nonspecific interactions in biological systems, but much additional work in-vitro and in-vivo is needed to understand their detailed interactions with proteins, membranes and cells. We investigated monodisperse (SD<5%), single-crystalline and superparamagnetic magnetite nanoparticles of different core size and densely grafted with poly(ethylene glycol) (Mw=5kDa), with particular emphasis on their interaction with biological membranes. Membrane interactions will determine nonspecific recognition and uptake by cells. These nanoparticles demonstrated no cytotoxicity and low cell uptake in in-vitro culture of HeLa and HEK cell lines.

However, using Quartz Crystal Microbalance (QCM) a strong DLVO-type interaction could be demonstrated with anionic membranes that simulate eukaryote membranes. This interaction was only present in nonphysiological buffer with low ionic strength. Only low, weak and transient binding was observed to zwiterionic phosphocholine membranes. Core size seems to have an effect, with the smallest core size (3.3nm) yielding the strongest interactions while 8nm cores displayed almost no interaction. These results imply that dense polymer grafting and nanoparticle curvature are crucial parameters to control interactions between biomedical core-shell nanoparticles and their biomolecular environment, in particular cell membranes. The interaction between nanoparticle and membrane was furthermore shown to not perturb membrane structure by Differential Scanning Calorimetry (DSC).

References

[1] M. Mahmoudi; S. Sant; B. Wang; S. Laurent; T. Sen, Advanced Drug Delivery Review, 2011, 63, 24-46.

[2] A. Kumar; Gupta, M. Gupta, Biomaterials, 2005, 26, 3995–4021

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T15: Nelder-Mead algorithm based control strategy for CO_2 heat pump

Jan Glos¹, Pavel Václavek¹

¹CEITEC - Central European Institute of Technology, Brno University of Technology, Czech Republic

Carbon dioxide (CO₂), also known as a refrigerant R744, is nowadays becoming much more popular since European directives prohibit automotive usage of refrigerants with global warming potential higher than 150 ($\rm GWP_{R744} = 1$). Also ozone depletion potential is very important from environmental protection point of view ($\rm ODP_{R744} = 0$).

Nevertheless, very low critical point of CO_2 causes supercritical operation under certain conditions, which leads to deterioration of coefficient of performance (COP). Supercritical operation also allows a high side pressure control since the gas cooling temperature and pressure are independent.

Utilizing proper control strategy for R744 heat pump components the COP deterioration can be minimized and the performance of R744 heat pump can be compared to a conventional one. The first group of methods uses an approximate equation [1] or a table to determine the optimal high pressure, the second one utilizes real-time optimization methods [2].

We proposed a real-time optimum searching algorithm based on 1-dim Nelder-Mead simplex method to determine the optimal high side pressure. This solution is advantageous due to a resistance to the heat pump parameter changes and other disturbances, fast convergence to optimal point and quite simple implementation. The proposed method was successfully evaluated in simulations.

Acknowledgement

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References

[1] L. Yang, H. Li, S. W. Cai, L. L. Shao, and C. L. Zhang, "Minimizing COP loss from optimal high pressure correlation for transcritical CO₂ cycle," *Applied Thermal Engineering*, vol. 89, pp. 656–662, 2015.

[2] B. Hu, Y. Li, F. Cao, and Z. Xing, "Extremum seeking control of COP optimization for air-source transcritical CO2 heat pump water heater system," *Applied Energy*, vol. 147, pp. 361–372, 2015.

P31: Novel fully portable capillary electrophoretic instrument for analysis of low-sample volumes of biological fluids in point-of-care diagnostics

Michal Gregus^{1,2}, Frantisek Foret¹, Petr Kuban^{1,2}

¹Bioanalytical Instrumentation, CEITEC Masaryk University, Veveří 97, 602 00, Brno, Czech Republic ²Department of Chemistry, Masaryk University, Kotlářská 2, 611 37 Brno, Czech Republic

In here we present a novel, easy to use and portable capillary electrophoretic instrument for injection of small volumes of biological fluids. Instrument is equipped with contactless conductivity detection and all necessary parts including a tablet computer are accommodated in a plastic brief-case with total weight less than 5 kg and can continuously operate for at least 10 hours. The semi-automated hydrodynamic sample injection is accomplished via a specially designed PMMA interface that is able to repeatedly inject sample aliquots from a sample volume as low as 10 μ L, with repeatability of peak areas below 5%. The developed interface and the instrument were optimized for the injection of biological fluids. Practical utility was demonstrated on the determination of formate in blood serum samples from acute methanol intoxication patients and on the analysis of ionic profile (nitrosative stress markers, including nitrite and nitrate) in the exhaled breath condensate (EBC) from one single exhalation.

Acknowledgement

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P32: Synthesis of nanoparticles by electrical discharge in the liquid

Jakub Horak¹, Anton Nikiforov², Christophe Leys², Ke Vin Chan², Frantisek Krcma¹

¹Brno University of Technology, Faculty of Chemistry, Institute of Physical and Applied Chemistry, Brno, Czech Republic

²Ghent University, Faculty of Engineering and Architecture, Department of Applied Physics, Ghent, Belgium

Commercially offered nanoparticles suffer from agglomeration in time although they are kept in stabilizing solutions and therefore they are unsuitable for the long-term storage. The presented research deals with development of a new method for in-situ synthesis of silver and copper nanoparticles by electric discharge generated in the liquid phase. Nanoparticles are prepared by bottom-up process from silver nitrate and copper sulphate solutions of different concentrations by plasma treatment in a specially designed glass container. Newly constructed electrode systems for solution treatment are used where one of them is straight with argon gas feed and second one is L-shape curved without gas influx [1]. Plasma discharge generated directly in the inorganic salt solution at normal conditions (room temperature and atmospheric pressure) was used. Nanoparticles are prepared without use of any stabilization agent and they have nearly spherical shape with size below 100 nm. Nanoparticles prepared under various operational conditions were characterized by ultraviolet-visible spectroscopy (UV-Vis) and scanning electron microscopy (SEM). Future research will be focused on nanoparticle properties and quantity improvement. Prepared particles will be consequently used for further research of their antibacterial activity.

References

[1] F. Krčma et al. 2016 Plasma Medicine. 6(1) 21-31

P33: High cycle fatigue behaviour of different types of cast MAR-M 247 superalloy at high temperatures

<u>Vít Horník</u>¹, Miroslav Šmíd², Pavel Hutař², Karel Hrbáček³ ¹CEITEC IPM, Žižkova 22, Brno, 616 62, Czech Republic ²IPM, Žižkova 22, Brno, 616 62, Czech Republic ³První brněnská strojírna Velká Bíteš a.s., Vlkovská 279, 595 12 Velká Bíteš, Czech Republic

Cast polycrystalline nickel-based superalloy MAR-M 247 was subjected to fatigue tests at temperatures of 650 and 800 °C. The investigated alloy was available in several batches cast under different conditions and also into different types of molds. High cycle fatigue tests were conducted by 100 kN resonant testing machine under load control regime in fully reversed loading (R = -1). Significant differences in fatigue life time were observed among various batches. Obtained fatigue test results are showing the effect of the distribution and size of defects, average grain size and also experimental temperature. Fractured surface of tested specimens were studied by light and scanning electron microscopy. Typical features of fracture surfaces for the experimental temperatures were closely studied and main deformation mechanisms were described. Fatigue crack initiation sites were, in most of the cases, shrinkage pores inherited from casting at both temperatures.

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P34: Thin films prepared with a Kaufman broad ion-beam source for a top-down micromachining process of MEMS/NEMS resonators

Miloš Hrabovský¹

¹Department of Microelectronics, Faculty of Electrical Engineering and Communication – Brno University of Technology, Brno, 616 oo, Czech Republic

In this abstract we propose the fabrication of MEMS/NEMS piezoelectric resonators with precise and controlled steps. The main aim of this work is to prevent impurities which generally originate from oxidation and layer micromachining after each deposition step. Impurities in aluminum nitride (AIN) piezoelectric layers lead to decreasing piezoelectric coefficients and building-up residual stress which is unwanted for MEMS/NEMS resonator fabrication [1]. This fabrication process will be realized with bottom-up deposition of all functional layers in one process such as a bottom titanium (Ti) electrode, AIN piezoelectric layer and top Ti electrode. Properties of thin films such as crystallographic orientation, misorientation of individual crystallites, residual stress, roughness, etc. are strongly dependent on the underlying material and the process parameters of ion-beam deposition by a Kaufman broad ion-beam source [2,3]. These fabricated MEMS/NEMS resonators are significant due to their CMOS-compatibility and bio compatibility as a result of material choice [4].

References

[1] M. Dubois, P. Muralt, Journal of Applied Physics, 89, (2001) 6389-6395

[2] I. Gablech, V. Svatoš, O. Caha, M. Hrabovský, Journal of Material Science, 51, (2016) 3329-3336

[3] J. Xiong, H. Gu, K. Hu, M. Hu, International Journal of Minerals, Metallurgy, and Materials, 17, (2010) 98-103

[4] N. Jackson, L. Keeney, A. Mathewson, Smart Materials and Structures, 22, (2013), 115033

P35: Scan to map fitting for indoor localisation of mobile robots - vectorized approach

Ales Jelinek¹, Ludek Zalud¹

¹Central European Institute of Technology, Brno, Czech Republic

Localisation with a map and especially a simultaneous localisation and mapping (SLAM) has employed researchers in robotics for more than three decades [1]. Many great steps towards the truly autonomous mobile robots have been made so far, but there are still many problems to be solved. One of the main ones lies in the area of an artificial intelligence and consists in representation of a spatio-temporal memories of robot's surroundings as captured during its operation. Large amount of these data calls for an effective compression and generalizing technique, which would help to get rid of unnecessary measurements and distinguish between the signal and noise.

We believe, that such technique is vectorization, i.e. conversion of point-like data (originating from distance measurement) into more complex geometrical objects, such as line segments in two dimensional space, or triangle meshes in 3D. Memory savings are evident, since several thousands of points can be converted into a handful of these objects. Efficient conversion algorithm and the generalizing abilities were already presented in [2].

To prove a practical usability of our approach in the SLAM related problems, a scan to map registration procedure was devised. It is fast and gives optimal results, if correct correspondences are provided. Though we do not deal with the SLAM in its full complexity yet, the results confirm the vectorization to be a viable way forward and determine further direction of our research.

References

[1] S. Thrun, "Robotic Mapping: A Survey," in Exploring artificial intelligence in the new millennium, 2003.

[2] A. Jelinek, L. Zalud, and T. Jilek, "Fast total least squares vectorization," in Journal of Real-Time Image Processing, 2016.

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T16: Plasma deposition of antibiotics containing composite coatings

P. Jelínek^{1,2}, F. Palumbo⁴, C. Lo Porto³, L. Zajickova^{1,2}, G. Camporeale³, P. Favia³

¹Department of Physical Electronics Faculty of Science, Masaryk University, Brno, Czech Republic ²Plasma Technologies, CEITEC – Central European Institute of Technology, Masaryk University, Brno, Czech republic

³Department of Chemistry, Universita degli Studi di Bari, Bari, Italy 4 Instituto di Nanotecnologia, CNR, Universita degli Studi di Bari, Bari, Italy

This contribution presents our last outcomes on an atomizer-assisted atmospheric-pressure plasma process for the production of coatings obtained by the co-deposition of an organic precursor and water or water solution containing a bioactive molecule - vancomycin. The purpose of this process is to deposit a coating for the controlled release of the bio-active molecule. It has been demonstrated that changing the deposition parameters can tune the release at a certain extent.

The investigated method is based on a one-step deposition process in the DBD reactor. It consisted of two parallel plate silver electrodes, 5 mm apart, both covered by thick alumina sheets. Helium (carrier gas, 99.999% Air Liquide) and ethylene were fed into the chamber via electronic mass flow controllers (MKS Instruments).

Helium plasma was ignited in the inter-electrode gap, while ethylene was added in constant amounts and water Vancomycin aqueous solution droplets were injected through an aerosol generator (atomizer). An aerosol-assisted AP plasma deposition process has been used to deposite vancomycin/CHx coatings. It has been found that Vancomycin containing coatings were deposited using DBD in pulsed mode contains significantly higher amount of Vancomycin then layers with similar thickness deposited with continuous mode. It was also shown that the deposition of top coating, a pure CHx film, could influence release rate of vancomycin and, therefore, allow tuning the release rate in a specific way.

P36: Electrospun titanium oxide nanofibers

<u>Eva Jindrová</u>¹, Klára Částková¹, Jakub Němčovský² ¹CEITEC BUT, Brno University of Technology, Brno, Czech Republic ²Faculty of Mechanical Engineering, Brno University of Technology, Brno, Czech Republic

Low temperature titanium oxide phase (anatase) is known for its photocatalytic characteristics. It is obvious that this attribute is strongly connected with the surface area of the material [1]. Therefore, nanofibers are a very promising type of structure in this case – owing to their high specific surface area. Electrospinning is quite fast and simple method for the preparation of such nanofibers. The technique is based on effect of the electrostatic field on a conductive liquid (usually organic) precursor.

The precursor for preparation of the TiO₂ nanofibers was composed of tetraisopropyl orthotitanate, stabilized by acetyl acetone, dissolved in ethanol. The influence of various parameters (composition and viscosity of the precursor, spinning voltage, size of the spinning needle) on the fibers' morphology were investigated. Data were analyzed by non-parametrical statistical test revealing the most important parameters influencing the diameter of the TiO₂ nanofibers. The influence of the calcination temperatures on the phase composition of the fibers has been described, too.

References

[1] RAHIMI, Nazanin, Randolph A. PAX a Evan MacA. GRAY. Review of functional titanium oxides. I: TiO2 and its modifications. Progress in Solid State Chemistry. 2016, 44(3), 86-105. DOI: 10.1016/j.progsolidstchem.2016.07.002.

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P37: High-resolution transmission electron microscopy analysis of 1-D nanostructures

Lukáš Kachtík¹, Tomáš Pejchal¹, Miroslav Kolíbal^{1,2}, Tomáš Šikola^{1,2} ¹CEITEC - Central European Institute of Technology, Purkyňova 123, Brno 61200 Czech Republic ²Institute of Physical Engineering, Brno University of Technology, Technická 2, Brno 61669, Czech Republic

One-dimensional nanostructures exhibit many electronic and optical properties that are not seen in bulk. These properties, like bandgap and electron mobility, can be tuned by changing the nanowire composition and structural properties, which is given e.g. by the amount of dopants in the nanowire [1]. Highly doped nanowires are predicted to exhibit plasmonic resonance frequencies in the infrared region, which is suitable for their use for detection of biomolecules [2]. The most powerful instrument for nanostructure analysis is transmission electron microscope equipped with different spectroscopic techniques, offering resolution at the atomic level.

The aim of this study is to analyze the semiconductor nanowires and nanotubes (Fig. 1) providing the information about concentration of dopants, their position in nanowire and structural changes induced by dopant incorporation.

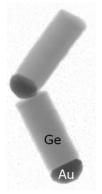


Fig. 1: STEM-EDX elemental map of Germanium nanowire.

References

[1] Stephanie L., et al., All-Semiconductor Plasmonic Nanoantennas for Infrared Sensing. Nano Letters. 2013, 13 (9), 4569-4574, DOI: 10.1021/nl402766t

P38: Computed tomography for inspection of inner structure of selected materials

<u>Dominika Kalasova</u>¹, Tomas Zikmund¹, Jozef Kaiser¹ ¹Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic

X-ray computed tomography (CT) is a nondestructive method for imaging of inner structure of materials. The sample is placed between the X-ray tube and the detector. A series of projections from different angles of rotation of the sample is recorded. From these projections, tomographic slices through the sample are reconstructed to get 3D data. Gray values in slices correspond to linear attenuation coefficient of material.

In this work, we show examples of application of CT from three different fields in material science.

- **Geology.** Knowledge of volume of different phases in meteorites found in different times after impact give information about rock original composition and erosion [1]. Volume of different phases can be determined by CT.
- **Civil engineering.** Fibre reinforced polymers are composite materials made of polymer matrix reinforced with fibres [2]. Fibre distribution in the bars can be controlled with CT.
- **Tissue engineering.** Collagen-based scaffold is an ideal substrate for cells seeding and growing. The optimum porosity and pore interconnectivity is essential for successful cell seeding. It can be measured and controlled by CT. [3]

References

[1] Kalasová, D., et. al., e-Journal of Nondestructive Testing. NDT. net, 2017. ISSN: 1435-4934.

[2] Girgle, F., etl al., KEY ENG MAR, 2017, 722 KEM, 2017. ISSN: 10139826.

[3] Žídek, J., et. al., J MATER SCI-MATER J, 2016, 27(6), 1-18. ISSN: 0957-4530.

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P39: Carbon nanotubes and its potential applications: A review

Preeti Kaushik^{1,2}, Lenka Zajičkova^{1,2}

¹Central European Institute of Technology, Masaryk University, Brno, Czech Republic. ²Department of Physical Electronics, Faculty of Science, Masaryk University, Czech Republic.

Carbon nanotubes (CNTs) have been used since past few years because of their unique electrical, mechanical and thermal properties [1]. These properties make them suitable for use in wide applications like nanotechnology, electronics, medical, optics and many more. Since their invention by lijima in 1991, CNTs have made a revolutionary change in different fields of science and technology. Synthesis of carbon nanotubes (CNTs) has always been the most influential process in the development of CNTs. Various techniques used for CNT growth arearc discharge, laser ablation and chemical vapor deposition (CVD).

Gas sensing has played a very important role as detection of various toxic gases from industries or other hazardous sources is a major research topic. CNT based gas sensors are more reliable as compared to metal oxide semiconductor based gas sensors as the later are more sensitive to environmental parameters. Recently, Bannov et al. developed highly sensitive ammonia gas sensors based on plasma treated carbon nanostructures [2].

High surface area and ability to recognize targets or analytes makes them suitable for biosensing [3]. Some of the emerging applications of CNTs in nanomedicine are tissue engineering, drug delivery and water purification [4]. Hu et al. have shown that vertically aligned carbon nanotube (VACNTs) array proved to be promising material for gecko adhesive [5]. One of the most important application of CNTs is in field emission which make them suitable for use in electronic displays and pressure sensors.

Despite of huge progress on CNTs over the years a lot is still to be done to improve the efficiency, quality and the cost of CNTs.

References

[1] M. S. Dresselhaus, G. Dresselhaus, J. C. Charlier, E. Hernández, *The Royal Society*, 2065–2098, 2004.

[2] Alexander G. Bannov, Ondřej Jašek, Anton Manakhov, Marian Márik, David Nečas, Lenka Zajíčková, *IEEE Sensors Journal*, Vol. 17, No. 7, April 1, 2017.

[3] Carmen-Mihaela Tîlmaciu and May C. Morris, Frontiers in Chemistry, Vol. 3, October 2015.

[4] H. Sadegh, R. Shahryari-ghoshekandi, Nanomed. J., 2(4): 231-248, 2015.

[5] Shihao Hu, Zhenhai Xia and Liming Dai, Nanoscale, 2013, 5, 475.

P4o: Fabrication and application of graphene-metal heterostructures in biosensing by surface enhanced raman spectroscopy

Martin Konečný^{1,2}, Veronika Hegrová¹, Pavel Procházka^{1,2}, Miroslav Bartošík^{1,2}, Jindřich Mach^{1,2}, Filip Ligmajer^{1,2}, Miroslav Kolíbal^{1,2}, Peter Varga^{1,2}, Tomáš Šikola^{1,2} ¹Institute of Physical Engineering, Brno University of Technology, Technická 2, Brno 629 00, Czech Republic ²CEITEC BUT, Brno University of Technology, Technická 10, Brno 616 00, Czech Republic

Graphene is two-dimensional material consisting of carbon atoms arranged in a hexagonal lattice. Due to its unique electrical and mechanical properties graphene has become one of the most intensively studied materials with wide range of possible application extending from electronics to biochemistry and biomedicine [1]. Due to its unique optical properties, graphene has attracted an attention also in the field of sensing, where it was shown to enhance the Raman scattering of molecules adsorbed on its surface [2]. This effect is the basis of so-called Surface-Enhanced Raman Spectroscopy (SERS). SERS traditionally requires special substrates, typically in the form of noble metal nanostructures (nanoparticles, nanodiscs, nanorods), and facilitates detection of molecules at extremely low concentrations. In this work, we present fabrication of novel type of SERS active substrates that combine promising properties of both metal nanostructures and graphene, and demonstrate their application in biosensing. The graphene-metal heterostructures are fabricated by depositing the graphene over gold colloidal nanoparticles assembled on silicon substrate. The usefulness of such heterostructures in SERS biosensing is tested upon detection of Rhodamine 6G molecules

References

[1] K. Novoselov, et al., A roadmap for graphene, *Nature* **490**, 192-200 (2012), DOI:10.1038/nature11458

[2] X. Ling et al., Can Graphene be used as Substrate for Raman Enhancement?, *Nano Letters* **10**, 553-561 (2010), DOI: 10.1021/nl903414x

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P41: Unexpected structural distribution of electroactive *ortho*-Iminoboronate in protic and aprotic solvents determined using MS

<u>Martin Konhefr</u>¹, Karel Lacina², Monika Skrutková Langmajerová¹, Zdeněk Glatz¹, Petr Skládal^{1,2}, Ctibor Mazal³

¹Department of Biochemistry, Faculty of Science, Masaryk University, Brno, Czech Rep. ²CEITEC, Masaryk University, Brno, Czech Rep. ³Department of Chemistry, Faculty of Science, Masaryk University, Brno, Czech Rep.

As metal cations are essential not only in biology, their selective sensing is important also from an industrial and environmental viewpoint. These diverse applications have lead to the development of many molecular sensor systems for cationic guest species. A wide variety of e. g. macrocycles with ferrocene unit as redox signaling group have been synthesized [1]. Generally, complexation by these ligands (cations) affects the electrochemistry of the redox center, particularly when the guest molecule is either in close proximity to the iron atom or coordinated by functional groups that are conjugated with the ferrocene system. This configuration is observable in the aminoferrocene derivatives.

As we studied the structure of novel iminoboronates bearing ferrocene as a signaling group for electrochemical sensing intended for a saccharide recognition [2], we realized that *ortho*-positioned iminoboronate in MS formed stable sodium and potassium chelates. These complexation properties were observed only in a protic solvent (methanol). None complexes were detected in an aprotic solvent (acetonitrile). Thus, methanol–hydroxyle rearrangement on boron atom in the proximity of imino group plays the most important role in the forming of an ideal surrounding (host) around complexed cation (guest) to form the chelate. In addition, this interacting site is close to redox-active center of ferrocene which reports on the binding event. Contrary to the protic solvent, stable *ortho*-iminoboronate anhydrides were observed in the aprotic solvent without any tendency to form chelates with cations.

References

[1] Beer, P. D. et al.; J. Am. Chem. Soc., Chem. Commun., 1989, 23, p. 1831.
[2] Konhefr, M. et al.; Monatshefte für Chemie, 2017 (submitted).

T17: Singular stress field near the sharp material inclusion tip and crack onset assessment

Ondrej Krepl¹, Jan Klusak¹

¹CEITEC IPM, Institute of Physics of Materials AS CR, Zizkova 22, Brno 616 62, Czech Republic

Composite materials find application in a variety of engineering components and structures. Desired material properties which can only be achieved by combination of 2 or more homogenous materials bring solution for high demands given by contemporary advanced technologies. However, the very nature of composites also causes difficulty in their assessment in terms of fracture mechanics. In the case of particle reinforced composites, where the particles are in a form of sharp material inclusions, singular stress concentration exists on each inclusion's tip. This is due to the geometric and material discontinuities between matrix and particle. These points of singular stress concentration are susceptible of crack initiation and thus responsible for failure of whole component or structure. Stress, energy or coupled criterion can be employed in order to predict crack onset conditions. The stress field near such singular stress concentrator is described by an asymptotic series. In a majority of fracture mechanics applications, only the singular terms of the series are used. Increased precision in stress field description is achieved by using both singular and non-singular terms. This can lead to improved estimation of the critical failure load for component failure prevention or composite design optimization.

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P42: About graphene oxide

Ondrej Kubesa¹, Karel Lacina², Veronika Horackova², Petr Skladal^{1,2}

¹Department of Biochemistry, Faculty of Science, Masaryk University, Kamenice 5, Brno, 625 oo, Czech Republic

²CEITEC – Central European Institute of Technology, Masaryk University, Kamenice 5, Brno, 625 oo, Czech Republic

Popularity of graphene oxide (GO) became ballistic in the last few years – in short, it almost seems that now everyone wants to work with graphene. However, as the number of electrochemical papers about graphene grows, so is the amount of misinterpretations.

Our work was mostly focused on preparation of GO and its electrochemical properties, namely catalysis. Our results show, that chemical preparation of GO by strong oxidation is very tricky. Several approaches were tested, such as different sizes of graphite particles, oxidation mixtures, even repetitive preparations under the same conditions usually resulted in different products. Another big topic are impurities which are present due to natural occurrence in graphite and some of them might be also introduced during purification processes of GO [1]. This could also lead to often observed catalytic activity of graphene [2]–[4]. From our observations neither selectivity nor significant electrocatalytic abilities of the reduced graphene oxide modified electrodes towards H2O2 were observed [5]. Moreover, our results suggest that GO does not need linking molecules in order to be deposited onto the surface of the electrodes, since it attaches spontaneously and keeps its electrochemical properties.

Graphene is usually reported as a superior material, but its proper description and characterization should not be underestimated.

References

[1] M. Pumera, Electrochem. Commun., vol. 36, pp. 14–18, Nov. 2013.

[2] Y. Shao, Electroanalysis, vol. 22, no. 10, pp. 1027–1036, Květen 2010.

[3] W. Chen, The Analyst, vol. 137, no. 1, pp. 49–58, 2012.

[4] J. Jiang and X. Du, Nanoscale, vol. 6, no. 19, pp. 11303–11309, Jun. 2014.

[5] K. Lacina and O. Kubesa, Electrochimica Acta, vol. 223, pp. 1–7, Jan. 2017.

P43: Simulation studies of receiver domain of cytokinin receptor CKI1RD from *Arabidopsis thaliana*

Sudhir Kumar Pal, Zuzana Jaseňáková, Lukáš Žídek, Jan Hejatko, Jozef Hritz Central European Institute of Technology, Masaryk University, Kamenice 5, CZ-62500 Brno, Czech Republic

The Two-Component-based Signaling (TCS) pathways (which are critical for hormonal regulation viz cytokinin signaling) in plants are mediated via a modified system known as MultiStep Phosphorelay (MSP) [1]. The signal transfer in Arabidopsis MSP from sensor Histidine Kinase (HK) to nuclear response regulators is redirected via histidine phosphotransfer proteins (AHP1–AHP5). The C-terminal receiver domain of HK CKI1 (CKI1RD) was proved to be responsible for the recognition of CKI1 downstream signaling partners; interacting specifically with AHP2, AHP3 and AHP5 with different affinities. Mg2+ acts as a co-factor necessary for signal transduction via MSP and its binding induces the rearrangement of some residues around the active site of CKI1_{RD} (which shares high similarities with the only known structure of plant HK, ETR1 RD, having main differences in loop L3) [2]. Recent NMR studies also suggested that out of these three only AHP3 complexed with CKI1RD. To support these experiments, we are studying the behaviour of L₃ loop and its role in MSP signaling mechanism through molecular modelling and dynamics simulations studies. As far according to the obtained results, we observe interesting stabilization of L₃ loop with MG₂+ and phosphorylation in action. Long range interactions are speculated to play vital role in the side chain motion of Met-1053 and Met-1056 in the direction of force of interaction, leading to different L₃ conformation than ETR1. More information is needed to make a strong conclusion.

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P44: Highly-ordered gold nanoparticle surfaces for localized surface plasmon resonance sensors

Tomáš Lednický¹, Attila Bonyár²

¹Central Institute of Technology (CEITEC), Brno University of Technology, Purkynova 123, 61200 Brno, Czech Republic

²Department of Electronics Technology, Budapest University of Technology and Economics, Budapest, Hungary

We present a technology to fabricate highly-ordered gold nanoparticle surfaces and to transfer them onto transparent polymer substrates. The nanoparticles are synthetized by utilizing the solid-state dewetting process of thin gold layers over nanodimpled aluminum templates. These templates were prepared by a porous anodizing of an aluminium sheet followed by selective etching of formed porous anodic alumina. Nanoparticles formed this way were directly transferred to a PDMS (polydimethylsiloxane) blocks, where their localized surface plasmon resonance (LSPR) transmittance spectra were measured. It was proved, that the presented fabrication technology enables proper control over the nanoparticle size and interparticle distance over a large surface area (cm² range), and that it is possible to synthetize nanoparticle arrangements, where the size/separation ratio can be tune to yields enhanced plasmonic sensitivities due to interparticle coupled plasmon effects.

P45: Rheological investigation into bottom-up solution blending preparation route of polymer nanocomposites

Petr Lepcio¹, František Ondreáš¹, Marek Zbončák¹, Josef Jančář¹ ¹Central European Institute of Technology, Brno, Czech Republic

An enormous scientific effort has recently focused on enhancement of thermomechanical, optical, electromagnetic, and barrier properties of polymers by nanoparticles (NPs). The resulting material is usually referred to as a polymer nanocomposite (PNC) and its extraordinary properties are inherited from interactions at vast NPs-polymer interface. Several strategies have been developed to control structural organization of NPs in polymer matrix, solution blending being one of them. It takes advantage of high mobility of species in low viscous media and various solvation, adsorption and specific interaction effects to fabricate nanocomposite assemblies of nanoparticles and polymer chains. The process is generally termed as a "bottom-up" technique since the bulk is constructed out of individual building components.

The aim of this study is to investigate a solution blending route of polymer nanocomposites through the means of rheology. The rheological response of various model systems was recorded and related to the structure. While rheology is not capable to provide a direct structural information and has to be combined with additional techniques (e.g. electron microscopy, SAXS), it proves as a great source of indirect evidence sensitive to structural features of nanocomposite suspensions.

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T18: Plasmonic hot carriers in photoelectrochemistry

Filip Ligmajer^{1,2}, Lukáš Kejík^{1,2}, Aleš Daňhel³, Miroslav Kolíbal^{1,2}, Miroslav Fojta^{3,4}, and Tomáš Šikola^{1,2} ¹CEITEC BUT, Brno, Czech Republic ²Institute of Physical Engineering, BUT, Brno, Czech Republic ³Institute of Biophysics of the CAS, v.v.i., Brno, Czech Republic ⁴CEITEC MU, Brno, Czech Republic

When metallic nanostructures are illuminated by light, collective oscillations of their electrons coupled to the electromagnetic field are excited. These excitations, called surface plasmon polaritons (SPPs), are the fundamental building block of many modern technologies based on subwavelength light confinement and enhanced light-matter interactions [1]. During the non-radiative decay of SPPs so-called hot charge carriers are produced - i.e. electrons and holes with energy and momentum much higher than that corresponding to the purely thermal Fermi distribution. Although inherently short-lived, these hot carriers can reach the metal surface and sensitize the conduction band of nearby semiconductors or facilitate chemical reactions that would otherwise not proceed. In our contribution, we will explain the main physical phenomena that govern the formation of hot carriers and also their useful extraction in terms of electromotive force or in chemical reactions. We will also describe a prototypical hot carrier system consisting of semiconducting tungsten disulfide nanotubes decorated by gold nanoparticles. This hybrid material can act as a nanostructured electrode when immobilized on the surface of transparent conductive oxide and the hot carrier effects can thus be studied using common electrochemical methods.

Acknowledgement

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References

[1] S. A. Maier, Plasmonics: Fundamentals and Applications, Springer, 1st ed. (2007).

P46: Maleic anhydride and acetylene plasma co-polymers for the development of effective QCM and SPR immunosensors

<u>E. Makhneva</u>^{1,2}, Z. Farka^{2,3}, A. Obrusnik^{1,2}, M. Michlicek^{1,2}, P. Skládal^{2,3}, L. Zajíčková^{1,2}

¹RG Plasma Technologies, European Institute of Technology (CEITEC), Masaryk University, Brno, Czech Republic

² Faculty of Science, Masaryk University, Brno, Czech Republic

³RG Nanobiotechnology, CEITEC, Masaryk University, Brno, Czech Republic

Biosensors have been extensively developed and applied for biomedical and environmental studies. Quartz crystal microbalance (QCM) and surface plasmon resonance (SPR) biosensors appeared to be very promising tools regarding the sensitive determination of various antigens and bacteria.

For the development of effective biosensors, the immobilization of the biorecognition biomolecules onto the sensor surface is always required. Carboxyl-rich films are of high interest for bio-applications thanks to their high reactivity allowing the formation of the covalent linkages between biomolecules and a surface. For most applications, a sufficiently high surface concentration of functional groups as well as the layer stability in different buffers is required.

In our work, stable carboxyl-rich plasma polymers (COOH PPs) were deposited using plasma co-polymerization of maleic anhydride and acetylene at atmospheric pressure using dielectric barrier discharge (DBD). Concentrations of carboxyl groups were determined by well-known derivatization with trifluoroethanol (TFE).

For the immunosensors development 20 nm thick COOH PPs were deposited onto the gold surfaces of QCM electrodes and SPR chips. Then the monoclonal antibody AL-01, specific to human serum albumin (HSA) was immobilized using EDC/NHS activation of COOH groups in the polymer. Performance of QCM and SPR immunosensors was evaluated by the immunoassay flow test.

P47: On properties and penetration depth of maleic anhydride-acetylene plasma co-polymer into elecrospun polycaprolactone nanofibers

Miroslav Michlíček^{1,2}, Anton Manakhov², Eva Kedroňová^{1,3}, Lenka Zajíčková^{1,2}

¹Department of Physical Electronics, Faculty of Science, Masaryk University, Kotlářská 2, Brno 611 37, Czech Republic

² Plasma Technologies, CEITEC – Central European Institute of Technology, Masaryk University, Kotlářská, 2, Brno 611 37, Czech Republic

³Department of Chemistry, Faculty of Science, Masaryk University, Kamenice 753/5, Brno 625 oo, Brno, Czech Republic

In this work, we present the deposition of the reactive carboxyl and anhydride groups performed by co-polymerization of maleic anhydride and acetylene using atmospheric pressure dielectric barrier discharge (AP-DBD). This low cost plasma process allowed the deposition of stable carboxyl and anhydride coatings onto the polycaprolactone nanofibers. Using the derivatization with trifluoroethylamine we determined that up to 6 at.% of reactive anhydride groups can be deposited at the nanofibrous substrate surface. The chemistry and homogeneity of the plasma coating was studied by Scanning Electron Microscopy, Infrared and X-ray Photoelectron Spectroscopies and Secondary Ion Mass Spectrometry. Analysis of the coated PCL meshes revealed that the deposition depth of coating exceeds tens of micrometers and traces of the coating are present more than about a hundred micrometers deep.

Acknowledgement

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P48: Nanoindentation of magnetron sputtered hard W-B-C coatings

<u>Saeed Mirzaei</u>, Mostafa Alishahi, Pavel Souček, Lukáš Zábranský, Vilma Buršíková and Petr Vašina

Department of Physical Electronics, Faculty of Science, Masaryk University, Brno, Czech Republic

In the present work, the microstructure and mechanical properties of new boron carbide family WBC thin films were studied, experimentally. A series of nanostructured W-B-C films were deposited by magnetron co-sputtering of W, B4C and C targets. The mechanical behavior of prepared films was analyzed by different nanoindentation techniques. Their composition has been analyzed by using Energy-dispersive X-ray spectroscopy (EDS) and Rutherford backscattering spectrometry (RBS). The incorporation of tungsten and carbon content in the films were in the range of 41-60.9 and 7.1-27.7 at.%, respectively. It is illustrated that, as the carbon to tungsten ratio (C/W) increases, the growth of the films experiences a transition from the granular structure into porous columnar one which accompanies by initiation of cracks first in the corner then on the side of the indents. In addition, as a result of increasing in the C/W content, the hardness and reduced elastic modulus of the coatings are increasing considerably from 21.6 to 28.4 GPa and 299 to 346 GPa, respectively.

¢° ⊂ EITEC

P49: Fabrication of germanium nanostructures using wet chemical etching

Tomáš Musálek¹, Tomáš Šikola^{1,2} and Miroslav Kolíbal^{1,2}

¹Institute of Physical Engineering, Brno University of Technology, Technická 2, 616 69 Brno, Czech Republic ²CEITEC BUT, Brno University of Technology, Technická 10, 616 oo Brno, Czech Republic

Nowadays, novel approaches in electronics, plasmonics and photonics aim to improve current technologies with the use of precisely grown semiconductor nanowires. Semiconductor nanowires prepared by vapor-liquid-solid method normally grow under precisely defined angles given by the substrate crystallography. For the production of complex structures combining two or more nanowire types, it is needed to pattern the substrate. One of the solutions to modify the substrate is anisotropic etching. Anisotropic wet etching of silicon has been exploited and successfully used in many different commercial applications. However, on the topic of anisotropic etching of germanium only a small amount of publications exists.

Two different approaches to the formation of anisotropic etch pits on germanium are shown in this work. First one includes atomic layer deposition (ALD) and electron beam litography (EBL) methods for preparation of mask followed by wet etching in H_2O_2 : H_3PO_4 : C_2H_5OH - 1:1:1 solution [1]. The other method to etch pits in germanium substrate incorporates deposition of metal micro and nano particles onto the germanium surface. After deposition of such particles the substrate is immersed into water. Consequently, an accelarated oxidation occurs under deposited metal particles and thus and an etch pit is created [2].

References

[1] Leancu, R. et al., Anisotropic etching of germanium, Sensors and Actuators A: Physical., 46.1-3 (1995): 35-37

[2] Kawase, T. et al., Metal-assisted chemical etching of Ge(100) surfaces in water towards nanoscale patterning, *Nanoscale research letters*. 8.1 (2013): 151

T19: Encapsulation of potential ruthenium prodrugs into macrocyclic carriers

Martin Novák¹, Petra Munzarová¹, Jan Chyba¹ and Radek Marek^{1,2} ¹CEITEC MU, Kamenice 5, Brno, Czech Republic ²Department of Chemistry, Masaryk University, Kamenice 5, Brno, Czech Republic

Only a handful of metallodrugs based on platinum is currently approved for treatment of patients. Although they are efficient against many types of cancer, they induce severe side effects. One of the most widely used alternative metal is ruthenium for its favorable characteristics. lt has two relevant oxidation states, favorable ligand-exchange dynamics and octahedral coordination sphere making it a very versatile scaffold for coordination chemistry and prime candidate for in vivo testing.

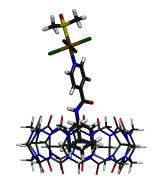


Fig. 1: Ruthenium prodrug in CB[7].

Ruthenium complexes are efficient against many cancerous cell cultures. However, clinical trials on humans were canceled due to the small impact of the treatment on cancerous tissue in save dosages. The primary goal of this project is computationally design, synthesize, encapsulate and, test the biological activity of novel coordination compounds based on ruthenium. The design is performed using quantum mechanical tools, the affinity of metallodrugs towards macrocyclic cavities, specifically cucurbit[7]uril (CB[7]) and β -cyclodextrin (β -CD) is estimated using DFT. Promising candidates are synthesized and the encapsulation is characterized by NMR, MS, X-Ray and, ITC.

Currently, the supramolecular assemblies between three derivatives of octahedral ruthenium complexes bearing the adamantyl moiety with β -CD and CB[7] are characterized. Experimental data show that the association constant is approximately 10^7 M^{-1} for complexes with CB[7] and 10^4 M^{-1} for β -CD. The encapsulation manifests itself by inducing secondary NMR chemical shifts on both partners which are further analysed using DFT.

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P50: Thermo-mechanical response of polymer nanocomposites with governed structure

<u>František Ondreáš</u>¹, Petr Lepcio¹, Marek Zbončák¹, Josef Jančář¹ ¹Central European Institute of Technology, Brno, Czech Republic

Polymer nanocomposites are a promising group of next-generation materials thanks to their advanced properties. However, their performance depends strongly on nanoparticle spatial organization in polymer matrix. Self-assembly process was controlled by adjusting preparation protocol conditions on a model system of bare nanosilica and polymethylmethacrylate (PMMA). Three basic dispersion states of nanofiller in polymer matrix were obtained out of different solvents: good dispersion state (steric stabilization), clustered state (bridging/tele-bridging), and aggregated state (contact aggregation). The ability to prepare various nanostructures at constant composition allowed to investigate their influence on final thermomechanical properties. Dependence of glass transition temperature (Fig. 1), entanglement network density, and stiffness on structure type was determined.

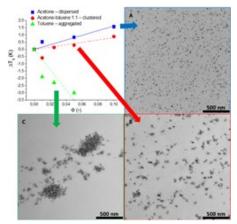


Fig. 1: Dependence of glass transition on volume fraction of various nanostructures

T20: Detection of *Salmonella* in milk using enzymatic precipitation enhanced SPR immunosensor

Matěj Pastucha^{1,2}, Zdeněk Farka¹, Tomáš Juřík^{1,2}, Petr Skládal^{1,2}

¹CEITEC, Masaryk University, Kamenice 5, 625 oo Brno, Czech Republic ²Department of Biochemistry, Faculty of Science, Masaryk University, Kamenice 5, 625 oo Brno, Czech Republic

Foodborne bacterial pathogens represent a serious threat for public health. Recent outbreaks have demonstrated the need for rapid and reliable screening techniques. As an alternative to the conventional methods, various biosensors have been developed. They offer fast, sensitive, specific, robust and cost effective analysis and have a potential for point-of-care application [1]. In this work, we have developed a surface plasmon resonance (SPR) immunosensor for the detection of *Salmonella Typhimurium* in powdered milk. In addition to a conventional label-free SPR assay, an enzymatic enhancement step was incorporated. The method did not require any pre-enrichment, making the assay fast and simple. After *Salmonella* was bound by capture antibody immobilized on the sensor surface, solution of detection antibody labelled by horseradish peroxidase was injected. The bound enzymatic label in turn catalyzed the precipitation of 4-chloro-1-naphthol to insoluble benzo-4-chlorocyclohexadienone. The formation of precipitate on the sensor surface provided substantial signal enhancement.

The precipitation-based assay format improved the sensitivity 40 times compared to the label-free format. The limit of detection of $10^2 \text{ CFU} \cdot \text{mL}^{-1}$ in buffer and $10^3 \text{ CFU} \cdot \text{mL}^{-1}$ in milk was achieved and the whole analysis time was below 60 min. The interaction of the bacteria with the sensor surface and the formation of the precipitate were studied in detail by optical and atomic force microscopy. The developed method represents a suitable approach for routine testing of food contamination [2].

Acknowledgement

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References

[1] Farka Z., Juřík T., Pastucha M., Kovář D., Lacina K., Skládal P. Electroanalysis 2016, 28(8), 1803–1809.

[2] Farka Z., Juřík T., Pastucha M., Skládal P. Anal. Chem. 2016, 88(23), 11830–11836.

\$\$ CEITEC

P51: Ge nanowire growth orientation and kinking

Tomáš Pejchal¹, Miroslav Kolíbal^{1,2}, Tomáš Šikola^{1,2}

¹CEITEC, Brno University of Technology, Brno, Czech Republic ²Institute of Physical Engineering, Faculty of Mechanical Engineering, Brno University of Technology, Brno, Czech Republic

The morphology of semiconductor nanowires is important for future biosensing and electronic applications. Controlling the growth direction of Vapour-Liquid-Solid (VLS)-grown nanowires and its manipulation is a complex task attracting a lot of attention. Germanium nanowires grown from a vapour phase (using hydride precursors and H2 as a carrier gas) exhibit <111> growth direction. On the other hand, Ge nanowires grown from atomic vapour (in Molecular Beam Epitaxy, MBE) tend to grow in <110> direction preferentially.

In this contribution, Ge nanowires were grown from atomic vapour with an optional supply of atomic hydrogen. Our results reveal the essential role of atomic hydrogen adsorption and gold outdiffusion from the catalyst droplet in determining the nanowire sidewall orientation and consequently, the growth direction of Ge nanowires. Our results stress the role of the catalyst material and surface chemistry in determining the nanowire growth direction and provide additional insights into a kinking mechanism, allowing to inhibit or intentionally initiate spontaneous kinking.

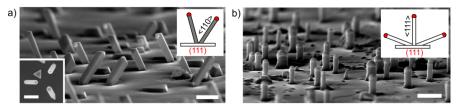


Fig. 1: Germanium nanowires of different morphology grown using Au catalyst droplet (a) in ultra-high vacuum, growth direction <110>, (b) in atomic hydrogen, growth direction <111>. Scale bars, 400 nm. [1]

References

[1] M. Kolíbal, T. Pejchal, T. Vystavěl and T. Šikola, Nano Letters 16 (2016), 4880.

P52: On a chip electrophoresis immunoassay with quantum dots

Jelena Pejovic Simeunovic^{1,2}, Jaromír Hubálek^{1,2}

¹Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic ²Faculty of Electrical Engineering and Communications, Department of Microelectronic, Brno University of Technology, Brno, Czech Republic

Antibodies with high targeting capability after being labelled by quantum dots (QDs) have been of a great importance for in vitro diagnostic, cellular imaging and in vivo theranostic applications. However, such a work concentrating on the conjugation of QDs and antibodies is still insufficient [1].

In the present work, CdTe QDs were conjugated with anti-human serum albumin antibody (Ab). The bioconjugation reaction has been optimized with respect to the pH, reaction time and ionic strength of buffer. Detection and separation of Ab-QDs conjugates was done in microfluidic system, with on a chip electrophoresis and optical detection. For application in capillary electrophoresis immunoassays QDs were further conjugated with human serum albumin via antigen-antibody reaction. QDs and QDs-antibody-antigen conjugates were successfully separated using on a chip CE within 10 minutes. The immunoassay application of quantum dots in CE offers considerable advantages and can be readily applied to other large bio-molecules.

References

[1] B. Zhang, J. Yu, C. Liu, J. Wang, H. Han, P. Zhang, et al., *RSC Advances*, vol. 6, pp. 50119-50127, 2016.

¢° ⊂ EITEC

P53: Damage evolution in thermomechanical loading of Sanicro 25 stainless steel

Roman Petráš^{1,2}, Jaroslav Polák^{1,2}

¹Institute of Physics of Materials ASCR, Brno, Czech Republic ²CEITEC, Institute of Physics of Materials ASCR, Brno, Czech Republic

In-phase and out-of-phase thermomechanical fatigue (TMF) loading experiments were performed with austenitic stainless Sanicro 25 steel. Four different amplitudes of mechanical strain and the changes of the temperature in the range of 250 to 700°C were applied to standard cylindrical specimens. Mechanical response was recorded and fatigue lives were obtained. Early fatigue damage has been studied by means of scanning electron microscopy (SEM) combined with focused ion beam (FIB) cutting and electron backscatter diffraction (EBSD) imaging. TMF loading resulted in the development of the thin oxide layer in the first place. During in-phase loading fatigue cracks start in grain boundaries by preferred oxidation and cracking of the oxide. Cracks grew preferentially along grain boundaries which resulted in rapid crack initiation and low fatigue life. Multiple cracks perpendicular to the stress axis have first arisen in oxide layer during out-of-phase TMF loading. The cracked oxide layer resulted in the development of local oxidation and later in crack initiation of cracks perpendicular to the loading axis. The crack grew trans-granularly. The delayed fatigue crack initiation and trans-granular growth led to longer fatigue life. The effect of grain boundary oxidation, grain boundary orientation and the development of cracks in surface oxide on the early fatigue damage and resulting fatigue life were discussed.

P54: Residual stress in polyethylene pipes

Jan Poduška¹, Pavel Hutař¹, Andreas Frank², Jaroslav Kučera³ ¹Institute of Physics of Materials, Brno, Czech Republic ²Polymer Competence Center Leoben, Leoben, Austria ³Polymer Institute Brno, Brno, Czech Republic

Polyethylene pipes have been used for many types of applications (mainly transportation of drinking water and natural gas under pressure) for the last 50 years approximately. Producers of plastic pipes have been continuously improving their properties and performance. The guaranteed lifetime of plastic pipes should be at least 50 years. However, the expected lifetime of the newest polyethylene pipes is 100 years. It is quite difficult to perform such test that would prove operating life this long. The common hydrostatic pressure testing and other test methods are ineffective in the case of the new pipe grades, because achieving the final failure is difficult and seriously time consuming. However, the most of the pipe failures are the result of a mechanism called slow crack growth (or sometimes creep crack growth), during which the crack grows guite slowly in the radial direction through the pipe wall from the inner towards the outer surface. The plastic zone at the crack tip is very small, which enables the application of linear elastic fracture mechanics to describe the crack growth. The total lifetime of polyethylene pipe under service conditions can be assessed using this approach, but many input parameters are needed including residual stress distribution and magnitude in the pipe wall. The residual stress is a result of solidification after extrusion of the pipe. The experimental determination of residual stress magnitude and distribution is described in this contribution. The influence of residual stress on the lifetime and other performance parameters of polyethylene pipe is discussed.

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P55: Atmospheric plasma treatment of ITO/PET foil for flexible electronics

Masoud Shekargoftar¹, Richard Krumpolec¹ and Tomas Homola¹

¹R&D Centre for Low-Cost Plasma and Nanotechnology Surface Modifications (CEPLANT), Department of Physical Electronics, Faculty of Science, Masaryk University, Kotlářská 267/2, 611 37 Brno, Czech Republic

Recently, flexible electronic devices received a significant attention because of their low-cost, light weight and ability to be manufactured in a roll-to-roll fashion.[1] The materials used for flexible electronics cannot be processed at high temperatures (>150 °C), because the polymeric flexible substrates are thermally sensitive and can be damaged. Low temperature large area roll to roll processing (activation, cleaning) of flexible materials and functional coatings is therefore still challenging. In this work we used proprietary large-area ambient air plasma called diffuse coplanar surface barrier discharge to modify the ITO coating on PET substrate. ITO is often used as an electrode on PET, however electronic properties of ITO are significantly influenced by its surface properties. We found that ambient air plasma treatment of ITO led to higher surface energy. The cause of the higher surface energy is the fast decrease of carbon contaminants and increase of oxygen polar groups, which was found using the X-ray photoelectron measurement. Atomic force microscopy showed no damage on the ITO surface, however the overall roughness of the surface slightly decreased. Afterwards, a hybrid SiO_2/TiO_2 mesoporous semiconductive material [2] was deposited by plasma treated ITO/PET using inkjet printing. We found that ITO surfaces treated in plasma for two seconds provide excellent surface properties and SIO₂/TiO₂ coating has high quality.

References

[1] K. N. Kim, S. M. Lee, A. Mishra, G. Y. Yeom, *Thin Solid Films* 2016, 598, 315.
[2] T. Homola, P. Dzik, M. Veselý, J. Kelar, M. Černák, M. Weiter, *ACS Appl. Mater. Interfaces* 2016, DOI 10.1021/acsami.6b09556.

T21: Electrochemical analysis of unnatural base pair content in DNA prepared in semi-synthetic organism

Jan Špaček¹, Yorke Zhang², Floyd Romesber², Miroslav Fojta¹

¹Dept. of Structure and Interaction of Biomolecules at Surfaces, CEITEC MU, Kamenice 753/5, 625 oo Brno, Czech Republic

²Dept. of Chemistry, The Scripps Research Institute, 10550 North Torrey Pines Road, La Jolla, CA 92037, USA

A semi-synthetic organism (SSO) which is able to replicate DNA containing man-made unnatural base pair (UBP) was created [1]. More recently the method was further optimized and SSO capable of stable replication and virtually unlimited retention of multiple UBPs was developed [2]. Yet unpublished results indicate that SSO can transcribe and translate expanded genetic code into unnatural proteins (proteins with content of unnatural amino acids), which could be used among other in pharmacology.

Electrochemical approach, which is faster, more facile and less costly than currently used methods, is sensitive enough for quantitative analysis of (less than) single UBP pair per plasmid. Our electroanalytical method is already helping Romesberg's team with development of SSO, which indicate large potential for application of the developed method in the future, further developing SSO(s), but also in applied research related to this topic.

References

[1] Malyshev, D. A.; Dhami, K.; Lavergne, T.; Chen, T.; Dai, N.; Foster, J. M.; Correa, I. R., Jr.; Romesberg, F. E. *Nature* 2014, 509, 385-388.

[2] Zhang, Y.; Lamb, B. M.; Feldman, A. W.; Zhou, A. X.; Lavergne, T.; Li, L.; Romesberg, F. E. *Proc Natl Acad Sci USA* 2017.

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P56: Novel hydrogels based on natural polysaccharides for 3D bioprinting applications

Marija Stojic¹, Edgar Benjamin Montufar Jimenez¹, Lucy Vojtová^{1,2} ⁷Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic ²Faculty of Chemistry, Brno University of Technology, Brno, Czech Republic

Bioprinting technology requires a hydrogel with specific properties, including a fast gelling time (<2 minutes) with a crosslinking mechanism suitable for *in situ* gelation. The hydrogel must not only be non-immunogenic, but also able to support appropriate cellular activity. Biological hydrogels composed of polysaccharides and/or proteins are a class of materials that are challenging to 3D print because they must first be gelled *in situ* during the fabrication process and then supported so that they do not collapse or deform under their own weight. Here, we would like to stress on natural hydrogels which are based on modified version of biological materials found in human and animal extracellular matrix (ECM) such as alginate, gym karaya and carrageenan. The goal of the work is to design and develop biological hydrogels which will be used as bio ink for 3D bioprinting of tissues and organs.

Acknowledgement

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T22: Temperature mapping apping and heat balance monitoring of living cells

Vojtech Svatos^{1,2}, Pavel Neuzil^{1,2}

 ¹Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic
 ²Faculty of Electrical Engineering and Communications, Department of Microelectronic, Brno University of Technology, Brno, Czech Republic

A real-time cell energy balance and temperature mapping inside a living cell would enable to reveal details of complex biological processes inside the cell. Thermal energy readily diffuses into surrounding areas thereby making temperature mapping extremely challenging [1-3]. Combination of micromachining techniques, nanotechnology, and molecular and cell biology can create a tool with total volume of only 50–100 fL with a minute heat capacity. This tool will then be used to gain new insights into vast array of both fundamental as well as highly specific processes within a living cell. This allows to study mechanism of apoptosis to improve cancer understanding, cell responses to induced drugs etc. A unique microcalorimeter is presented in this abstract. The novel approach for temperature mapping and heat balance monitoring of living cells is proposed and discussed.

References

[1] Forrest, W. W. & Walker, D. J. Thermodynamics of Biological Growth. *Nature* **196**, 990-991 (1962).

[2] Vetrone, F. et al. Temperature Sensing Using Fluorescent Nanothermometers. *ACS Nano* **4**, 3254-3258, doi:10.1021/nn100244a (2010).

[3] Yue, Y. & Wang, X. Nanoscale thermal probing. *Nano Reviews & Experiments* **3** (2012) incl Supplements (2012).

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P57: Machinable porous hydroxyapatite scaffolds prepared by gel-casting method using an epoxide as a gelling agent

Přemysl Šťastný¹, Martin Trunec¹, Dominika Kalasova¹, Tomáš Zikmund¹, Zdeněk Chlup²

¹CEITEC BUT, Brno University of Technology ²CEITEC IPM, Institute of Physics of Materials, Academy of Sciences of the Czech Republic

Porous calcium phosphate (CaP) scaffolds have been used in medicine for bone regeneration in a case of a bone tissue loss for decades [1, 2]. Owing to specific shapes of voids there is a need for implants' individualization. We investigate a possibility of employing CAD/CAM technology and a multi axis milling of porous ceramics in the field of a preparation of individualized bioceramic scaffolds.

Hydroxyapatite foams with a proper structure were prepared by combination of a direct foaming method and an epoxy gel-casting [3]. Rheology of suspensions as well as mechanical and structure characterizations of HA foams were described. A machinability of HA foams in a different stage of a heat treatment were evaluated both qualitatively using a uniform milling pattern and quantitatively correlating the mechanical characteristics to machining results. Improvement of the machinability by temporary saturation of the structure by a paraffin wax was investigated as well. Practical usage of the multi axis milling has been demonstrated at the example of mandibular defect.

References

[1] HENCH, Larry L. Bioceramics: From Concept to Clinic. *Journal of the American Ceramic Society*. 1991, **74**(7), 1487-1510.

[2] ELSALANTY, Mohammed a David GENECOV. Bone Grafts in Craniofacial Surgery. *Craniomaxillofacial Trauma and Reconstruction*. 2009, **2**(03), 125-134.

[3] XIE, Rui, Kechao ZHOU, Xueping GAN, Dou ZHANG a J. SMIALEK. Effects of Epoxy Resin on Gelcasting Process and Mechanical Properties of Alumina Ceramics. *Journal of the American Ceramic Society*. 2013, **96**(4), 1107-1112.

P58: High frequency vibrations in teeth for the structural identification of supporting substances with a piezo-device

Hector A. Tinoco^{1,2}, Juan P. Gomez²

¹Institute of Physics of Materials, Academy of Sciences of Czech Republic-CEITEC, Brno, Czech Republic ²Department of Oral Health, Universidad Autónoma de Manizales, Colombia.

In this study, an experimental method is described to identify and differentiate materials that act as supporting substances in human teeth. The procedure of implanting teeth in different materials is supposed to mimic the bone as supporting structure. To measure the parameters of supporting substance inside the tooth, a sensor system was developed. The sensor device is composed of a stainless steel bracket bonded to a steel wire attached to two piezoelectric patches that act as a sandwich structure. The device contains a concentrated mass in the end of the wire. High frequency voltage (between 5-10 KHz) is applied through the piezo-transducers, which generate tooth motions by means of vibration in the wire. This procedure generates high frequency mechanical vibrations that allow the assessment of the mechanical response from the supporting substances. To quantify the mechanical responses associated to the support, the electrical impedance of the piezo-transducers is determined applying the electromechanical impedance technique (EMI). For the experiments, the device is coupled to the crown of the teeth, which is fastened by the root portion when these are embedded in the supporting substance. In a window observation in frequency (5-10 KHz), it was possible to identify a similar trend in the derivatives of the electrical impedance obtained for different materials; using a molar and canine tooth as interface. Results suggest that the measurements are invariant to the interface (in this case, the tooth) that couples supporting substances.

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P59: Processing of aerogel coatings on bulk materials substrates

Jorge Alberto Torres Rodríguez¹, Edgar B. Montúfar¹, Karel Slamecka¹, Martin Papula¹, Ladislav Čelko¹, Jozef Kaiser¹

¹Central European Institute of Technology (CEITEC), Brno, Czech Republic.

Thermal barrier coatings (TBC's) are widely used in the hot gas path of advanced aeroengines and land-based turbines. They protect the engine blades and vanes from exceeding their maximum use-temperature. They also improve the operation lifetime while increase the turbine efficiency. The TBC system consist of a ceramic top coat that reduces the metal temperature, a metallic bond, and a thermally grown oxide. Yttria stabilized zirconia containing 6-8 wt% Y_2O_3 (7YSZ) is the most widely used material for the TBC top coat because of its low thermal conductivity, high melting point, etc. The current TBC system suffers of a number of limitations and 7YSZ has reached its maximum temperature that it can be exposed (1300 °C) without incurring in phase changes.

The requirement for future engine designs is driving a search for materials that reduce the temperature of the metal surface while concomitantly facilitate increased gas path temperatures. A new generation of TBC material deposited in a manner that maximizes their lifetime during aircraft engine operation is required. Aerogels are materials with the lowest thermal conductivity and density due to its high porous structure (up to 99%). Thus, the aerogels can be considered as potential materials applied in TBC top coating. The aim of this work is to replace the typical 7YSZ top coat by an aerogel coating made of different ceramic compounds (SiO₂, ZrO₂, Al₂O₃) that can help to reduce in a great manner the thermal gradient between front and back part of the TBC, and therefore its effectivity.

P6o: Correlative probe and electron microscopy of threading dislocations in III-nitrides

Petr Vacek¹

¹Central European Institute of Technology – Brno University of Technology, Purkynova 123, 612 oo Brno, Czech Republic

Correlative imaging is an emerging paradigm which allows simultaneous capture of surface signals from two different probes - typically surface topography from SPM (scanning probe microscopy) and the complementary signal from SEM (scanning electron microscopy). Various signals from SEM can be used, e.g. secondary and backscattered electrons, cathodoluminescence or electron beam induced current. There is also option to use other SPM modes like Kelvin probe, conductive, magnetic, electrostatic and others. By correlating images from SPM and SEM you can compare information from the very same spot on the sample surface. Therefore, you are able to associate topography features with physical properties.

I am going to use this technique to study threading dislocations in Ill-nitrides. AlN, GaN and InN are promising materials for optoelectronic applications, high-frequency short-wavelength UV devices for water purification and in long lifetime lasers for high-density data storage. Due to high density of lattice defects originating from lattice mismatched substrates, the internal quantum efficiency of these devices is significantly lowered. By reducing density of defects, it is possible to create new generation of more efficient devices. This shows the importance of studying the properties, origin and behavior of these defects.

P61: Completing the process of foot posture index objectivization using the microsoft kinect: talar head position assessment

Olga Vallová¹, Luděk Žalud^{1,2}, Veronika Svozilová³, Kateřina Bucsuházy⁴

¹CEITEC - Central European Institute of Technology, Brno University of Technology, Purkynova 123, 612 oo Brno, Czech Republic.

² Faculty of Electrical Engineering and Communication, Brno University of Technology, Technicka 3058/12, 616 oo Brno, Czech Republic.

³Brno University of Technology, Department of Biomedical Engineering, Technicka 12, Brno 61200, Czech Republic

⁴Brno University of Technology, Institute of Forensic Engineering, Purkynova 464-118, Brno 61200, Czech Republic

Foot Posture Index (FPI) as a novel, observational scoring system improves the comprehensive evaluation of the static foot position, but is seriously handicapped by its subjective character and unsatisfactory five-point scale. In an effort to eliminate these disadvantages the optical motion sensor the Microsoft KinectTM were used for objectivization and better quantification. But not all of the six original items of FPI were monitored by Kinect [1]. The aim of the study is to find appropriate approach how to assess the last item of FPI, talar head position, using the Microsoft KinectTM and entirely finalize the objectivization of the original Foot Posture Index method. The result should offer a novel solution to the problem of unsatisfactory subjective testing technique based on the FPI-6, specifically talar head position evaluation. The proposed approach should provide indisputable advantages such as the objectivization and quantification of the measured parameters, easy operation, low purchase price, negligible space requirements, and portability.

Acknowledgement

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References

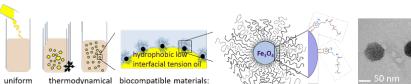
[1] Mentiplay BF, Clark RA, Bartold S, Paterson K. Reliability and validity of the Microsoft Kinect for evaluating static foot posture. Journal of Foot and Ankle Research 2013;6.

T23: Self-assembled, superparamagnetic, nanoparticle stabilized delivery vehicles for hydrophobic compounds

Iris Vonderhaid¹, Erik Reimhult¹

¹Institute of Biologically inspired Materials, Department of Nanobiotechnology, University of Natural Resources and Life Sciences, Vienna, Austria

Today, the main problem in treatment of severe diseases, such as cancer, is often less a problem of lack of efficient compounds than inefficient delivery of these compounds to the target site. Many of the most efficient drugs are poorly water soluble and can only be made water soluble at high cost and at the expense of reduction in binding affinity to its target and thereby reduced efficiency. We present a novel, non-toxic drug delivery carrier consisting of a ioo nm fatty acid droplet decorated by PEG-grafted iron oxide superparamagnetic nanoparticles. Uniquely, these nanoscale Pickering-type emulsions form through spontaneous emulsification to a uniform nanoscale size suitable for drug delivery. The deformable and highly hydrated PEG-brush, stealth coating on the particle surface, is believed to be crucial to the emulsification process and the colloidal stability also in cell culture. We investigate the emulsion formation and stability with a combination of dynamic light scattering, particle tracking and transmission electron microscopy, as function of concentration and incubation conditions. Finally, cell uptake and toxicity was tested.



uniform stable droplets form after core shell nanoparticle mixing NP solution and oil; (SPION) decorated oil periods solely by irreversible oil adsorption of NPs to oil water interfacial interface without additional interface (decanoic acid) surfactants

tension

hydrophilic monodisperse (σ < 5%) TEM imag of two NP superparamagnetic magnetite core stabilized droplets; stabilisation over long time droplet; use of an limpid shell NPs (SPION); shell consists of 35 days old providing a low thermoresponsive polymer brush

(crosslinkable or non crosslinkable)

with to NP irreversibly bound

nitrodopamine anchor



Fig. 1: Schematics of the self-formation of pickering nanoemulsions

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P62: Solvothermal synthesis of metal nanoalloys

Vit Vykoukal^{1,2}, Jiri Pinkas^{1,2}

¹Masaryk University, Faculty of Science, Department of Chemistry, Kotlarska 2, 611 37 Brno, Czech Republic ²Masaryk University, CEITEC MU, Kamenice 5, 625 oo Brno, Czech Republic.

Studies on new nanomaterials and especially their preparation by bottom-up methods is a very progressive field of materials research. The synthesis of nanoalloys is one integral part of nanoscience. Development of efficient preparative methods is a challenging task due to their chemical, phase, and morphological variability.

Nanoparticles of metal alloys exhibit many interesting properties, such as depression of melting point [1], plasmon resonance, catalytic activity [2] and phase separation [3]. Nanoalloys can be prepared by many routes, but the solvothermal synthesis, specifically in oleylamine is highly advantageous [2,4]. Hot injection technique should ensure homogeneous conditions for nanoparticle nucleation and growth.

AgNi and AgCu nanoparticles were prepared by injection of an oleylamine solution of metal precursors (different molar ratios) to a mixture of oleylamine and octadecene at 230 °C. After 10 minutes, the reaction mixture was cooled down to room temperature in a water bath. Nanoparticles were isolated, purified, dispersed in hexane and characterized by Dynamic Light Scattering (DLS), Transmission Electron Microscopy (TEM), Elemental analyses (ICP OES), and Small-Angle X-ray Scattering (SAXS) analyses. Plasmon resonances were also observed. Phase separation was followed by High Temperature X-Ray Diffraction (HT-XRD) technique and was confirmed by Scanning Electron Microscope (SEM) and by measuring of magnetic properties during heating.

References

- [1] Takagi, M. J. Phys. Soc. Japan 1954, 9 (3), 359–363.
- [2] Somorjai, G. A. J. Phys. Chem. C 2008, 112 (32), 12092–12095.
- [3] Sopousek, J.; Vykoukal, V.; Pinkas, J.; Phys. Chem. Chem. Phys. 2015 17 (42), 28277-28285.

P63: Fabrication of 3D cobalt nanostructures using FEBID

Ondřej Vyroubal¹, Miroslav Kolíbal^{1,2}, Michal Urbánek^{1,2}

¹Institute of Physical Engineering, Brno University of Technology, Technická 2, 616 69 Brno, Czech Republic ²CEITEC BUT, Brno University of Technology, Technická 10, 616 oo Brno, Czech Republic

Functional nanostructures fabricated by focused electron and ion beam induced processing (FEBIP/FIBIP) open a promising route for applications in nanoelectronics [1]. In particular, we have studied a viability of Focused Electron Beam Induced Deposition (FEBID) for preparation of shape-specific cobalt nanostructures using octacarbonyl dicobalt Co2(CO)8 precursor. FEBID is a direct-write resistless lithography technique that allows the definition of patterns on a substrate using solely an e-beam allowing growth of 3D nanostructures. In our experiment, we started with preparation of thin squares and discs on which we measured standard Landau and vortex patterns (respectively) by Magnetic Force Microscopy verifying the magnetic properties, the purity of our deposit was also verified by EDX to be up to 75%. Then we followed with preparation of more demanding 3D objects, with the goal of depositing cobalt spheres of submicron size towards measurement of 3D vortex states [2]. Deposition of the bottom part of the sphere presented a difficult task which we overcame by milling a template into the substrate by focused ion beam. Afterwards the material was successfully deposited. Cross-sections of prepared spheres are shown in Fig. 1.

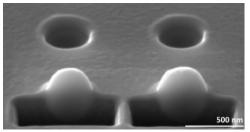


Fig. 1: Cross-section of cobalt spheres deposited by FEBID into the template prepared by FIB.

References

[1] I. Utke et al., Gas-assisted focused electron beam and ion beam processing and fabrication, J. Vac. Sci. Technol. B 26, 1197 (2008)

[2] R. Boardman et al., Micromagnetic simulation studies of ferromagnetic part spheres, J. Appl. Phys. 97, 10E305 (2005)

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T24: Magnetic field directed self-assembly as route for biomimetics

<u>Marek Zboncak</u>¹, Frantisek Ondreas¹, Petr Lepcio¹, Josef Jancar^{1,2} ¹Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic ²Faculty of Chemistry, Brno University of Technology, Brno, Czech Republic

Nature has unique ability to bottom-up build-up very complex structures. Examples of such structures can be found around various hard tissues such as abalone, shells, bones, tooth, claws, beaks, deep sea sponges, wood, bamboo and others. Beside their geometry, inner multi-level hierarchical structure is source of their unique properties. The structure on the individual hierarchical level is preciously engineered to offer best performance to withstand various deforming loads applied on the organism during its life. Build-up process of these composites begins at nanoscale from inorganic nanoparticles and biological macromolecules such as polysaccharides or proteins. These two primary building blocks are specifically arranged into more complex reinforcement units. In comparison with man-made composites that offer either mechanical strength enhancement or toughness enhancement, natural composites possess these two commonly contradictory properties together. Reasons of this unique balance of mechanical properties are various reinforcing and toughening mechanisms operating on various length scales presented in the system. In past few years various experimental and laboratory scale techniques have been developed to mimic natural structures employing wide range of materials. Directed self-assembly of magnetic nanoparticles by magnetic field into anisotropic structures is driven by the particle dipole-dipole interactions. We utilize this force-assembly approach as novel biomimetic method with magnetic nanoparticles as stiff primary building block and photopolymer as soft and tough building block mimicking the bottom-up build-up process found in natural composites.



Agriculture and food science

Students' abstracts



P64: High pressure enzyme assisted extraction of phenolics in brown algae *Cystoseira* abies-marina

<u>Romana Bačová</u>¹, Miguel Herrero², Jose Mendiola², Elena Ibañez², Dalibor Húska¹, Bořivoj Klejdus¹ ¹Mendel university in Brno, Brno, Czech Republic ²Institute of Food Science Research (CIAL), Madrid, Spain

Polyphenols of edible seaweeds recently raised attention for the potential health benefits coupled with abilities displayed as radical scavengers. According to this, they became interesting candidates for food and pharmaceutical products as additives [1]. Enzyme assisted extraction (EAE) of seaweed *Cystoseira abies marina* in combination with supercritical fluid extraction (SFE) and pressurized liquid extraction (PLE) was investigated. However, these compounds are bound inside of the cell walls, what makes the extraction more complicated. To provide higher total phenolic content it may be advisable to use suitable enzymes. To our knowledge, we are first, who deals with extraction of polyphenols in brown macroalgae *Cystoseira abies*, using enzyme assisted extraction (EAE) with pressure enhancement. In this study we are testing efficiency of this environmentall impact for the determination of natural bioactive compounds. Optimization of the process parameters are crucial for better understanding the chances of the process scale-up, and this has been the current stage of efforts in the area.

References

 P. B. Andrade, M. Barbosa, R. P. Matos, G. Lopes, J. Vinholes, T. Mouga a P. ValentÃo, Valuable compounds in macroalgae extracts, 2013, Food Chemistry 138, pp. 1819-1828.
 E. Deniaud-Bouet, N. Kerverec, G. Michel, T. Tonon, B. Kloareg, C. Herve, 2014, Annals of Botany, 114, p. 1203 - 1216

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P65: Effect of selenium dietary supplementation on shell eggs

Miroslava Fasiangova^{1,2}, Gabriela Borilova^{1,2}, Dana Kumprechtova³

¹Department of Meat Hygiene and Technology, Faculty of Veterinary Hygiene and Ecology, Veterinary and Pharmaceutical Sciences Brno, Palackeho tr. 1946/1, 612 42 Brno, Czech Republic

²CEITEC - Central European Institute of Technology, University of Veterinary and Pharmaceutical Sciences Brno

³Institute of Animal Science Praha Uhrineves, Czech Republic

Nowadays, consumers pay close attention to their nutrition in their effort to live healthier. Selenium supplementation of feed for food animals used to increase human selenium intake has showed significant progress in the area of healthy nutrition. Results of various studies show that there are possibilities to use eggs with defined selenium content as a new potential source this scarce element in human nutrition.

Additionally, it has been proven that the antioxidant function of selenium is likely to be contributive to the retardation of aging of animal products. The aging of eggs can be monitored by assessment of malondialdehyde content - lipid oxidation product.

The aim of our research was to determine the effect of different kind of selenium dietary supplementation on selenium accumulation in eggs and on the oxidative stability of egg yolk. Supplementation of either form of selenium significantly increased the selenium concentration in egg components. Selenium in the organic form increased selenium in the egg albumen compared to inorganic form (p<0.001). The concentration of malondialdehyde in egg yolk was significantly lower in supplemented groups compared to the control group (p<0.001).

T25: Proteome profiling by a large-scale targeted analysis – a new approach to detect and quantify lower abundant proteins

<u>Hana Habánová</u>¹, Miroslav Berka¹, Martin Černý¹, Břetislav Brzobohatý¹ ¹Department of molecular biology and radiobiology, Faculty of AgriSciences, Mendel University in Brno, Brno, Czech Republic

Germination is a crucial step in plants' life and its timing and progress significantly affect their future growth and development. Proteomics analysis provides a novel and promising approach to analyze seed germination, but the composition of a seed proteome reduces the achievable detection limits. The ratio between three major seed components - lipids, carbohydrates and proteins - may vary Our data revealed that the proteome of high-protein-content significantly. seeds is predominantly formed by only a few cupin-like seed storage proteins [1]. In contrast, the barley seed proteome (a low-protein-content seed) contains a relatively high number of abundant proteins belonging to different protein families, the depletion of which is complicated and ineffective. Here, we demonstrate a new approach to analyze complex seed proteome, which is based on a spectral library and an SRM-based targeted analysis. We utilized complementary proteome fractionation techniques and build the barley seed proteome spectral library that contains spectra for over 4,000 seed proteins. As a proof of concept, we employed this library in an SRM-based targeted analysis of barley embryo response to hydrogen peroxide. We compared the results with that of a standard LC-MS/MS analysis and show that this library can be used to increase proteome coverage and detect and guantify low-abundant proteins without the need of pre-fractionation.

References

[1] Habánová, H., Saiz Fernandez, I. 2016: Seed Storage Proteins in Four Contrasting Plant Species. In MendelNet 2016: Proceedings of International PhD Students Conference, p. 978-982. Mendel University in Brno. ISBM 9788075094438.

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P66: The effects of addition of technical hemp (*Cannabis sativa L*.) on performance parameters of broiler chickens

Lucie Kratochvílová

Mendel University, Brno, Czech Republic

The aim of this experiment was to evaluate the effects of addition of technical hemp (*Cannabis sativa L.*) on performance parameters of broiler chickens. A total of 105 broilers Ross 308 were divided into three groups. The groups were identified: Cannabis 5, Cannabis 15 and Control. Group of Cannabis 5 was fed with 5% hempseed cake in feed composition, analogically group of Cannabis 15 was fed with 15% hempseed cake and the Control's group feed composition was not changed. As a response criterion the weight gain, feed conversion, lean meat, carcass yield and blood liver enzymes concentrations were chosen.

We finally focused on assessing the impact of hempseed cakes on weight gain, feed conversion and the carcass yield. The increment in body weight was significantly changed in the Cannabis 5 group (P < 0.05). Broilers fed with 15% hempseed cake in feed composition grew slower than Control group and had worse feed conversion during the experiment that lasted 37 days. Carcass yield was not affected (P > 0.05) by the content of the hempseed cake in feed composition in both tested groups. Measured concentrations of enzymes in groups were not affected by the contents of hempseed cake.

References

[1] C. Callaway, 2004, Hempseed as a nutritional resource: An overview, Euphytica, 140: 65-72,

[2] R.U. Khan et al., 2009, Effect of Cannabis sativa on muscle growth and visceral organs of broiler chicks, Inter J Biol Biotech, 4 (1): 79-81

P67: Bacterial community dynamics in a rumen fluid bioreactor under various conditions

Martina Zapletalová, Andrea Smejkalová, Jitka Kašparovská, Ludmila Křížová, Tomáš Kašparovský, Jan Lochman

Department of Biochemistry, Faculty of Science, Masaryk University, Kotlářská 267/2, 611 37 Brno, Czech Republic

In vitro techniques realize more controlled and reproducible conditions compared to *in vivo* experiments so they are widely used to study the various processes in the rumen. Mostly, only the parameters like pH changes, volatile fatty acids content or metabolite production are monitored. In our previous publication [1], we highlighted the major influence of the low redox potential on rumen microbiome during in vitro cultivation. Low redox potential is caused by strict anaerobic conditions in the bioreactor, so we decided to increase it to the physiological value by using air supply. In this study we focus on mapping the rumen microbiome during cultivations with and without air inflow by using various types of feed. To ensure stable conditions we monitored pH values, redox-potential values and VFA content throughout the ten-day cultivation run. The bacterial community of rumen fluid was monitored by 16S rRNA sequencing. Under conditions of micro aeration, we found much more stable microbiome during the ten days of cultivation, compared with cultivation without air intake. Moreover, we found that the stability and richness of microbiome are largely influenced by the type of feeding. Ascertain differences may partly explain the problems with acidosis in cattle under diet changes conditions.

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References

[1] M. Zapletalová, J. Kašparovská, L. Křížová, T. Kašparovský, O. Šerý, and J. Lochman, "Bacterial community dynamics in a rumen fluid bioreactor during in-vitro cultivation," *J. Biotechnol.*, vol. 234, pp. 43-49, Sep. 2016.

List of students with contact information

Genomics and biomedicine

Balakhonova, Veronika (CEITEC MU), Po1, 🖾 450883@mail.muni.cz Bencurova, Petra (CEITEC MU), To1, 🛱 petra.bencurova@ceitec.muni.cz Boudný, Miroslav (University Hospital Brno and Faculty of Medicine), Po2, miroboudny@seznam.cz Bucsuházy, Kateřina (Brno University of Technology), Po3, 🗢 k.bucsuhazy@gmail.com Černá, Kateřina (Masaryk University), NONE, 🗟 katerina.cerna384@gmail.com **Covelo Molares, Helena** (CEITEC MU), **To2**, **A** hcovemoo@mail.muni.cz Cuyacot, Abigail (CEITEC MU), Po4, 🛱 abigail.cuyacot@ceitec.muni.cz Grioni, Andrea (CEITEC MU), Po5, 🖾 grioni.andrea@gmail.com Horský, Vladimír (LCC NCBR CEITEC), To3, 🛱 vladimir.horsky@mail.muni.cz Janoš, Pavel (Masaryk University), To4, 🛱 janpav@mail.muni.cz Jian Bagherpoor, Alireza (Institute of Biophysics), Po6, 🕿 ajbagherpoor@yahoo.com Kastl, Tomáš (Veterinary Research Institute), Tos, 🛱 kastl@vri.cz khan, Anzer (CEITEC MU), Po7, 🛱 anzer.khan@ceitec.muni.cz Kočková, Helena (CEITEC MU), Po8, 🛱 helena.kockova@gmail.com Kohoutková, Marcela (Marcela Kohoutková), P70, 🛱 maca.kohoutkova@seznam.cz Kosařová, Zdeňka (Masaryk University), Pog, 🛱 zdenka.kosarova@gmail.com Kozlová, Veronika (CEITEC MU), P10, 🛱 kozlovaveronica@gmail.com **Krepl, Miroslav** (Institute of Biophysics), **To6**, **a** krepl@seznam.cz Kseniya, Timofeyenko (CEITEC MU), P11, 🛱 ksenitim@gmail.com Kutilová, Iva (University of Veterinary and Pharmaceutical Sciences Brno), To7, kutilova.iva@gmail.com Lobello, Cosimo (CEITEC MU), P12, 2 lobello.cosimo@gmail.com Machálková, Markéta (Masaryk University), P13, 🛱 marketa.machalkova@gmail.com Mucha, Ján (Brno University of Technology), P14, 🗟 muchajano@phd.feec.vutbr.cz **Oppelt, Jan** (NCBR & CEITEC MU), **To8**, **b** jan.oppelt@mail.muni.cz Pál, Karol (CEITEC MU), P15, 🛱 karolpal.jr@gmail.com Peltanova, Barbora (Masaryk University), P16, Speltanova@seznam.cz Pešková, Marie (Masaryk University), P69, 🛱 252574@mail.muni.cz Pokorný, Daniel (National Centre for Biomolecular Research), P17, 🖾 pokec@mail.muni.cz Pravda, Lukáš (CEITEC MU), P18, 🖾 lukas.pravda@ceitec.muni.cz Schořová, Šárka (Masaryk University), Tog, 🛱 sarka.schorova@gmail.com Šeda, Václav (CEITEC MU), T10/P68, 🛱 vasek.k.seda@gmail.com Tanasa, Sorin (CEITEC MU), T11, 🛱 sorin.tanasa@ceitec.muni.cz

Tom, Nikola (CEITEC MU), T12, 🏝 1nikola.tom@gmail.com Vojáčková, Eva (CEITEC MU), P19, 🏝 EvaVojackova@email.cz Zemlianski, Viacheslav (CEITEC MU), P20, 🏝 viacheslav.zemlianski@yandex.ru Zemlyanskaya, Elena (CEITEC MU), P21, 🏝 yolka28@gmail.com Zigackova, Dagmar (CEITEC MU), P22, 🏝 zigackova.d@gmail.com Žufanová, Zuzana (Masaryk University), T13, 🏟 zufinka@mail.muni.cz

Material science and biosensing

Babrnáková, Johana (CEITEC BUT), P23, 🛱 Johana.Babrnakova@ceitec.vutbr.cz Badin, Viktor (Brno University of Technology), P24, 🛱 viktor.badin@vutbr.cz Bertolla, Luca (Institute of Physics of Materials), P25, 🛱 bertolla@ipm.cz Bora, Pankaj Lochan (CEITEC MU), P26, 🖾 pankajlbora@gmail.com Brinek, Adam (CEITEC BUT), P27, 🛱 Adam.Brinek@ceitec.vutbr.cz Casas Luna, Mariano (CEITEC BUT), P28, 🛱 mariano.casasluna@ceitec.vutbr.cz Fojtů, Michaela (Masaryk University), P29, 🛱 michaelafojtu@gmail.com Gablech, Imrich (CEITEC BUT), T14, 🛱 imrich.gablech@ceitec.vutbr.cz Gal, Noga (University of Natural Resources and Life Sciences Vienna), P30, noga@groupwise.boku.ac.at Glos, Jan (CEITEC BUT), T15, 🖾 jan.glos@ceitec.vutbr.cz Gregus, Michal (Masaryk University), P31, 🖨 gregus@mail.muni.cz Horák, Jakub (Brno University of Technology), P32, 🛱 xchorakj@fch.vut.cz Horník, Vít (CEITEC IPM), P33, 🛱 hornik@ipm.cz Hrabovský, Miloš (Brno University of Technology), P34, 🛱 xhrabo11@stud.feec.vutbr.cz Jelinek, Ales (CEITEC BUT), P35, 🛱 ales.jelinek@ceitec.vutbr.cz Jelínek, Petr (Masaryk University), T16, 🛱 324198@mail.muni.cz Jindrová, Eva (CEITEC BUT), P36, 🛱 Eva.Jindrova@ceitec.vutbr.cz Kachtík, Lukáš (CEITEC BUT), P37, 🛱 lukas.kachtik@ceitec.vutbr.cz Kalasová, Dominika (CEITEC BUT), P38, 🛱 dominika.kalasova@ceitec.vutbr.cz Kaushik, Preeti (Masaryk University), P39, 🛱 piscian.preeti@gmail.com Konečný, Martin (Inst. of Physical Engineering), P40, 🛱 martin.konecny@ceitec.vutbr.cz Konhefr, Martin (Masaryk University), P41, 🖾 379848@mail.muni.cz Krepl, Ondrej (CEITEC IPM), T17, 🛱 krepl@ipm.cz

Kubesa, Ondrej (Masaryk University), P42, 🛱 ondrej@kubesa.cz Kumar Pal, Sudhir (CEITEC MU), P43, 🛱 sudhir.kumar@ceitec.muni.cz Lednický, Tomáš (CEITEC BUT), P44, 🛱 tomas.lednicky@ceitec.vutbr.cz Lepcio, Petr (CEITEC BUT), P45, 🛱 petr.lepcio@ceitec.vutbr.cz **Ligmajer, Filip** (CEITEC BUT), **T18**, **b** filip.ligmajer@ceitec.vutbr.cz Makhneva, Ekaterina (CEITEC MU), P46, 🛱 potakesst@mail.ru Michlíček, Miroslav (CEITEC), P47, 🛱 michlicekm@mail.muni.cz Mirzaei, Saeed (Masaryk University), P48, Smirzaei.phy@gmail.com Musálek, Tomáš (Brno University of Technology), P49, 🛱 musalekt@gmail.com Novák, Martin (CEITEC MU), T19, 🛱 323460@mail.muni.cz **Obrusnik, Adam** (Masaryk University), **NONE**, **A** adam.obrusnik@gmail.com **Ondreáš, František** (CEITEC BUT), **P50**, **D** frantisek.ondreas@ceitec.vutbr.cz Pastucha, Matěj (CEITEC MU), T20, 🛱 mpastucha@mail.muni.cz Pejchal, Tomáš (CEITEC BUT), P51, 🛱 tomas.pejchal@ceitec.vutbr.cz **Pejovic-Simeunovic, Jelena** (CEITEC BUT), **P52**, **A** jelena.pejovic@ceitec.vutbr.cz **Petráš, Roman** (Institute of Physics of Materials), **P53**, **A** petras@ipm.cz Poduska, Jan (Institute of Physics of Materials), P54, 2 poduska@ipm.cz Shekargoftar, Masoud (CEPLANT), P55, 🛱 mshekargoftar@mail.muni.cz Spacek, Jan (IBP and CEITEC MU), T21, 🛱 j.h.spacek@gmail.com Šťastný, Přemysl (CEITEC BUT), P57, 🛱 Premysl.Stastny@ceitec.vutbr.cz Stojic, Marija (CEITEC BUT), P56, 🛱 Marija.Stojic@ceitec.vutbr.cz Svatos, Vojtech (CEITEC BUT), T22, 🖾 vojtech.svatos@ceitec.vutbr.cz Tinoco Navarro, Hector Andres (CEITEC IPM), P58, 🛱 andrest149@gmail.com Torres Rodríguez, Jorge Alberto (CEITEC BUT), P59, SorgeAlberto.TorresRodriguez@ceitec.vutbr.cz Vacek, Petr (Brno University of Technology), P60, 🛱 Petr.Vacek@ceitec.vutbr.cz Vallová, Olga (CEITEC BUT), P61, 🛱 olga.vallova@ceitec.vutbr.cz Vonderhaid, Iris (Institute of Biologically inspired Materials), T23, iris.vonderhaid@boku.ac.at **Vykoukal, Vít** (CEITEC MU), **P62**, **A** vit.vykoukal@gmail.com Vyroubal, Ondřej (Brno University of Technology), P63, 🛱 ovyroubal@gmail.com **Zboncak, Marek** (CEITEC BUT), **T24**, **A** marek.zboncak@ceitec.vutbr.cz

Agriculture and food science

Bačová, Romana (Mendel University in Brno), P64, A RomanaBacovaa@gmail.com
Fasiangova, Miroslava (University of Veterinary and Pharmaceutical Sciences Brno), P65, A FASIANGOVAM@VFU.cz
Habánová, Hana (CEITEC MENDELU), T25, A habanova.ha@gmail.com
Kratochvílová, Lucie (Mendel University in Brno), P66, A xkratoc3@mendelu.cz
Zapletalová, Martina (Masaryk University), P67, A 357594@mail.muni.cz

















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