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Frontiers in Material and Life Sciences

Book of Abstracts

CEITEC Annual Conference
"Frontiers in Material and Life Sciences"

21-24/10/2014

Brno, Czech Republic

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WELCOME ADDRESS

Dear Participants of the CEITEC Annual Conference Brno 2014,

It is a great pleasure to welcome you to the **Frontiers in Material and Life Sciences** in Brno, Czech Republic. CEITEC is becoming more internationally recognized, as such, this conference will bring more than 40 international scientists as speakers to Brno. This meeting is designed to bring experts and students together from different disciplines to stimulate the formation of new collaborations. Also, this conference will provide a platform to showcase the science from the Brno academic community.

The major goal of this year's conference is to establish a forum to allow scientists and stakeholders in the fields that broadly encompass Material and Life Sciences to examine new approaches in answering important scientific questions. In particular, scientific areas which utilize advanced technologies to address problem solutions would be highlighted. The conference will cover a breath of subjects including: nanotechnology and its applications to biosensors development and medicine, advances in imaging technologies from cells to organs, regenerative medicine and materials utilized in biomedical application, as well as fundamental studies of genome biology, plant biology, and infectious agents.

As a social highlight, we will have a gala dinner in the spectacular Moravian Gallery on 23 October to encourage interdisciplinary scientific discussion and take in the current art display in a relaxed atmosphere. This will also give us the opportunity to celebrate the winners of the poster competition and to taste wines from the South Moravian region.

We wish you a wonderful stay during the CEITEC Annual Conference 2014.

Markus Dettenhofer
Executive Director

GENERAL INFORMATION

» Internet Facilities
Wi-Fi connection is available in the Hotel International Brno free of charge.

» Time Zone
The local time in the Czech Republic at the time of the conference will be GMT +2 due to Summer Daylight Saving Time.

» Electricity
The Czech Republic uses a 220 volt 50 Hz system.

» Emergency Telephone Numbers
The emergency phone number is 112.

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

» Taxi Service
We recommend using taxi service of the following reliable company:
City taxi plus s. r. o. +420 542 321 321 or use hotel taxi.

REGISTRATION AND INFORMATION

Registration desk is located in the foyer of the Hotel

» Opening hours

Tuesday, October 21, 2014	14:00 – 18:30
Wednesday, October 22, 2014	08:00 – 18:00
Thursday, October 23, 2014	08:00 – 18:00

» Registered and confirmed participant is entitled to:
» Admission to all scientific sessions
» Admission to the poster sessions
» Congress materials
» Coffee breaks during the congress
» Lunch

CONFERENCE INFORMATION

» Badges

Participants and accompanying persons will receive a name badge upon registration. Everyone is kindly requested to wear his name badge when attending the meeting. Only participants who are wearing their name badge will be admitted to the lecture halls.

Name badges have been colour-coded as follows:

- Orange: Speakers
- Grey: Visitors
- Green: Active participants
- Blue: Organizers

» Official Language

The official language of the conference is English.

» Programme Changes

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

SOCIAL EVENT

» Conference Dinner sponsored by CTP

» Thursday, October 23, 2014
» Starts at 20:00
» Moravian Gallery - Museum of Applied Arts
Husova 14, 662 26 Brno

POSTER SESSION AND COMPETITION

All participants of the CEITEC Annual Conference are invited to submit a poster which will be displayed during the whole conference. PhD students'and Postdocs' posters are automatically included into the CEITEC Poster Competition awarding the best posters. The competition has two categories, each eligible for valuable prizes – PhD students and Postdocs.

» Judging for the competition

A panel of judges will review the poster presentations. The judges are selected by Conference Organizational Committee among all speakers at conference.
Each judge will rate each entry on a point scale. The final ranking of entries will be based upon the mean of the resulting distribution of scores.

» Requirements

The participant is responsible for making sure that the poster display fits on the display board, and is completely responsible for attaching the individual elements to the display board according to our instructions.
All participants of the competition are obliged to be available to answer the questions about their research in the specific timeslots (**Wednesday** – lunch break, **Wednesday** – evening poster session).
The winners of the competition will be announced on **Thursday 23rd** and will give a short presentation about the research at the conference on Thursday evening.

PROGRAMME OF THE POSTER SESSION

Wednesday 13:30 – 14:20
POSTER SESSION in hotel lobby / The participants will be present to answer questions.
Wednesday 19:00 – 21:00
POSTER SESSION in hotel lobby / The participants will be present to answer questions.
Thursday 18:00
Presentation of the winners of the posters – Conference Hall 1

PROGRAMME

Tuesday, October 21

14:00 Registration

17:30 Welcome Mixer & Snacks
Welcome speeches: Conference Hall 1
18:00 **Mikuláš Bek** (*Rector of Masaryk University*)
18:10 **Petr Štěpánek** (*Rector of Brno Technical University*)
18:20 **Markus Dettenhofer** (*Executive Director of CEITEC*)

Keynote lecture: Conference Hall 1
Chair: Pavel Plevka *(CEITEC)*
18:35 **Jack Johnson** *(The Scripps Research Institute)* - Biophysical studies of non-enveloped virus maturation: insights into elegantly programmed nano-machines

Keynote lecture: Conference Hall 1
Chair: Peter Varga *(CEITEC)*
19:35 **Jochen Feldmann** *(Ludwig Maximilians-Universtity Munich)* - Nanoplasmonics – from biosensing to biomonitoring

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Wednesday, October 22

Plenary I: Genome Biology: Conference Hall 1
Chair: Vladimír Sklenář *(CEITEC)*

09:00 **Steven Buratowski** *(Harvard Medical School)* - Shaping the eukaryotic transcriptome with chromatin and non-coding RNA
09:30 **Edward N. Trifonov** *(University of Haifa)* - Strong nucleosomes: from chromatin to chromosome structure
10:00 **Dirk Eick** *(Ludwig Maximilians-Universtity Munich)* - The RNA polymerase II carboxy-terminal domain (CTD) code

10:30 Coffee Break

Plenary II: Materials in Nanotech in Biomedcine: Conference Hall 1
Chair: Tomáš Šikola *(CEITEC)*

11:00 **Jakub Dostálek** *(Austrian Institute of Technology)* - Fluorescence biosensors with plasmonically amplified signal
11:30 **Romain Quidant** *(ICREA, Barcelona)* - Novel nano-optical tools for the detection and manipulation of biomolecules
12:00 **Rainer Hillenbrand** *(CIC nanoGUNE, San Sebastian)* - Nano-FTIR spectroscopy of individual protein complexes

12:30 Lunch - Restaurant Lucullus / Restaurant Plzeňka
13:30 **POSTER SESSION I**

	Workshop – Hall 1 **A: Imaging** Chair: Radim Chmelík *(CEITEC)*	**Workshop** – Hall 2 **B: MEMS and biosensing** Chair: Petr Skládal *(CEITEC)*	**Workshop** – Hall 3 **C: Veterinary Sciences** Chair: Vladimír Celer *(CEITEC)*
14:30	**Katarina Wolf** *(RIMLS, Nijmegen)* - 4D imaging of cancer cell invasion in vitro and in vivo	**Maria Isabel Pividori** *(Autonomous University of Barcelona)* - Magnetic carriers in biosensing and bioassays	**Zdeněk Hubálek** *(Academy of Sciences of the Czech Republic, v. v. i.)* - West Nile virus - an emerging mosquito-borne agent
14:50	**Petr Strnad** *(EMBL)* - Novel selective plane illumination microscope (SPIM) to study mouse pre-implantation development	**Jan Přibyl** *(CEITEC)* - Atomic force microscopy as a tool to study mechanobiological properties of human cardiomyocytes	**Udeni Balasuriya** *(University of Kentucky)* - Equine Infectious Diseases in the Genomic Era: Equine CXCL16 Associated with EAV Carrier State in the Stallion
15:10	**Eishu Hirata** *(Cancer Research UK)* - IIntravital imaging reveals how BRAF inhibition generates drug tolerant microenvironments with high integrinβ1/FAK signaling	**Pavel Neužil** *(CEITEC)* - Lab-on-a-Chip for Point of Care Applications	**Ivan Rychlík** *(Veterinary Research Institute)* - Interactions of Salmonella with its host

15:30 **Alan Boyde** *(Queen Mary University of London)* - 3D microscopy of bone and bone cells: new views of bone wounds by looking directly at the implant interface

15:50 **Daniel Zicha** *(London Research Institute)* - Potential of quantitative interferometry in personalised cancer treatment

16:10 **Tomáš Slabý** *(Brno University of Technology)* - Coherence-controlled holographic microscopy: a new technique for quantitative phase imaging

Fred Lisdat *(Technical University Wildau)* - Strategies for coupling enzymes to electrodes by means of nanoparticles and polymers

Antje Baeumner *(University of Regensburg)* - New concepts for lab-on-a-chip systems using electrospun nanofibers

Jenny Emnéus *(Technical University of Denmark)* - Three-dimensional (3D) Polymer and Carbon Scaffolds for Future Cell Replacement Therapy and Bioartificial Organ-on-a-Chip Systems

David Modrý *(CEITEC)* - How much do we share with our closest relatives: parasite communities of humans and great apes

16:30 Coffee Break

Plenary III: Molecular Oncology and Immunology: Conference Hall 1
Chair: Sarka Pospisilova *(CEITEC)*

17:00 **Marek Mráz** *(CEITEC)* - The role of microRNAs in the B cell receptor signalling and microenvironmental interactions in B cell malignancies
17:30 **Lumír Krejčí** *(Masaryk University)* - DNA repair as a therapeutic target
18:00 **Shona Murphy** *(University of Oxford)* - Control of transcription elongation by pol II CTD kinases

18:30 Snacks and Informal break
19:00 **POSTER SESSION II** – Lobby Bar

Thursday, October 23

Plenary IV: G. J. Mendel Session: Conference Hall 1
Chair: Jiří Fajkus *(CEITEC)*

09:00 **Charles I. White** *(GReD, Clermont-Ferrand)* - Recombination - Genetic instability versus genome maintenance

09:30 **Ingo A. Schubert** *(IPK - Gatersleben)* - DNA double-strand breaks in barley are repaired by diverse pathways, primarily involving the sister chromatid

10:00 **Jiří Friml** *(CEITEC)* - Auxin-mediated polarity and patterning in plant development

10:30 Coffee Break

	Workshop – Hall 1 **A: Plasmonic in Biomedicine** Chair: Jakub Dostálek *(AIT)*	**Workshop** – Hall 2 **B: Cell Regulations** Chair: Martin Trbušek *(CEITEC)*	**Workshop** – Hall 3 **C: Regenerative Medicine** Chair: Josef Jančář *(CEITEC)*
10:55	**Daniel Aili** *(Linköping University)* - Polypeptide-modified gold nanoparticle for colorimetric detection of hydrolytic enzymes	**Damien Hermand** *(University of Namur)* - Promoter nucleosome dynamics regulated by signaling through the RNA Polymerae II CTD	**Chris Holland** *(University of Sheffield)* - Turning a silk purse into a sows ear: the importance of processing when using silk in regenerative medicine
11:15	**Georg Ramer** *(Vienna University of Technology)* - Time Resolved Photothermal Expansion Infrared Nanoscopy	**Eric Eldering** *(AMC, Amsterdam)* - Impact of SF3B1 mutations in CLL on the DNA damage response	**Claudio Migliaresi** *(University of Trento)* - Silk protein based constructs for tissue engineering
11:35	**Tomáš Šikola** *(CEITEC)* - Plasmonic antennas and nanocomposite particles for detection of biomolecules	**Dalibor Blažek** *(CEITEC)* - Ovarian carcinoma CDK12 mutations deregulate DNA repair genes via deficient formation and function of the CDK12/CycK complex	**Alan J Lesser** *(University of Massachusetts Amherst)* - Effects of Physical Aging and Rejuvenation on the Mechanical Behavior of Polymer Glasses

11:55 **Giuseppe Spoto** *(University of Catania)* - Nanoparticle-enhanced SPR imaging detection of nucleic acids

Jan Trka *(Charles University)* - Childhood Leukaemia Investigation; Ontogeny of childhood acute lymphoblastic leukaemia

Jose M. Kenny *(ICTP-CSIC, Madrid)* - Processing and applications of multifunctional nanostructured polymeric biomaterials

12:15 **Emiliano Descrovi** *(Polytechnic University of Turin)* - Light management in photonic crystals for fluorescence biosensing

Petr Müller *(MMCI, Brno)* - Alteration of chaperones in cancer

Leon Govaert *(Eindhoven University of Technology)* - Current options for fast evaluation of the long-term performance of load-bearing thermoplastics

12:35 **Sabine Szunerits** *(IRI, Villeneuve-d'Ascq)* - Pathogen detection of grapheme-coated SPR surfaces

Martin Trbušek *(CEITEC)* - Innovative therapies for high risk B-cell malignancies

Daniel Hanoch Wagner *(Weizmann Institute of Science)* - The future of structural materials: Teachings from nature

13:00 Lunch - Restaurant Plzenka / Restaurant Lucullus

Plenary V: Advanced Ceramic Materials: Conference Hall 1
Chair: Jaroslav Cihlář *(CEITEC)*

14:30 **Zhijian Shen** *(Stockholm University)* - Spark plasma sintering: advantages and limitations
15:00 **Heinz-Christoph Schröder** *(Johannes Gutenberg University Mainz)* - Morphogenetically active inorganic polymers for bone tissue engineering
15:30 **Chantal Guillard** *(IRCELYON)* - Mechanism of photocatalytic inactivation of microorganisms

16:00 Coffee Break

Plenary VI: Infection Biology: Conference Hall 1
Chair: Petr Hořín *(CEITEC)*

16:30 **Johannes Langedijk** *(Crucell)* - The fusion protein of Respiratory Syncytial Virus stabilized in the prefusion conformation is a potent vaccine candidate
17:00 **Alessandra Carattoli** *(ISS)* - Mobile DNA: antibiotic resistance touring in Gram-negative bacteria
17:30 **Luis Enjuanes** *(CNB-CSIC, Madrid)* - Coronavirus zoonosis, virulence and protection

18:00 **PRESENTATION OF THE WINNERS OF POSTERS**

20:00 Conference Dinner

Friday, October 24

Plenary VII: Brain and Mind: Conference Hall 1
Chair: Alexandra Šulcová *(CEITEC)*

09:00 **Thomas M. Tzschentke** *(Grünenthal GmbH)* - Analgesics as rewards and the relation to ongoing pain: positive and negative reinforcement mechanisms
09:30 **Hilleke Hulshoff Pol** *(UMC Utrecht)* - Network connectivity changes in schizophrenia: advances of (ultra) high field magnetic resonance imaging and spectroscopy
10:00 **Anna Devor** *(UC San Diego)* - Tools for high resolution optical imaging of neuronal, glial, vascular, and metabolic activity for neuroscience studies in vivo

10:30 Coffee Break

Plenary VIII: Integrative Structural Biology: Conference Hall 1
Chair: Richard Štefl *(CEITEC)*

11:00 **Antony W. Oliver** *(University of Sussex)* - Assembling the DNA damage checkpoint machinery in S.pombe
11:30 **Evžen Bouřa** *(IOCB AV CR)* - Assembling multi-protein complexes by simulation and experiment - combination of Xtal,SAXS, DEER and FRET data
12:00 **Pavel Plevka** *(CEITEC)* - Mechanisms of virus genome delivery into host cells

12:30 Lunch - Restaurant Plzenka / Restaurant Lucullus

ABSTRACTS OF SPEAKERS

Polypeptide-modified gold nanoparticle for colorimetric detection of hydrolytic enzymes

D. Aili
Division of Molecular Physics, Department of Physics, Chemistry and Biology (IFM), Linköping University, Sweden

Sensors capable of detecting biomolecular recognition events are invaluable tools for medical diagnostics and drug development. Gold nanoparticle-based colorimetric sensors can transduce molecular interactions by an optical signal that can be observed by the naked eye or a simple and cheap optical detector, e.g. a smart phone camera. They are thus well suited for applications where a more complex and expensive biosensor either is not required, not affordable or not practical to use. The recognition strategy employed is critical in order to fully exploit the unique optical properties of this nanomaterial in bioanalytical applications and to achieve a selective and sensitive detection of target analytes.

In this talk, I will describe a system of polypeptide-modified gold nanoparticles with highly tunable assembly properties, for detection of a range of different hydrolytic enzymes, including proteases[1], phosphatases[2], and phospholipases[3]. Aggregation of the nanoparticles is mediated by dimerization and folding of the immobilized polypeptides and results in a distinct colorimetric shift. Numerous factors influence the folding of the polypeptides, either directly or indirectly, which in turn affects the colloidal stability of the nanoparticles. This possibility to control and tune the stability of the gold nanoparticles with high precision enables decoupled analyte recognition and signal transduction, which facilitates development of very flexible biodetection strategies.

[1] P. Chen, R. Selegård, D. Aili, B. Liedberg, Nanoscale, **2013**, 5, 8973-8976.
[2] R. Selegård, K. Enander, D. Aili, 2014, Nanoscale, **2014**, DOI: 10.1039/C4NR02791D.
[3] D. Aili, M. Mager, D. Roche, M. M. Stevens, Nano Lett., **2011**, 11, 1401-1405.

New concepts for lab-on-a-chip systems using electrospun nanofibers

A. J. Baeumner[1, 2], L. Matloc-Colangelo[2], A. Georgescu[1, 2], M. W. Frey[3]

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[2] *Department of Biological and Environmental Engineering, Cornell University, Ithaca, NY, USA*

[3] *Department of Fiber Science and Apparel Design, Cornell University, Ithaca, NY, USA*

Microfluidic biosensors, labs-on-a-chip and lateral flow assays for the detection of viable organisms, toxins, and clinically relevant markers have been successfully developed in our research group including analytes such as *B. anthracis, C. parvum*, dengue virus, ***E. coli, S. pyogenes***, cholera toxin, CD4+ T-lymphocytes, thrombin and myoglobin.

Recently, we initiated the study of electrospun nanofibers and their potential to enhance bioassays in paper-based lateral-flow assays (LFA) and in polymer-based microfluidic devices by adding functionalities to the formats otherwise not available. In the case of the LFA format we successfully demonstrated the de novo fabrication of nanofiber-mats as membrane material enabling immobilization of biorecognition elements, adding novel surface chemistries and preventing non-specific binding without the use of blocking reagents.

Nanofiber-enhanced microfluidic devices provide additional degrees of freedom for bioassay designs, as nanofibers with various surface chemistries are electrospun into distinct locations in the microfluidic channels. As the resulting fiber mats can be of varying density and size and hence generate a 3D-structure within the channels intimate contact with the sample is guaranteed throughout the channel volume. Current investigations study these systems for sample preparation, as mixers, and as concentrators where, for example, a concentration of *E. coli* cells by a factor of 20, 000 has already been demonstrated. We also develop a nanofib er modelling software that enables fluid dynamic studies to investigate mixing capabilities within our microfluidic devices.

Equine Infectious Diseases in the Genomic Era: Equine CXCL16 Associated with EAV Carrier State in the Stallion

U. Balasuriya, E. Bailey, Y. Y. Go, L. Chelvarajan, J. Eberth, S. Mondal, S. Sarkar, B. Nemec, S. Artiushin, F. Cook, K. Shuck, P. Timoney
Maxwell H. Gluck Equine Research Center, Department of Veterinary Science, University of Kentucky, Lexington, KY, USA

Recently, we reported evidence that $CD3^+$ T cells from equine arteritis virus (EAV) carrier stallions were susceptible to in vitro EAV infection. In contrast, EAV seropositive stallions not possessing the $CD3^+$ T cell susceptible phenotype had not become carriers of the virus. A genome wide association study (GWAS) using the Illumina Equine SNP50 chip revealed that a gene for the trait was located on chromosome 11 (ECA11). Its distribution was consistent with a dominant mode of inheritance. Here we describe work to identify the gene and mutations responsible. The genomes of two susceptible (one Thoroughbred [TB] and one Standardbred [STB]) and one resistant horse (TB) were sequenced (30x NextGen) and the sequences compared for genetic variants within the target region. The two susceptible horses differed from the resistant horse at four sites within exon 1 of CXCL16. These four mutations altered the amino acids at positions 40, 50, 51 and 53 of the mature CXCL16 protein. Further investigation of 57 TB, STB and Quarter horses confirmed the two sequences were associated with in vitro susceptibility or resistance of $CD3^+$ T cells to EAV. Only two alleles of CXCL16 were observed. Subsequent investigation of the sequences among 64 stallions of diverse breeds characterized as being semen shedders or non-shedders of EAV demonstrated a strong association of the genotypes with the shedding trait ($P<0.000001$). When the published sequence for the CXCL16 exon 1 was compared among other species, this region was found to be highly variable. The region was sequenced for other members of the family Equidae. The in vitro $CD3^+$ resistant haplotype was found only among the horse species (Equus caballus and E. przewalskii) while the susceptible haplotype was found for domestic horses (E. caballus), zebras (E. zebra, E. grevyi and E. quagga burchelli), an onager (E. hemionus onager) and an ass (E. asinus), but not in the E. przewalskii sample. This suggests that the susceptibility genotype is ancestral to the resistant genotype. The CXCL16 is the ligand for the chemokine receptor CXCR6 expressed on $CD4^+$ and $CD8^+$ T cells, NKT cells and NK cells. Thus, equine CXCL16 and CXCR6 may be the two major cellular proteins associated with EAV carrier state in horses. In summary, this work demonstrated that development of persistent infection and semen shedding of EAV is strongly influenced by genetic factors on ECA11, most likely a variant of CXCL16 and its receptor CXCR6.

Ovarian carcinoma *CDK12* mutations deregulate DNA repair genes via deficient formation and function of the CDK12/CycK complex

K. M. Ekumi[a, 1], H. Paculova[b, 1], V. Pospichalova[c], C. A. Bosken[d], V. Bryja[c, e], M. Geyer[d], D. Blazek[b, 2], M. Barboric[a, 2]

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[e] *Institute of Biophysics, Academy of Sciences of the Czech Republic, 61265 Brno, Czech Republic*

[1] *K.M.E. and H.P. contributed equally to this work.*

[2] *D.B and M.B. contributed equally to this work, senior co-authors*

The CDK12/CycK complex promotes gene expression by phosphorylating the C-terminal domain of RNA polymerase II. In previous work we found that CDK12/CycK complex maintains genomic stability via regulation of expression of several key DNA damage response genes such as BRCA1, ATR, FANCD2 or FANCI. CDK12 is among only nine genes with recurrent somatic mutations in high-grade serous ovarian carcinoma. However, the influence of these mutations on the CDK12/CycK complex and their link to cancerogenesis remain ill-defined. Here, we show that most mutations interfere with the CDK12/CycK complex formation, rendering the kinase inactive. By examining the mutations within the CDK12/CycK structure, we find that they likely provoke structural rearrangements detrimental to CDK12 activation. Our mRNA expression analysis of the patient samples containing the CDK12 mutations identifies coordinated down-regulation of key DNA damage response genes. Accordingly, we observed that the mutant CDK12 proteins fail to promote the repair of DNA double strand breaks via homologous recombination. Together, we provide the molecular basis of how mutated CDK12 ceases to function in ovarian carcinoma. We propose that CDK12 is a tumor suppressor of which the loss-of-function mutations may elicit defects in multiple DNA repair pathways, leading to genomic instability that underlies the genesis of the cancer.

Assembling multi-protein complexes by simulation and experiment - combination of Xtal, SAXS, DEER and FRET data

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[2] *Institute of Physics, Polish Academy of Sciences, Al. Lotnikow 32/46, 02-668 Warsaw, Poland*

Among the proteins that are most difficult to characterize structurally are those which which have several large domains connected by long, flexible polypeptide segments. Although abundant and critically important in biological cells, such proteins have proven intractable by conventional techniques. This gap has recently led to the advancement of hybrid methods that use state-of-the-art computational tools to combine complementary data from various high- and low-resolution experiments. Here the EROS (Ensemble Refinement of SAXS) method will be presented. EROS combines high-resolution crystallographic data, low-resolution small angle X-ray scattering (SAXS) data, and coarse-grained molecular simulations. This method allows us to construct conformational ensembles of partially flexible proteins, which are physically meaningful and consistent with available experimental data. We have also extended the EROS method to include in the ensemble refinement procedure data from electron paramagnetic resonance (EPR) and single-molecule Förster resonance energy transfer (smFRET) experiments, and used it to obtain detailed representations of the structures and motions in systems ranging from the ESCRT membrane-protein trafficking system to multi-domain protein kinases and kinases in dynamic complexes with phosphatases.

3D microscopy of bone and bone cells: new views of bone wounds by looking directly at the implant interface

A. Boyde
Dental Physical Sciences, Barts and The London School of Medicine and Dentistry, Queen Mary University of London, London E1 4NS, UK

Large numbers of studies of implants in bone have been made to assess bone bonding to a variety of materials, but especially titanium and in the context of dental implants, and nearly always employing sections of the implant to bone interface. The present report focuses on novel approaches in which we either separate the boundary and look straight at it, as against sectioning it, or look through the surrounding tissue straight at the implant with 3D light microscopy: in both cases we see a much greater proportion of the interface.

Cylindrical or screw Ti implants, 3.2 mm diameter, were placed through the proximal medial tibial plateau in three month old rabbits, and retrieved at intervals from 7 to 365 days (1). Bones were fixed in glutaraldehyde, embedded in PMMA and implants sectioned longitudinally. Tissues near implants were studied using reflected light microscopy, confocal LM and, after carbon coating, compositional contrast BSE SEM and quantitative BSE (qBSE). Suitable blocks were prepared to permit study of the implant surface through the surrounding PMMA-embedded bone or marrow environment using direct view 3D LM methods and confocal LM. We also studied Ti framed glass window implants used for intra-vital microscopy retrieved at up to two years, prepared by PMMA embedding or maceration for SEM (2).

Later, hemisectioned implants were removed from the PMMA and the half beds recoated for BSE SEM. PMMA facing more difficult-to-remove implants was sectioned again to produce quarter beds, imaged using BSE SEM of uncoated samples at 50Pa chamber pressure before and after staining with ammonium tri-iodide for cells and soft tissues.

Plasma ashing was used to remove PMMA, cells and matrix from implant beds to visualise the nearest mineralised tissue surface with 3D BSE SEM. Conversely, sequential HCl and NaOCl etching was used to remove all bone components and produce a non-bone space cast for study of osteocytes and their canalicular processes.

The relatively smooth finish of Ti implants mostly permitted separation of PMMA with all included tissue elements, the resin exactly replicating original machining marks. Direct viewing of the bed showed the exact extent of true bone contact, varying from very small areas with reticular, immature, highly cellular woven bone in early healing to large tracts of mature bone at 6 and 12 months - with the greatest coverage on smooth cylinder forms. Extensive bone-free patches bordered by scalloped osteoclastic resorption lacunae outlines indicated recent removal: similarly sized bone-covered patches had a lower mineralisation density, indicating recent re-formation, i.e., remodelling-turnover. Mature bone contained oriented osteocyte lacunae with canaliculi near the implant demonstrating growth from the implant.

Tri-iodide staining additionally disclosed both osteoid and cells contacting the implant, with large numbers of multinucleated osteoclasts where bone was missing. Some regions showed direct contact of adipocytes and small blood vessels.

Highly mineralised acellular material was often found in contact with the implant. Whereas some of this might be categorised as cement line matrix, we believe that we demonstrate the occurrence of an undescribed repair phase involving a type of dystrophic calcification akin to the cleft or gap sealing and healing seen elsewhere in bone and cartilage (3, 4).

References

1. Boyde A, Wolfe LA, Bone Engineering, (Davies JE ed.), em squared Inc, Toronto, 321-331, 2000.
2. Boyde A, et al. Scanning 17, 72–85, 1995.
3. Boyde A, J Anat., 203,173-189, 2003.
4. Boyde A et al., J Anat., 225, 436-446, 2014.

Shaping the eukaryotic transcriptome with chromatin and non-coding RNA

S. Buratowski
Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Boston, MA, USA

My lab studies the events that make up the RNA polymerase II transcription cycle. Each step - initiation, elongation, and termination - is carefully orchestrated and coordinated with other processes such as mRNA processing and chromatin modification. A major component of this coupling is the C-terminal domain (CTD) on the Rpb1 subunit of RNA polymerase II. The changing CTD phosphorylation pattern serves to mark the position of the transcription complex relative to the transcription start site. Various factors bind to specific phosphorylation configurations, resulting in sequential recruitment of mRNA processing and chromatin modifying enzymes at the appropriate times.

I will present our recent work addressing the mechanisms by which the CTD phosphorylation patterns changes during transcription, how various factors recognize these patterns, and what the downstream effects are on gene expression. In particular, we have found that a great deal of non-coding transcription in yeast affects expression of nearby coding gene, not through any activities of the RNAs themselves, but by changing the chromatin modification patterns at promoters that are overlapped by non-coding transcription.

Mobile DNA: antibiotic resistance touring in Gram-negative bacteria

A. Carattoli
Istituto Superiore di Sanità, Rome, Italy

Antimicrobial resistance in pathogenic bacteria is a global problem. Studies on mechanisms mediating the spread of antimicrobial resistance genes generate valuable information that is helpful to curb further dissemination of resistance. A multidisciplinary approach is currently applied to study the antimicrobial resistance mechanisms occurring in clinically-relevant bacteria: the established surveillance is implemented by molecular characterization of the strains by genotyping, identification of the resistance gene types, virulence profile analysis and replicon typing of the circulating plasmids. However, the genomic approach is currently the most innovative method to fully characterize the globally prevalent resistant bacterial clones. Whole genome sequencing allows the identification of the genetic determinants implied in the horizontal transfer of resistance genes. These elements include transposable elements, integrons, plasmids, prophages, constituting the so called mobilome (all mobile genetic elements in a genome). These successful elements represent the vehicles for transferring and mediating the horizontal acquisition of a variety of resistance genes (resistome) among bacteria of different origin, source, species and genera. In several bacterial clones the mobilome evolves as an integral part of the bacterial genome and is associated with specific traits, imparting to the bacterial host virulence, resistance, and persistence in a given environment or reservoir. The recognition of successful clones and mobile elements is an essential first step that will ultimately inform the design of intervention strategies aimed at preventing their spread.

Light management in photonic crystals for fluorescence biosensing

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In an attempt to improve the performances of fluorescence-based biosensing schemes, the use of photonic crystals (1DPCs) offers the advantage of low losses, strong field intensity enhancement, high resonance quality factors, and the absence of fluorescence quenching. When Bloch Surface Waves (BSW) on one-dimensional photonic crystals are concerned, the overall fluorescence signal intensity can be increased by the fluorescence excitation via a BSW-resonant laser illumination. In addition, the near-field coupling of emitters to BSW allows for control angular distribution of the emitted fluorescence intensity. These two mechanisms are exploited in assays for detection of molecular analytes and shown to efficiently push the Limit of Detection of assays to smaller concentrations.

Compared to the widespread use of surface plasmons in biosensing, the implementation of BSWs as an optical transducer provides important advantages, such as spectral and polarization tunability. Taking advantage of the available growth technologies, periodic stacks of dielectric layers having different refractive indices can be easily obtained, wherein BSW can be coupled in a broad spectral range. Recently, surface modes on 1DPC have been used for demonstrating label-free detection based on spectral/angular resonance shift , or to improve fluorescence-based detection. To this extent, 1DPCs sustaining BSWs represent a powerful platform combining most of the sensing possibilities offered by conventional SPR and photonic-crystal based fluorescence detection[1].

The photonic crystal can be used in the Kretschmann configuration as a means to enhance a specific fluorescence detection of IgG. When the 1DPC surface is patterned with a diffraction grating, the BSW-coupled fluorescence can be more efficiently extracted, according to a beaming effect. The improvement in fluorescence collection can be appreciated by using a low Numerical Aperture imaging system wherein labelled IgGs are incubated on the 1DPC surface. Enhancement factors up to 10-20 can be easily obtained with no critical alignment issues, thus making this approach quite suitable also in consolidated microarray architectures.

[1] See www.biloba-project.eu for reference.

Tools for high resolution optical imaging of neuronal, glial, vascular and metabolic activity for neuroscience studies in vivo

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What is the first association that comes to your mind when you hear "fiber optics"? Connecting one side of the Atlantic Ocean to the other or high-definition TV? For sure, it would not be manipulation of neuronal firing … unless you are a neuroscientist. While neuroscience has relied on optics to power discovery from the beginning of times, brain imaging remains an exotic application in the mainstream optics and photonics. In US, the Presidential BRAIN Initiative, launched last year, targets this disconnect by encouraging "hard core" technology experts to step up to the neuroscience quest. In Czech Republic, synergistic efforts are on the go under the umbrella of CEITEC.

The goal of the BRAIN Initiative is to develop new technologies driven by the needs of basic and translational neuroscience – easy to say but difficult to do. The first challenge for the technology expert is to understand the needs of the endpoint application; for the neuroscientist – to keep up with the rapidly developing technology and recognize its strengths and limitations.
Generally speaking, brain tissue is an unfriendly environment for optics. Light is scattered by microscopic structures with different refractive indices and absorbed by natural chromophores. Red blood cells fly through capillaries, scattering and absorbing light along the way. The brain moves (slightly but noticeably on a microscopic scale) with respiration and heartbeat beyond what can be corrected for by rigid body registration. Further, the duration of an action potential is only 1-2 ms, which imposes stringent constraints on the acquisition speed required for measurements of brain activity. In addition, brain tissue is sensitive to heating and photodynamic damage, placing limits on photon flux.

Despite these limitations, consider this: arguably the most significant breakthroughs in neuroscience have been powered by advancements in optical technology, from the discovery of synaptic junctions by the father of modern neuroscience Ramón y Cajal in 1880s, to the contemporary advances in cell- and system-level neuroscience through utilization of 2-photon microscopy, optogenetics, and superresolution microscopy.

In this talk, we will consider the current state-of-the-art of a number of key microscopy technologies that now power high-resolution mechanistic in vivo neuroscience studies and review example applications from our work where the use of these novel technologies has been instrumental for biological discoveries. These endpoint biological insights are a product of long-term multidisciplinary collaboration enabling application-driven comprehensive technological developments. In future, the brain application of optics and photonics will become a little less exotic. However, the excitement will only grow as application-driven technology and technology-powered neuroscience evolve in a continual dialog.

Fluorescence biosensors with plasmonically amplified signal

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Fluorescence represents (arguably) the mostly spread readout method for the analysis of chemical and biological species in important areas of medical diagnostics (biomarkers and drugs), food control (pathogens and toxins), as well as in life sciences. Recent rapid advancements in plasmonics – a branch of nanophotonics that investigates tightly confined electromagnetic fields of surface plasmons on surfaces of metallic films and metallic nanostructures – offers efficient means for the amplification of fluorescence signal and thus advancing performance characteristics of fluorescence–based analytical technologies [1].

This paper will introduce several assays that utilize plasmon-enhanced fluorescence (PEF) for rapid detection of minute amounts of analytes in realistic samples such as blood serum. Examples of PEF biosensors for detection of analytes including inflammation and cancer biomarkers with the limit of detection at low femtomolar concentrations will be presented [2, 3]. In general, PEF enables enhancing the fluorescence signal-to-noise-ratio by the combination of increased excitation rate, improved quantum yield of emitter, and by directional emission. Firstly, there will be discussed PEF sensors that rely on propagating surface plasmons coupled to the far field by attenuated total reflection method or by diffraction gratings that offer the enhancement factor of around 10^2. Afterwards, there will be investigated possible routes for even stronger enhancement $>10^3$ through the probing by more tightly confined electromagnetic fields of lattice localized surface plasmons [4] and by using enzymatic post-amplification. Approaches relying on plasmonic structures that can be prepared by mass production-compatible methods such as laser interference lithography or nanoimprint lithography will be particularly addressed.

Acknowledgments
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References
[1] M. Bauch, K. Toma, M. Toma, Q. Zhang, J. Dostalek, Plasmonics (2014), in press.
[2] Y. Wang, A. Brunsen, U. Jonas, J. Dostalek, W. Knoll, Analytical Chemistry, (2009) 81, 23, 9625-9632.
[3] M. Bauch, S. Hageneder, J. Dostalek, Analytical Chemistry, in preparation.
[4] M. Bauch, J. Dostalek, Optics Express (2013) 21(17) pp 20470-20483.

The RNA polymerase II carboxy-terminal domain (CTD) code

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In eukaryotes, an unusual carboxy-terminal domain (CTD) is crucial for the function of RNA polymerase II (Pol II) in transcription. Mammalian CTD consists of 52 heptad-repeats with the consensus repeat Tyr1-Ser2-Pro3-Thr4-Ser5-Pro6-Ser7. Specific post-translational modifications in CTD appear to fulfil specific tasks during the transcription process. Biochemical and genetic studies have shown CTD modifications to be essential for multiple steps in the regulation of gene expression, from initiation of transcription on chromatin templates to splicing and processing of nascent RNA. To gain deeper insight into the function of CTD we produced monoclonal antibodies (mAbs) directed towards specific modifications in CTD. We show that all potential phosphorylation sites in a consensus heptad-repeat are phosphorylated in vivo and that CTD phosphorylation regulates general and gene specific functions. Methylation of lysine and arginine residues of CTD in non-consensus repeats add further layers of gene regulatory mechanisms.

Antibodies are useful tools for the detection of Tyr1-P, Ser2-P, Thr4-P, Ser5-P, and Ser7-P marks in CTD, but fall short to detect combinations of CTD-marks. In addition, antibodies do not discriminate between phospho-marks occurring in heptad-repeats of the proximal, middle, or distal part of CTD. To overcome these limitations we created a set of Pol II mutants to make the entire CTD sequence accessible to mass spectrometry analysis and to mapping of signatures of CTD phosphosites. The mutants achieved full CTD sequence coverage in mass spectrometry experiments. We found that (i) almost all 239 potential phosphorylation sites in CTD are target for phosphorylation in vivo, (ii) two-fold phosphorylated heptads occur in all possible combinations, (iii) and that different phosphorylation signatures can occur in adjacent CTD heptads. Our work provides first insights into phosphorylation signatures of Pol II CTD in vivo and allows the mapping of modification signatures along the entire CTD molecule.

Impact of SF3B1 mutations in CLL on the DNA damage response

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Mutations or deletions in TP53 or ATM are well-known determinants of poor prognosis in Chronic Lymphocytic Leukemia (CLL), but account for approximately 40% of chemo-resistant patients. Genome-wide sequencing has recently uncovered novel mutations in CLL that are linked with poor prognosis. Of these, mutations in the splicing factor SF3B1 have attracted attention, also because the pathological mechanism is as yet unexplained. Therefore, we performed detailed genetic and functional analyses in a CLL cohort (n=105) containing ATM, SF3B1 and TP53 gene defects. Here, we focus on comparative functional analyses in the ten patients where single SF3B1 (sSF3B1) lesions were identified.

Aberrant splice products of various candidate causative genes, although elevated in SF3B1 mutated compared to WT cases, were expressed at a fraction of normal transcript levels. Surprisingly, sSF3B1 resembled ATM mutated CLL in displaying defective ATM/p53 transcriptional and apoptosis response to various DNA-damaging regimens. Just as in ATM mutated cases, sensitivity to fludarabine in sSF3B1 cases could be restored by the MDM2 inhibitor nutlin-3a. Nevertheless, ATM kinase function remained intact. Finally, γH2AX focus formation as marker for DNA damage was increased both at baseline and upon irradiation in SF3B1 mutated cases.

In conclusion, our data suggest that alternative splicing is not the primary basis for the observed phenotype, and that single mutations in SF3B1 have an impact on the DNA damage response. Combined, our observations suggest an explanation for the poor prognosis of affected patients, and may lead to new treatment strategies.

Three-dimensional (3D) Polymer and Carbon Scaffolds for Future Cell Replacement Therapy and Bioartificial Organ-on-a-Chip Systems

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A range of different materials is currently being explored for building three-dimensional (3D) scaffolds for tissue engineering and biomedical research. The 3D environment is envisaged to provide a better mimic of the natural in vivo environment, leading to more physiologically realistic and reliable biomedical research tools than currently used standard 2D formats. Polymeric microtowers and superhydrophobic silicon micropillars have proven to provide optimal 3D microenvironment for neuron-astrocyte co-cultures, promoting formation of neuronal networks. 3D peptide nanowire scaffolds have boosted rodent stem cell differentiation into dopaminergic phenotype. Carbon nanotubes (CNT) have been used to mechanically stabilize commonly used "soft" scaffold materials such as hydrogels and fibrous scaffolds. Electrically conductive CNT hydrogel composites of these otherwise non-conductive scaffolds, has enabled electrical stimulation of neural stem cells. Recently, graphene foam was suggested as a new promising conductive scaffold that may incorporate, in the same structure, topographical, chemical and electrical cues. In this talk, I describe our work towards the development of perfusable, scalable, structured and/or conductive scaffolds in polymer or carbon.

Polymer scaffolds were fabricated using a combination of 3D printing and polymer casting techniques. The scaffolds were designed with: (a) Primary structured perfusable channel network that allows flow through the material enabling delivery of necessary nutrients and oxygen to the interior of the scaffolds. (b) A secondary more arbitrary random porous network that optionally can enclose a hydrogel phase with a "nearby" source of important cell factors, supporting the growth and differentiation of cells. The preliminary application of these polymer scaffolds for the development of a bioartificial liver support system will be presented.

Conducting carbon scaffolds were obtained through pyrolysis of polymer scaffold molds at 900 °C under 100 % nitrogen atmosphere. The molds were fabricated through photolithographic micro patterning or in similar fashion as the polymer scaffolds described above. Depending on the precursors used for fabricating the polymer mold, the conductivity and properties of the carbon could be tuned. Here, the application of a conductive carbon scaffold material is demonstrated to boost the differentiation of human neural stem cells into mature dopaminergic neurons, with the degree of maturity confirmed by amperometric detection of exocytotically released dopamine directly at the carbon scaffold backbone.

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Coronavirus zoonosis, virulence and protection

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Coronaviruses (CoVs) have frequently crossed species barriers and have recently caused important zoonosis. In fact, in 2002-03 a previously unknown human and animal CoV, the severe and acute respiratory syndrome virus (SARS-CoV) emerged in South East China, infected around 8000 people and killed around ten per cent of them. More recently, in the summer of 2012, the Middle East syndrome CoV (MERS-CoV) emerged in the Arabian Peninsula and has since then caused more than more than 837 hospitalizations and around 300 deaths. In 2010 a highly virulent PEDV emerged in China. Three years later, this virus re-emerged in the USA and spread to more than 20 North American States. In February 2014 this virus has also been diagnosed in Canada. Interestingly, a new CoV causing the same type of disease than PEDV and TGEV, but highly different in sequence from them, has been detected in February 2014 in Ohio (USA). This virus is closely related to one that was isolated in Hong Kong in 2013. This is a new virus that probably is more widely distributed in the World, but that has not been identified before due to its previously unknown identity. Whereas TGEV and PEDV have been classified as members of CoV genus α, the new CoV belongs to genus δ and has been named swine delta CoV (SDCV).
We will address two objectives: (i) the generation of vaccines to protect against emerging CoVs, and (ii) the identification of antivirals for controlling coronavirus infections. The identification of the genes involved in CoV virulence and in signaling pathways contributing to pathogenesis has been addressed using SARS- and MERS-CoVs. SARS-CoV non-essential genes have been deleted using a reverse genetics system. Among them, deletion of E gene led to the most attenuated phenotype (SARS-CoV-ΔE). Stress response and unfolded protein response genes were upregulated in cells infected by SARS-CoV-ΔE in relation with those infected by SARS-CoV with E protein. The expression of proinflammatory cytokines was reduced in the lungs of mice infected with a mouse adapted SARS-CoV-MA15-ΔE compared to lungs infected with the wild type virus. In infections by SARS-CoV with and without E protein, NF-κB was the only proinflammatory pathway differentially activated. Interestingly, the addition of an inhibitor of NF-κB led to a reduced inflammatory response after SARS-CoV infection and to an increase in mice survival. Therefore, these inhibitors could serve, in principle, as antivirals. A reduction in neutrophil migration to lung-infected areas was observed in mice infected with SARS-CoV-MA15-ΔE, probably contributing to the lower degree of inflammation detected and to SARS-CoV-ΔE attenuation. SARS-CoV E protein is a viroporin with three domains: amino terminus, transmembrane and carboxy-terminus. The role of the different domains of E protein in SARS-CoV virulence, including its ion channel activity and a PDZ binding domain mapping at the most carboxy-terminus of this protein has been evaluated. Alteration of these domains attenuated the virus, and the mechanisms of attenuation have been studied. These attenuated mutants provided long-term protection both in young and elderly mice against the challenge with pathogenic SARS-CoVs. Deletion of E gene in MERS-CoV using a reverse genetics system, led to a replication-competent propagation-defective virus that is a safe vaccine candidate. These data indicated that SARS-CoV and MERS-CoV with E protein deleted or modified are promising vaccine candidates.

Nanoplasmonics – from biosensing to biomonitoring

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I will report on our recent efforts to utilize some of the unique plasmonic properties of noble metal nanoparticles for sensing and controlling nano- and microscale processes in aqueous solution. The use of optical forces and of local optothermal heating has been in the focus of our investigations. Examples range from fast optothermally controlled DNA analysis and amplification, controlled laser printing experiments for cell transfection up to direct optical monitoring of flow generated by bacterial flagellar rotation.

Most relevant publications:

Hierarchical assembly of metal nanoparticles, quantum dots and organic dyes using DNA origami scaffolds
R. Schreiber, J. Do, E. Roller, T. Zhang, V. Schüller, P. Nickels, J. Feldmann, and T. Liedl Nature Nanotechnology 9, 74 (2014)

Direct optical monitoring of flow generated by bacterial flagellar rotation
S. Kirchner, S. Nedev, S. Carretero-Palacios, A. Mader, M. Opitz, T. Lohmüller, and J. Feldmann Appl. Phys. Lett. 104, 9 (2014)

Tuning DNA Binding Kinetics in an Optical Trap by Plasmonic Nanoparticle Heating
L. Osinkina S. Carretero-Palacios, J. Stehr, A. Lutich, F. Jäckel, J. Feldmann Nano Lett. 13, 3140 (2013)

Optically trapped gold nanoparticle enables listening at the microscale
A. Ohlinger, A. Deak, A. Lutich, and J. Feldmann Phys. Rev. Lett. 108, 018101 (2012)

Gold NanoStoves for Microsecond DNA Melting Analysis
J. Stehr, C. Hrelescu, R. A. Sperling, G. Raschke, M. Wunderlich, A. Nichtl, D. Heindl, K. Kürzinger, W. J. Parak, T. A. Klar, and J. Feldmann Nano Lett., 8 (2), 619 (2008)

Auxin-mediated polarity and patterning in plant development

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Plants and animals live different lives. Whereas animals typically react with a behavioural response manifested by fight or flight, plants display adaptive and flexible development that optimally adjusts their phenotype to the environment. Post-embryonic growth involving the activity of meristems, tissue regeneration, de novo organ formation and tropistic growth responses are unique examples of ways, in which plants shape their form to environmental changes.

Therefore, in plants, more than in other eukaryotes, (re)establishment of cell and tissue polarities along with patterning of different cell types are major developmental themes. The connection between cellular polarizing events and macroscopic manifestation of polarity such as specification of different cell types along the axis, depend on an action of the signalling molecule auxin. Auxin is a prominent intercellular signal in plants and acts as a versatile trigger of developmental change in multitude of processes. Directional, active transport between cells mediates so called auxin gradients that underlie many patterning processes, including apical-basal axis formation during embryogenesis, organogenesis, vascular tissue formation and tropisms. Environmental and endogenous signals can be integrated into changes in auxin distribution through their effects on auxin transport, specifically on the cellular distribution of PIN auxin transporters. Different PIN proteins, each with specific polar, subcellular localization form a network mediating directional auxin fluxes through different tissues for formation of auxin activity gradients. Within cell, PIN proteins undergo constitutively cycles of a clathrin-dependent endocytosis and recycling to the plasma membrane. Various endogenous and external signals can regulate this subcellular dynamics, thus changing polarity of PIN localization and controlling their directional activity. In this view, the PIN-dependent auxin transport network provides one of the key mechanisms underlying the plasticity and adaptability of plant development.

Current options for fast evaluation of the long-term performance of load-bearing thermoplastics

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Failure under static or dynamic loading conditions is a major concern in the application of polymers in load-bearing components: it is not the question *whether* it will fail, but rather on *what time-scale*. It is imperative that the ability to estimate the lifetime of polymer components under design-load specifications is essential in their design and optimization. With the increasing social demand for durable products with long service life, and a gradual shift to more critical applications, e.g. involving high loads and temperatures (e.g. under-hood automotive applications, domestic hot water systems), the need for such predictive methods is even more vital.

In the absence of reliable models, one is left with no other option than to revert to prototyping and full-product tests. Unfortunately, such trial and error approaches are time-consuming, costly and hamper flexible product development.

In my presentation I will give an overview of current options for modelling and accelerated characterization protocols concerning the long-term performance of load-bearing polymer systems.

Mechanism of photocatalytic inactivation of microorganisms

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The photocatalytic antimicrobial effects of TiO_2 on three yeast species *(S. cerevisiae, C. kruzei and R. glutinis)* and a filamentous fungus *(B. cinerea)* representative of distinct environments have been investigated. Antimicrobial effect of TiO_2 strongly depends on protective structures and molecules like pigmented cell walls, frequently associated fungal cell structure. Cell viability, loss of enzymatic activity, byproducts analysis such as small carboxylic organic acids, amino acids, inorganic ions and malondialdehyde (MDA), peripheric and intracellular proteins, superoxide radical ions (O_2°-) and transmission electronic microscopy were investigated. Moreover, our studies points out that.

Promoter nucleosome dynamics regulated by signaling through the RNA Polymerae II CTD

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The phosphorylation of the RNA polymerase II CTD plays a key role in delineating the transcribed regions within chromatin by recruiting histone methylases and deacetylases (HDAC). Using genome-wide nucleosome mapping, we show that CTD S2 phosphorylation controls nucleosome dynamics in the promoter of a subset of 346 genes including the regulators of cell differentiation ste11 and metabolic adaptation inv1. Mechanistic studies on these genes indicate that during gene activation the Set1 dependent methylation of histone H3 and the recruitment of specific HDACs through the phosphoS5 CTD is impaired by a local increase of phosphoS2 CTD nearby the promoter, leading to nucleosome depletion and efficient transcription. The early peak of phosphoS2 results from the phosphorylation of the CTD S2 kinase by MAP kinase in response to cellular signaling. Therefore, signaling through the CTD code regulates promoter nucleosomes dynamics during developmental switch and metabolic reprogramming.

Nano-FTIR spectroscopy of individual protein complexes

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We introduce the mapping of protein structure with 30 nm lateral resolution and sensitivity to individual protein complexes by infrared scattering-type scanning near-field optical microscopy (IR s-SNOM) and Fourier transform infrared nanospectroscopy (nano-FTIR). s-SNOM and nano-FTIR are based on recording the infrared light scattered by a metallized atomic force microscope tip probing the sample surface. We present and discuss local broadband spectra of individual viruses, vferritin complexes, purple membranes and insulin aggregates, which can be interpreted in terms of their alpha-helical and/or beta-sheet structure [1]. Applying nano-FTIR for studying insulin fibrils, we find clear evidence that 3-nm-thin amyloid-like fibrils contain a large amount of alpha-helical structure. Nano-FTIR spectra of one ferritin complex demonstrate extraordinary sensitivity to ultra-small amounts of material, about 1 attogram of protein, respectively 5000 C=O bonds. By further sharpening the tips and optimizing their antenna performance, we envision single protein spectroscopy in the future, paving the way to a new era in infrared bio-spectroscopy. We foresee manifold applications, such as studies of conformational changes in amyloid structures on the molecular level, the mapping of nanoscale protein modifications in biomedical tissue or the label-free mapping of membrane proteins.

[1] I. Amenabar, et al., Nature Commun. 4:2890 doi: 10.1038/ncomms3890 (2013)

Turning a silk purse into a sows ear: the importance of processing when using silk in regenerative medicine

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If we wish to harness the power of silk we must first understand it. Understanding means not only knowing the relevant proteins but also knowing their function and, importantly, their structure - property relationships. And here is a gap in our present knowledge. Silk proteins have been patented by many research groups and companies and been expressed in bacteria, plants and animals. However it is processing that defines a silk, for unlike all other biological materials they are spun, not grown.

Silks are biological polymers that have evolved to be processed by controlled protein denaturation, a process with many similarities to amyloidogenesis. But no one, to our knowledge, has succeeded in successfully configuring, i.e., spinning those proteins into anything resembling the natural fibre neither in its microstructure (which is rather complex) nor in its mechanical properties (which are outstanding). This presentation will provide an overview of Natures 400 million years of R&D into silk and our recent studies into the importance of processing in this fascinating material. Finally I will discuss silk's potential in medicine and how fundamental research is being translated into the development of implantable biomedical devices whose performance may be tuned in terms of both degradation rate and mechanical performance simply by altering the processing of the material.

West Nile virus - an emerging mosquito-borne agent

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West Nile virus (WNV) is a mosquito-borne flavivirus from the Japanese encephalitis antigenic group. While culicine mosquitoes are its invertebrate vectors, main amplifying vertebrate hosts are birds. Some mammals (e.g. horse, man) are also very susceptible to infection with WNV which can cause severe disease including encephalitis in them, but they are so-called „dead-end hosts" that do not represent a risk of infection for non-infected mammals. In this conribution, WNV history and geographic distribution will be briefly reviewed, with emphasis on the recent emergence and spread of the virus in southern and central Europe. Seroepidemiological and virological studies on WNV, carried out by Laboratory of Medical Zoology (Institute of Vertebrate Biology ASCR), including isolation of two WNV lineages - 3 (Rabensburg) and 2 - in Czechland, will be presented.

Network connectivity changes in schizophrenia: advances of (ultra) high field magnetic resonance imaging and spectroscopy

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UMC Utrecht

Schizophrenia is characterized by loss of brain volume, which may represent an ongoing pathophysiological process. This loss of brain volume may be explained by reduced neuropil rather than neuronal loss, suggesting abnormal synaptic plasticity and cortical circuitry. A possible mechanism implicates altered glutamate and GABA levels and changes in connectivity. Using magnetic resonance brain imaging at high field (3 Tesla) and ultra high field (7 Tesla) it is now possible to measure structural and functional brain network connectivity and brain metabolite levels in patients with schizophrenia and healthy control individuals. Findings from these studies support a mechanism involving altered structural and functional network connectivity in schizophrenia. In addition, findings suggest a mechanism involving altered GABA levels distinguished from glutamate levels in the medial prefrontal cortex in schizophrenia, particularly in high functioning patients. I will discuss the implications of these imaging findings to gaining new insight into brain functioning in health and in psychiatric disease.

Biophysical studies of non-enveloped virus maturation: insights into elegantly programmed nano-machines

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Virus particles with quasi-equivalent surface lattices (i.e. identical gene products in different quaternary structure environments) often assemble as a fragile, spherical shell in which subunits are properly positioned on the surface lattice with differences in the environments minimized. Quasi-equivalent subunit contacts then differentiate during particle maturation, creating a robust, faceted particle with subunits in dramatically different local environments. Nudaurelia Capensis W Virus (NWV) is a eukaryotic, quasi-equivalent virus, with a T=4 surface lattice, where maturation is dramatic (a change in particle size of 100Å) and is novel in that it can be investigated in vitro. We used X-ray crystallography, molecular genetics, biochemistry, computational chemistry, small Angle X-ray scattering, and electron cryo-microscopy and image reconstruction (CryoEM), to characterize the kinetics of morphological change, maturation intermediates, an associated auto-catalytic cleavage, and to demonstrate that regions of NWV subunit folding are maturation-dependent and occur at rates determined by their quasi-equivalent position in the capsid.

Processing and applications of multifunctional nanostructured polymeric biomaterials

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The promising perspectives of nanostructured biomaterials based on biodegradable polymers and their relevance in different application sectors with particular focus on tissue engineering and food packaging are reported. Regarding the first sector, the effects of polymeric nanoparticles, nanocomposites and nanotopography development are here extensively investigated and reported in terms of polymer properties and cell response. A number of recent advances developed in our laboratories, concerning the synthesis of polymeric nanoparticles and nanoshells, the processing of polymeric multifunctional nanocomposites and surface modification techniques will be highlighted. Polymeric nanoparticles with well-defined size and morphology were produced by double emulsion and this method was used for protein encapsulation, offering interesting possibilities for revolutionary improvements in tissue engineering, diagnosis and targeted drug delivery systems. On the other hand, nanocomposite scaffolds were produced by casting and electrospinning combining the biodegradable polymer with functional nanostructures, while surface properties were modulated by radiofrequency plasma treatments. The physical properties as well as the chemical properties of materials, including size, shape, mechanical properties, surface texture, etc. can regulate biological responses and provide mechanical stimuli to stem cells. Traction forces generated by cells may markedly influence many biological processes such as self-renewal and differentiation. The possibility to control specific cell functions by modulating the polymer scaffold properties, represents a key point of material science in tissue engineering applications. Finally, the development of biodegradable polymer blends, copolymers and their nanocomposites with shape memory behavior and potential applications as biomaterials will be also discussed. Regarding the second application sector, food packaging, the development of innovative multifunctional formulations based on biodegradable polymers with different nanofillers (nanocellulose, silver nanoparticles) with the support of different compatibilizers, surfactants and plasticizers will be reported in terms of thermal, mechanical and migration properties. Nanostructured biodegradable materials are ready for take-off and certainly promise an exciting future at the interface of chemistry, biology and material science.

DNA repair as a therapeutic target

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Cellular DNA is constantly exposed to various types of stresses resulting in damaged DNA. Complex networks of redundant surveillance mechanisms, namely evolutionarily conserved DNA damage response (DDR), maintain genomic integrity following various genomic insults. Defects in DNA repair pathway often lead to cancer predisposition as a consequence of genomic instability and accumulation of chromosomal mutations. However, the same mechanisms also increase cancer cell viability and facilitate resistance to radiation and chemotherapy. Several key components involved in DDR and genome surveillance pathways represent druggable targets for pharmacological intervention. Their role and regulation will be discussed in more details together with our approach to identify novel inhibitors. These should provide new chemical biological probes to dissect molecular mechanism of corresponding pathways as well as their redundancy. We will also explore their synthetic lethal interactions with possible therapeutic applications.

The fusion protein of Respiratory Syncytial Virus stabilized in the prefusion conformation is a potent vaccine candidate

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Background

The fusion protein F of RSV is the principal target for induction of neutralizing antibody (Nab) responses. RSV-F drives membrane fusion by irreversible protein refolding from the highly labile prefusion conformation, via several steps into the highly stable postfusion conformation. Both conformations can induce and bind NAbs but the prefusion conformation binds a broader spectrum of Nabs. Therefore the prefusion conformation is a preferred vaccine candidate, however development of a prefusion F-based RSV subunit vaccine has been hampered by the metastable nature of F.

Methods and Results

The 3D structure of prefusion RSV-F clarifies obvious structural instabilities that drive protein refolding after triggering. Introduction of unique single point mutations close to heptad repeat A (HRA) at the top can arrest this movement by stabilizing the region. In combination with a mutation that stabilizes the movement of HRB at the bottom of the protein, a stable prefusion F protein was constructed with high expression levels in mammalian cells. The stabilizing effect of the new mutations that are based on the obstruction of movement and hinging, suggest a new path to stabilization that does not need introduction of disulfides or cavity filling. The protein binds a panel of monoclonal antibodies specific for the prefusion conformation. The conformation is maintained upon freeze/thaw cycles, a wide range of pH, osmolarities, temperatures and adjuvant formulations. EM averaging with and without Fabs show that it consists of a single species of prefusion trimers. Stabilized prefusion F was subsequently evaluated as candidate vaccine in the mouse and cotton rat model. Balb/C mice immunized with the prefusion protein induced neutralizing antibody titers that were higher than titers induced by postfusion RSV-F protein. Immunization of cotton rats with the prefusion F protein conferred complete protection in lung and nose after challenge, whereas postfusion F or unadjuvanted prefusion conferred partial protection.

Conclusion

With a minimal number of unique amino acid substitutions stable prefusion RSV-F protein was constructed that could be produced with high yield in mammalian cells. Prefusion F elicited NABs and induced full protection against viral challenge in cotton rats.

Time Resolved Photothermal Expansion Infrared Nanoscopy

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Recent developments in the field of scanning probe microscopy have made infrared imaging and spectroscopy below the diffraction limit possible. Spatial resolutions below 50 nm can now be reached for mid-infrared (MIR) wavelengths using either scattering scanning near field optical microscopy (sSNOM) [1,2] or photothermal expansion microscopy (atomic force microscope induced resonance, AFMIR) [3,4]. Both methods have in common that they use a tuneable MIR laser as a light source. This use of a monochromatic source makes imaging of the absorption at a single wavelength across the sample during a standard topographic scan of the surface easily possible. However, it also implies that to acquire a full spectrum the laser has to step through all the wavelengths that are to be recorded. Accordingly, the time it takes to acquire one spectrum with a commercially available infrared nanoscopy instrument is in the range of a minute or more.

Here we present our first steps towards time resolved infrared spectroscopy over a broad wavelength range (> 100 cm^{-1}) using photothermal expansion microscopy. Through the use of a Daylight solutions external cavity quantum cascade laser (EC-QCL) which can be quickly sweeped across its tuning range a time resolution in the range of 10 s or better can been achieved. We explain the working principle of this method and demonstrate how it can be used to analyze changes in the secondary structure of proteins over time.

1. N. Ocelic, A. Huber, and R. Hillenbrand, Appl. Phys. Lett. **89**, 101124 (2006).
2. F. Huth, A. Govyadinov, S. Amarie, W. Nuansing, F. Keilmann, and R. Hillenbrand, Nano Lett. **12**, 3973 (2012).
3. A. Dazzi, R. Prazeres, F. Glotin, J. M. Ortega, M. Al-Sawaftah, and M. de Frutos, Ultramicroscopy **108**, 635 (2008).
4. F. Lu, M. Jin, and M. A. Belkin, Nat. Photonics **8**, 307 (2014).

Effects of Physical Aging and Rejuvenation on the Mechanical Behavior of Polymer Glasses

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Recent years have shown an increased interest into the underlying mechanisms that govern the time-dependent changes that occur in the nonlinear properties of polymer glasses. It is well established that changes in thermal history (processing conditions) alter the mechanical response but new process methods including mechanical rejuvenation are not so well understood. This seminar will present results that contrast aging kinetics in polymeric glasses subjected to different thermal histories to those exposed to mechanical rejuvenation. Additional results will be shown how molecular additives affect the aging kinetics after the rejuvenation process on model polymer networks.

Strategies for coupling enzymes to electrodes by means of nanoparticles and polymers

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The combination of proteins and electrodes is still a highly innovative research field. Considerable progress has been achieved in constructing artificial signal chains allowing defined analyte detection. Direct electron transfer from enzymes to electrodes is particularly advantageous for such systems, but several problems are limiting the practical application[1, 2]. One research trend can be seen in the formation of multiple protein layers which are able to take part in the signal generation process. This results in tunable sensing systems. The redox protein cytochrome c has been found to be a valuable building block for such arrangements[3] which can be formed by the layer-by-layer technique. Different materials have been applied for this purpose including nanoparticles[4]. Based on this catalytically active bi-protein assemblies have been developed, e.g. with billirubin oxidase[5], sulfite oxidase[6] or cellobiose dehydrogenase[7]. These systems follow natural examples of signal chains and have been further developed to a switchable entity with three proteins immobilised together and allowing a defined electron exchange in the surface confined state[8].
Another direction of research can be seen in an advanced design of the electrode interface. Here nanomaterials and polymers provide a wide and adoptable repertoire of interfacial properties. We have been using different kinds of carbon nanotubes and have modified the CNT-surface with P-stacking compounds or polymers from the group of sulfonated polyanilines. Thus, enzymes such as billirubin oxidase and PQQ-dependent glucose dehydrogenase can be directly coupled to electrodes[9-11]. For the polymer-GDH interaction the reaction has been first studied in solution and then transfered to surfaces[12]. Here different strategies can be demonstrated such as multilayer formation or the modification of a mesoporous ITO electrode with the polymer/enzyme [13]. Application is also devoted to biofuel cells. For example, vertically aligned CNTs have been used as basic material for enzyme electrode construction[14]. Current densities up to the mA/cm^2 level can be achieved.

[1] E. E. Ferapontova et al, Perspectives in Bioanalysis, Vol. 1, Elsevier, 2005, p. 517
[2] F. Lisdat et al, Chem. Com. 3 (2009) 274
[3] M.K. Beissenhirtz et al., Anal. Chem. 76 (16) (2004) 4665
[4] S. C. Feifel, F. Lisdat, J. of Nanobiotech. (2011) 9:59
[5] R.V. Dronov et al, JACS 130 (4) (2008) 1122
[6] R.V. Dronov et al., Angew. Chem. 47 (16) (2008) 3000
[7] S. C. Feifel et al., RSC Advances 3 (2013) 3428
[8] S. C. Feifel et al., Angew. Chem. 53(22) (2014) 5676
[9] K. Schubert et al., Electrochim. Acta 54 (11) (2009) 3033
[10] G. Göbel, F. Lisdat, Electrochem. Com. 10 (11) (2008) 1691
[11] I. W. Schubart et al., Electrochim. Acta 82 (2012) 224
[12] D. Sarauli et al., Acta Biomaterialia 9 (9) (2013) 8290
[13] D. Sarauli et al., J. of Mat. Chem. B 2 (21) (2014) 3151
[14] V. Scherbahn et al., Biosens. Bioelectr. 61 2014 631

Silk protein based constructs for tissue engineering

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Nature evolved a number of strategies (material-structure relationship) to create outstanding functional properties with "cheap based materials". Composition and structure of natural materials, assembled by different organisms, are tailored by obtain multi-functional, adaptive and self-healing structures. Many natural materials possess unique biological properties that makes them ideal materials for biomedical applications and namely for tissue engineering and regenerative medicine.

The lecture will be focused on main concepts on the use of natural polymers for the fabrication of bioactive scaffolds for tissue engineering applications, and specific examples will be presented on silk-derived constructs. Silk, a family of biopolymers is an excellent example of structural material derived from self-assembly of relative simple molecular building blocks. Several Insects, Arthropods and mollusks produce silks, as building material for constructs with different uses. Silks are also synthetized to be assembled in composite materials (collagen, chitin, inorganic nanocrystals) with unique structural and mechanical. However, silks produced by Bombicidae are mainly used in the biomedical field. Silk-derived proteins are been used as the starting material to prepare a wide range of systems with tuned mechanical properties, degradability, geometry, water content, and processing-related bioactivity.

How much do we share with our closest relatives: parasite communities of humans and great apes

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Among the infectious diseases of humankind, most can be classified as zoonoses, shared between humans and animals. Most of these diseases were acquired during and after the Neolithic revolution, being usually associated with abrupt changes in human ecology. However, part of these pathogens coevolved with anthropoid primates and can be traced deeply into the evolutionary history of primates. Phylogenetic proximity of humans and (mainly) African great apes (chimpanzees and gorillas) eases the exchange of broad range of pathogens, often with serious consequences. Recent epidemics of ebola in W Africa is a prominent example of such a disease. Part of the research carried out by RG Parasitology of CEITEC VFU addresses the epidemiology of diseases and molecular diversity of pathogens of free ranging great apes, including malaria, trypanosomoses and gastrointestinal protists and helminths. Part of the results of our team work will be presented, divided into three model cases: (i) diversity of gastrointestinal ciliates and their association with the diet; (ii) diversity of malaria in lowland gorillas and possible transmission of P. ovale; and (iii) the exchange of GI nematodes between indigenous people and lowland gorillas. Presented data are outcomes of HPI-lab, co-financed from European Social Fund and state budget of the Czech Republic (project OPVK CZ.1.07/2.3.00/20.0300)

The role of microRNAs in the B cell receptor signalling and microenvironmental interactions in B cell malignancies

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We and others have shown that expression of certain miRNAs associates with disease activity in B cell malignancies (Calin et al. NEJM, 2005; Mraz et al. Blood, 2012; Mraz et al. Leukemia, 2009). However, the functional significance of miRNAs is largelly unknown. Recently, we have demonstrated that miRNAs regulate B cell receptor /BCR/ signalling pathway in malignant B cells, and thus contribute to the onset and progression of these malignancies. We will review and discuss these observations and novel data suggesting that non-coding RNAs contribute to other microenvironmental interactions in normal and malignant B cells.

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Alteration of chaperones in cancer

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Molecular chaperones (heat-shock proteins, Hsps) are proteins that maintain intracellular homeostasis through folding and stabilization of conformation of other proteins. Molecular chaperones are critical for survival of cells that undergo cellular stress due to their ability to guard the proteome against misfolded proteins and aggregation. In addition to their canonical role in basic cellular homeostasis and protection against external stress, several molecular chaperones play fundamental role in malignant cell transformation. The level of molecular chaperones is increased in many solid tumours and haematological malignancies. The increased activity of Hsps in cancer cells reflects the ability of chaperones to compensate the stress caused by hypoxia, increased protein turnover and presence of numerous mutated unstable proteins. In addition, chaperone molecules allow tumour cells to tolerate genetic alterations by stabilizing tertiary structure of mutated unstable proteins – typically oncoproteins that would otherwise be lethal. From this perspective, chaperones mediate the phenotypic expression of oncogenic mutations and contribute to all hallmarks of cancer cell.

The work was supported by the Czech Science Foundation (Projects No. P206/12/G151 and P301/11/1678) and by the European Regional Development Fund and the State Budget of the Czech Republic (RECAMO, CZ.1.05/2.1.00/03.0101).

Control of transcription elongation by pol II CTD kinases

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Transcription through early-elongation checkpoints requires phosphorylation of negative transcription elongation factors (NTEFs) by the cyclin-dependent kinase (CDK)9. Using CDK9 inhibitors and global run-on sequencing, we have mapped CDK9-sensitive checkpoints genome-wide in human cells. Our data indicates that early-elongation checkpoints are a general feature of RNA polymerase (pol) II-transcribed human genes and occur independently of polymerase stalling. Pol II that has negotiated the early-elongation checkpoint can elongate in the presence of inhibitors but, remarkably, terminates transcription prematurely close to the terminal polyadenylation (poly(A)) site. Our analysis has revealed a hitherto-unsuspected poly(A)-associated elongation checkpoint, which has major implications for the regulation of gene expression. Interestingly, the pattern of modification of the carboxyl-terminal domain (CTD) of pol II terminated at this novel checkpoint largely mirrors the pattern normally found downstream of the poly(A) site, suggesting common mechanisms of termination.

Lab-on-a-Chip for Point of Care Applications

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An LOC system suitable for point-of-care applications will be presented. It is portable, battery-operated, application-specific lab-on-a-chip (ASLOC) system that can be easily configured for a wide range of Lab-on-a-Chip applications. The system is based on multiplexed electrical current detection, which serves as the sensing system. I will show this LOC in different configurations such as PCR, LSPR, nanowires measurement, and electrochemical measurement. The complete system is controlled by a single chip and the collected information is stored in situ, with the option to transfer the data to an external display using an USB interface. In addition to providing a framework for truly portable real-life developments of LOC systems, we envisage that this system will have a significant impact in education especially as it can demonstrate the benefits of integrated microanalytical systems. Also a world smallest and cheapest real-time PCR system derived from the same basic platform will be shown.

Assembling the DNA damage checkpoint machinery in *S.pombe*

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The BRCT-domain protein, Rad4^{TopBP1}, facilitates activation of the DNA damage checkpoint in *S. pombe* by physically coupling the Rad9-Rad1-Hus1 clamp, the Rad3^{ATR} -Rad26^{ATRIP} kinase complex and Crb2^{53BP1} mediator. We have now determined crystal structures of the BRCT repeats of Rad4^{TopBP1}, revealing a distinctive domain architecture, and have characterized their phosphorylation-dependent interactions with Rad9 and Crb2^{53BP1}. We identify a cluster of phosphorylation sites in the N-terminal region of Crb2^{53BP1} that mediate interaction with Rad4^{TopBP1}, and reveal a hierarchical phosphorylation mechanism in which phosphorylation of Thr215 and Thr235 promotes phosphorylation of the non-canonical Thr187 site by scaffolding CDK recruitment. Finally we show that simultaneous interaction of a single Rad4^{TopBP1} molecule with both Thr187 phosphorylation sites in a Crb2^{53BP1} dimer, is essential for establishing the DNA damage checkpoint.

Magnetic carriers in biosensing and bioassays

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The lecture will be focused on one of the most promising materials for bioassays: the magnetic carriers[1]. Based on the concept of magnetic bioseparations, biologically modified magnetic particles offer some new attractive possibilities in biomedicine and bioanalysis since their size is ranged from few nanometers to micrometers comparable to those of cells. Moreover, they can be coated with a broad range of biological molecules (for instance DNA[2], antibodies[3], bacteriophages[4], among others), and they can also be manipulated by an external magnetic field gradient. As such, the biomaterial, specific cells[5] (as shown in Figure 1), proteins or DNA, can be selectively bound to the magnetic particles and then separated from its biological matrix by using an external magnetic field. Moreover, magnetic particles of a variety of materials and sizes, and modified with a wide variety of surface functional groups, are now commercially available. They have brought novel capabilities to electrochemical immunosensing[6, 7]. The magnetic particles have also been used in novel electrochemical genosensing protocols[8]. These approaches using magnetic particles have been combined with different strategies for the electrochemical or optical detection, such as label-free genosensing[9] or different external labels, such as enzymes[2-4], electrochemical indicators[10] or metal tags, e.g. gold or silver nanoparticles[11], and using different electrochemical techniques, such as DPV, potentiometric stripping analysis (PSA) or square wave voltammetry (SWV).

Instead of the direct modification of the electrode surface or the microplate, the biological reactions (as immobilization, hybridization, enzymatic labeling or affinity reactions) and the washing steps can be successfully performed on magnetic particles. After the modifications, the magnetic particles can be easily captured by magnetic forces onto the surface of the microplate or the GEC electrodes holding a small magnet inside (m-GEC). The application of these bioassays ranges from food safety (detection of food contaminants such as antibiotics[12], pesticides[6], and bacteria[3, 4], additives or food allergens[13]) to clinical bioassays for the diagnosis of infection diseases such as malaria[14], dengue, AIDS or TBC.

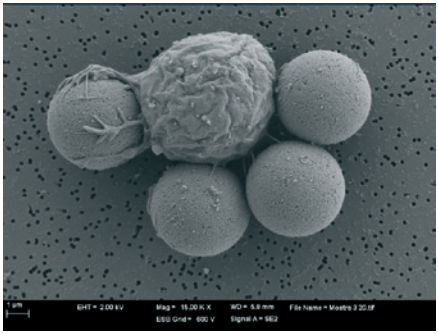

Figure 1. Evaluation of the immunomagnetic separation of CD4+ T lymphocytes cells from whole blood on magnetic particles by scanning electron microscopy. Acceleration voltage (15 KV) was used (Carinelli et al., unpublished material).

[1] Haukanes BI and Kvam C, 1993. Biotechnology 11:60–3
[2] Lermo A, Campoy S, Barbé J, Hernández S, Alegret S, Pividori MI, 2007. Biosens Bioelectron 22:2010-7
[3] Liébana S, Lermo A, Campoy S, Barbé J, Alegret S, Pividori MI, 2009. Anal Chem 81:5812–20
[4] Liébana S, Spricigo DA, Cortés MP, Barbé J, Llagostera M, Alegret S Pividori MI, 2013. Anal Chem. 85:3079-86
[5] Carinelli S, Xufré Ballesteros C, Martí M, Alegret S, Pividori MI, in preparation
[6] Zacco E, Pividori MI, Alegret S, Galve R, Marco MP, 2006. Anal Chem 78:1789-98
[7] Dequaire M, Degrand C, and Limoges B, 1999. Anal. Chem. 71:2571–7
[8] Palecek E, and Jelen F, Crit. Rev. Anal. Chem. 2002, 32, 261–270
[9] Wang J, and Kawde AN, 2002. Electrochem. Commun, 4:349–352
[10] Palecek E, Billova S, Havran L, Kizek R, Miculkova A, and Jelen F, 2002. Talanta 56:919–30
[11] Wang J, Xu D, and Polsky R, 2002. J. Am. Chem. Soc. 124:4208–9
[12] Lermo A, Fabiano S, Hernández S, Galve R, Marco MP, Alegret S, Pividori MI, 2009. Biosens Bioelectron 24, 2057–63
[13] Laube T, Kergaravat SV, Fabiano SN, Hernández SR, Alegret S, Pividori MI, 2011. Biosens Bioelectron 27:46-52
[14] De Souza Castilho M, Laube T, Yamanaka H, Alegret S, Pividori MI, 2011. Anal Chem 83:5570-7

Mechanisms of virus genome delivery into host cells

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Delivery of genome into a host cell cytoplasm is a necessary step in initiation of virus infection. This process involves release of the genome from virion and transfer of the nucleic acid across cytoplasmic membrane. Here we present cryo-electron microscopic study of the genome-injection process of bacteriophage phi812 that is a putative anti-Staphylococcus therapy agent. Furthermore, we used specific nano-gold labeling to identify genome release pore in human rhinovirus and honeybee virus.

Atomic force microscopy as a tool to study mechanobiological properties of human cardiomyocytes

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Stem cell-derived cardiomyocytes (SC-CMs) can be differentiated in vitro from human embryonic stem cells (hESCs) and human induced pluripotent stem cells (hiPSCs). Traditional suspension method for 3D cluster formation, so called embryoid bodies (EBs) results in heterogeneous EB population. Formation of EBs through forced aggregation of the cells allows controlling initial number of SCs and obtaining highly uniform EBs in size and shape.

Using a defined number (2000) of undifferentiated single cells in AggreWell plates, we formed homogeneous EBs in size and shape. The differentiation process was achieved using defined growth factors at different stages to enhance mesodermal differentiation and production of cardiac progenitors.

Atomic force microscopy was performed in Tyrode's solution and drugs modulating the beta-adrenergic receptors were applied as well as caffeine.

Contrary to suspension method, the forced aggregation using AggreWell plates allows production of highly uniform-sized EBs with high percentage of contracting clusters. Produced hESC-CMs and hiPSC-CMs expressed cardiac specific markers. Using atomic force microscopy-based technique, we measured the beating properties of hESC-EBs and hiPSC-EBs. A slower beat rate in hESC-CMs was obtained when compared to hiPSC-CMs (51 ± 5 vs 74 ± 7 bpm), while contracting force remained similar (31 ± 7 vs 39 ± 9 nN). Both cell types respond comparably upon stimulation or inhibition of beta-adrenergic pathway and caffeine.

Our results indicate that the mechano-biological properties of homogenous beating EBs, containing SC-CMs can be investigated by atomic force microscopy and be used to test drugs on 3D homogeneous syncytium containing beating human cardiomyocytes.

References

[1] Pesl M, Acimovic I, Pribyl J, Hezova R, Vilotic A, Fauconnier J, Vrbsky J, Kruzliak P, Skladal P, Kara T, Rotrekl V, Lacampagne A, Dvorak P, Meli AC. Forced aggregation and defined factors allow highly uniform-sized embryoid bodies and functional cardiomyocytes from human embryonic and induced pluripotent stem cells. *Heart Vessels*. 2013
[2] Liu J, Sun N, Bruce MA, Wu JC, Butte MJ (2012) Atomic Force Mechanobiology of Pluripotent Stem Cell-Derived Cardiomyocytes. PLoS ONE 7(5): e37559.

Novel nano-optical tools for the detection and manipulation of biomolecules

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Nanoscale control of plasmonic fields in engineered metal nanostructures offers unique opportunities to boost the interaction of light with tiny amounts of matter, down to the molecular level. In this talk we discuss how such enhanced interaction can benefit the detection and non-invasive manipulation of biomolecules.

We first present an integrated analytical platform that combines the sensing capability of plasmonic antennas with advanced microfluidic technologies for the label-less detection of protein cancer markers in blood. Our platform offers parallel, real-time inspection of tens of sensing sites distributed across independent microfluidic channels with very high reproducibility. This enables us to test various sensing strategies for the detection of biomolecules. In particular we demonstrate the fast detection in human serum of relevant cancer biomarkers (AFP and PSA) down to concentrations of 500 pg/m.

A second technology exploits the optical properties of gold nanostructures to optically trap and 3D-manipulate single specimens as small as 10nm. In particular, a nano-optical trap is built by engineering a bowtie plasmonic aperture at the extremity of a tapered metal-coated optical fiber. Both the trapping operation and monitoring are performed through the optical fiber, making these nanotweezers totally autonomous and free of bulky optical elements. The achieved trapping performances allow for the trapped specimen to be moved over tens of micrometres over a period of several minutes with very low in-trap intensities.

Interactions of Salmonella with its host

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Chickens can be infected with *Salmonella enterica* at any time during their life. However, infections within the first hours and days of their life are epidemiologically the most important, as newly hatched chickens are highly sensitive to *Salmonella* infection. *Salmonella* is initially recognized in the chicken caecum by TLR receptors and this recognition is followed by induction of chemokines, cytokines and many effector genes. This results in infiltration of heterophils, macrophages, B- and T-lymphocytes and changes in total gene expression in the caecal *lamina propria*. The highest induction in expression is observed for matrix metalloproteinase 7 (MMP7). Expression of this gene is increased in the chicken caecum over 4000 fold during the first 10 days after the infection of newly hatched chickens. Additional highly inducible genes in the caecum following *S.* Enteritidis infection include immune responsive gene 1 (IRG1), serum amyloid A (SAA), extracellular fatty acid binding protein (ExFABP), serine protease inhibitor (SERPINB10), trappin 6-like (TRAP6), calprotectin (MRP126), mitochondrial ES1 protein homolog (ES1), interferon-induced protein with tetratricopeptide repeats 5 (IFIT5), avidin (AVD) and transglutaminase 4 (TGM4). The induction of expression of these proteins exceeds a factor of 50. Similar induction rates are observed also for chemokines and cytokines such as IL1β, IL6, IL8, IL17, IL18, IL22, IFNγ, AH221 or iNOS. Once the infection is under control, which happens approx. 2 weeks after infection, expression of IgY and IgA increases to facilitate *Salmonella* elimination from the gut lumen. This review outlines the function of individual proteins expressed in chickens after infection with non-typhoid *Salmonella* serovars.

Intravital imaging reveals how BRAF inhibition generates drug tolerant microenvironments with high integrinβ1/FAK signaling

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We use intravital imaging of BRAF-mutant melanoma cells containing an ERK/MAPK biosensor to study how the tumor microenvironment affects response to BRAF inhibition by PLX4720. Initially all melanoma cells respond to PLX4720, but rapid reactivation of ERK/MAPK is observed in areas of high stromal density. Melanoma-associated fibroblasts (MAFs) are sufficient to induce ERK/MAPK reactivation following PLX4720-treatment. This is linked to 'paradoxical' activation of MAFs by PLX4720 and the promotion of matrix remodeling leading to elevated integrinβ1/FAK/Src signaling in melanoma cells. The activation of this signalling pathway provides a BRAF independent mechanism for ERK activation. Consistent with these results, co-inhibition of BRAF and integrinβ1/FAK signaling abolished ERK compensation and effectively induced cell death in melanoma cells *in vitro* and suppressed tumor growth *in vivo*. We observe that this mechanism enables the survival of residual disease even in melanoma models that exhibit a clear response to PLX4720. Further, changes in the fibroblastic stroma and ECM are observed in Vemurafenib-treated melanoma patients with progressive disease. We propose that paradoxically activated MAFs provide a 'safe haven' for melanoma cells to tolerate BRAF inhibition. This environment can then foster the emergence of genetically resistant melanoma.

Spark plasma sintering: advantages and limitations

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Spark plasma sintering as a unique technique for rapid densification of materials has been widely investigated during the last two decades. The intension of this presentation is not to give a fully review of this technique but to illustrate its advantages and limitations. Under a uniaxial pressure higher than what normally applied during conventional hot-press process what occurs during spark plasma sintering is not sintering by definition. The benefits brought in by the applied pressure should not be underestimated. Pressure promotes efficient particles sliding that favours the particles close packing thus rapid densification and sintering involves the final stage only. This obvious advantage generates further more advantages that encounter avoiding grain growth during densification thus the preparation of nanomaterials, retarding chemical reactions thus the preparation of composites or hybrid materials consisting of phases far away from thermodynamic equilibrium, or stimulate in-situ chemical reactions thus the preparation of dense materials of hard-to-sinter type. Another advantage of SPS is the efficient heat transfer occurring during this process, which favours the grain growth by dynamic ripening, or by superplastic deformation induced directional dynamic ripening. The limitations of the SPS process related also to the application of a uniaxial pressure, which sets the limit that only components with simple cylindrical geometries can be prepared, and the process is batch type. The demands of a combination of good electrical and thermal conductivity of the die and punches at high temperature that can withstand the applied uniaxial pressure sets another limitation that graphite contamination and partially reduced atmosphere are common for this process.

Morphogenetically active inorganic polymers for bone tissue engineering

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The development of rapid prototyping (RP) techniques has opened new possibilities not only in a variety of technical areas, but also in the biomedical field, in particular for the fabrication of custom-made scaffolds for bone regeneration and repair. However, there is an urgent need for suitable materials that can be introduced in the RP chain to enable a fast regeneration of bone and its integration in both the hard and soft tissues. Research on the lowest multicelluar organism, the sponges, revealed that Nature might offer such a material: biosilica, an enzymatically formed inorganic biopolymer consisting of a network of polycondensated silica units. This bio-inorganic material, biosilica, turned out to be morphogenetically active, i.e. it acts as inducer of a number of cytokines and growth factors that are involved in differentiation and control of the activity of cells that are involved in bone anabolism and catabolism, and also has self-adapting / self-repairing properties. Moreover, this bio-polymer can be used, together with a hydrogel, prepared of Na-alginate, as a matrix for 3D printing of bone-forming cells. Our results revealed that embedding of osteoblast-like SaOS-2 cells in a biosilica alginate hydrogel matrix not only maintains cell viability and supports cell growth but also causes the expression of genes that facilitate the release of the entrapped cells and of osteogenetically active silicate. In addition, another inorganic bio-polymer that can be introduced into the RP chain has turned out to be a promising candidate for a morphogeneticaly active scaffold material, polyphosphate. Both inorganic bio-polymers can be applied for preparation of customized materials, either alone or in combination, taken advantage of the partly complementary spectrum of biological activities of these polymers. Both polymers proved not only to positively and favorably influence osteoblasts growth and function, but also to be biocompatible and biodegradable, and finally they can be totally replaced by the body's own bone material.

References:

Neufurth M, Wang XH, Schröder HC, Diehl-Seifert B, Steffen R, Wang SF, Müller WEG. Biomaterials 35:8810-8819 (2014)
Schröder HC, Wang XH, Wiens M, Diehl-Seifert B, Kropf K, Schloßmacher U, Müller WEG. J Cell Biochem 113:3197-3206 (2012)
Wang XH, Schröder HC, Müller WEG. Trends Biotechnol 32:441-447 (2014)
Wang XH, Schröder HC, Schlossmacher U, Neufurth M, Feng QL, Diehl-Seifert B, Müller WEG. Calcif Tissue Int 94:495-509 (2014)
Wang S, Wang XH, Draenert FG, Albert O, Schröder HC, Mailänder V, Mitov G, Müller WEG. Bone 67:292-304 (2014)

DNA double-strand breaks in barley are repaired by diverse pathways, primarily involving the sister chromatid

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DNA double-strand break (DSB) repair mechanisms differ in requirement of a homologous repair template and in the accuracy of the result. We aimed to quantify the outcome of repair of a single targeted DSB in somatic cells of young barley (Hordeum vulgare L.) plants. Amplicon sequencing of three reporter constructs revealed 47 to 58% of reads as repaired via non-homologous end-joining (NHEJ) with deletions and/or small (1-3 bp) insertions. Alternative NHEJ revealed 2 to 5 bp microhomology (15.7% of cases), or new replication-mediated short duplications at sealed breaks. Although deletions outweigh insertions in barley, this bias was less pronounced, and deleted sequences were shorter than in Arabidopsis. Between 17 and 33% of reads likely represent restoration of the original sequence. Depending on the construct, 20 to 33% of reads arose via gene conversion (homologous recombination). Remarkably, <1 to >8% of reads apparently display synthesis-dependent strand annealing linked with NHEJ, inserting 4 to 61 bp, mostly originating from the surrounding of breakpoints. Positional coincidence of >81% of sister chromatid exchanges with target loci is unprecedented for higher eukaryotes and indicates that most repair events for staggered DSBs, at least in barley, involve the sister chromatid, and occur during S or G2 phase of the cell cycle. As expected, a single DSB did not increase the frequency of chromosome aberrations.

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Coherence-controlled holographic microscopy: a new technique for quantitative phase imaging

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Nowadays new techniques of quantitative phase imaging (QPI) are emerging and trying to attract attention of biomedical microscopists. It is mainly due to label-free imaging that provides quantitative information about cell mass and low phototoxicity. Here we present coherence-controlled holographic microscopy (CCHM) a new technique for QPI. It is based on off-axis holography adapted for incoherent illumination. The off-axis setup allows for single-shot imaging while the use of incoherent illumination provides high quality of recorded phase images without halo artefact. Next important attribute brought about by incoherent illumination is a coherence-gating effect. This enables CCHM to image objects immersed in scattering media by utilizing the ballistic light only.

CCHM imaging capability in different coherence states of illumination and effect of coherence-gated imaging will be shown as well as examples of QPI recording of living cells dynamics.

Nanoparticle-enhanced SPR imaging detection of nucleic acids

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Most of currently available methods for nucleic acids detection require sequences to be identified to be previously amplified and labeled with fluorophores. Such procedures involve the use of expensive reagents and pitfalls may occur due to contaminations or matrix effects. In addition, high-specificity and multiplexed capability are essential properties required for new devices to be used in genomic DNAs and RNAs detection. Ultrasensitivity is also required in order to avoid the sample PCR amplification.
Efforts have been recently made in identifying innovative DNA detection protocols which can be performed without PCR.[1] Most of them exploit strategies for signal amplification based on the use of enzymes or metallic nanostructures. In particular, peculiar chemical and physical properties of gold nanoparticles (AuNPs) have been exploited to obtain an ultrasensitive DNA detection.[2, 3]
Surface plasmon resonance imaging (SPRI) has emerged as a powerful optical method able to investigate interactions with biomolecules arrayed onto chemically modified metal surfaces. SPRI enables biomolecular interactions to be monitored in parallel, in real-time and without labels.[4]
We have demonstrated that an ultrasensitive detection of non-amplified genetically modified organism soybean genomic DNA and SNPs in non-amplified human genomic DNAs carrying the mutated β°39-globin gene sequence can be obtained by combining a nanoparticle-enhanced SPRI platform with peptide nucleic acid (PNA) probes.[5, 6] PNAs are DNA mimics in which the negatively phosphate deoxyribose backbone is replaced by a neutral N-(2-aminoethyl)glycine linkage. They have been shown to be able to improve both selectivity and sensitivity in targeting complementary DNA and RNA sequences. Possibilities offered by the nanoparticle-enhanced SPRI methods in microRNAs detection have been also investigated.

[1] G. Spoto, R. Corradini [Eds.], Detection of Non-Amplified Genomic DNA, Dordrecht, Springer, 2012.
[2] L. M. Zanoli, R. D'Agata, G. Spoto. Functionalized gold nanoparticles for the ultrasensitive DNA detection. Analytical and Bioanalytical Chemistry 402, 2012, 1759– 1771.
[3] G. Spoto, M. Minunni. Surface Plasmon Resonance imaging: What's next? The Journal of Physical Chemistry Letters, 3, 2012, 2682–2691.
[4] R. D'Agata, G. Spoto. Surface Plasmon Resonance Imaging for Nucleic Acid Detection. Analytical and Bioanalytical Chemistry, 405(2-3), 2013, 573-584.
[5] R. D'Agata, R. Corradini , C. Ferretti, L. Zanoli, M. Gatti, R. Marchelli, G. Spoto. Ultrasensitive detection of non-amplified genomic DNA by nanoparticle-enhanced Surface-Plasmon Resonance Imaging. Biosensors and Bioelectronics 25, 2010, 2095- 2100.
[6] R. D'Agata, G. Breveglieri, L.M. Zanoli, M. Borgatti, G. Spoto, R. Gambari. Direct Detection of Point Mutations in Non-amplified Human Genomic DNA. Analytical Chemistry. 83(22), 2011, 8711-8717.

Novel selective plane illumination microscope (SPIM) to study mouse pre-implantation development

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Light sheet microscopy is a technique where optical sectioning is achieved by restricting the excitation light specifically to the focal plane by illuminating the specimen from the side with a thin sheet of light. Its main advantages compared to a confocal microscope are reduced light dose and therefore lower photo-toxicity as well as high imaging speed. Although several configurations of light sheet microscopes have been developed, none of them is compatible with the stringent culture conditions required for robust long-term in vitro culture of mouse pre-implantation embryos. To overcome this limitation we have designed and built a novel inverted light-sheet microscope where the specimen is completely isolated from the immersion medium. The sample can be mounted without embedding in agarose allowing us to use a standard micro-drop embryo culture. In addition, multiple embryos can be placed next to each other for high-throughput multi-position imaging. Using this microscope we have succeeded to image and track chromosomes and kinetochores during the first mouse embryonic divisions. In addition, we have imaged and tracked nuclei during the entire mouse pre-implantation development and analysed patterns of cell divisions in the in toto reconstructed early mouse embryo lineage.

Pathogen detection of grapheme-coated SPR surfaces

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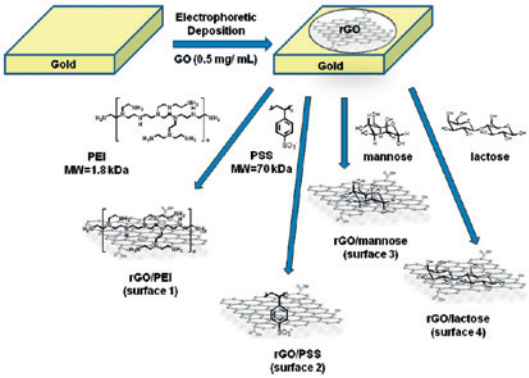
A variety of physical and chemical parameters are of importance for adhesion of bacteria to surfaces. In the colonization of mammalian organisms for example, bacterial fimbriae and their adhesins not only seek particular glycan sequences exposed on diverse epithelial linings, they also enable the bacteria to overcome electrostatic repulsion exerted by their selected surfaces. We have developed a novel experimental concept for the investigation of pathogen tropism in an easy manner. For this purpose, gold-based surface plasmon resonance (SPR) interfaces were coated with thin films of reduced graphene oxide (rGO) through electrophoretic deposition. The rGO matrix was post-modified with polyethyleneimine (PEI), poly(sodium 4-styrenesulfonate) (PSS), mannose, and lactose through ⑦-stacking and/or electrostatic interactions by simple immersion of the SPR interface into their respective aqueous solutions. The adhesion behaviors of one uropathogenic and two enterotoxigenic *Escherichia coli* clinical isolates, that each express structurally characterized fimbrial adhesins, were investigated. The strategy is however adaptable to any other bacteria and more specifically if one is interested in examining bacterial affinities to molecules on a surface and quantitative detection of pathogen in solution.



References

P. Subramanian, A. Lesniewski, I. Kaminska, A. Vlandas, A. Vasilescu, J. Niedziolka-Jonsson, E. Pichonat, H. Happy, R. Boukherroub, S. Szunerits, Biosensors and Bioelectronics, 2013 , 50, 239-243
Szunerits, S.; Maalouli, N.; Wijaya, E.; Vilcot, J. P.; Boukherroub, R., *Anal. Bioanal. Chem*. 2013,
P. Subramanian, F. Barka-Bouaifel, J. Bouckaert, N. Yamakawa, R. Boukherroub, S. Szunerits, ACS Mater.& Inter., 2014, 6, 5422-5431

Plasmonic antennas and nanocomposite particles for detection of biomolecules

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In the first part of the contribution the principles of localized surface plasmons (LSP) will be briefly discussed. Consequently, the sensing of biomolecules by plasmonic antennas utilizing LSP resonances will be explained and some examples given. In addition, selective determination of cysteine (Cys) using a CuO/ZnO nanoparticle nanocomposite as a dual colorimetric and fluorometric assay will presented.

Innovative therapies for chronic lymphocytic leukemia

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Chronic lymphocytic leukemia (CLL) is the most frequent leukemia affecting the adult people and still represents an incurable disease. Although a proportion of patients respond to initial therapy, relapse is virtually inevitable and frequently accompanied by the strong resistance to diverse types of treatment. Prognostic stratification is extremely important in this disease; the worst prognosis is connected with defects in the *TP53* gene and particularly *TP53* mutations are intensely selected by therapy. The current treatment options for *TP53*-affected patients are limited and involve only monoclonal antibody alemtuzumab or allogeneic stem cell transplantation. Also abnormalities in *ATM* gene (deletion 11q and/or mutations) are associated with unfavorable clinical course, namely large lymphadenopathies and short time to treatment. Experimental approach tested the inhibitor of poly-ADP ribose polymerase (PARP) and manifested promising effects in the *ATM*-affected CLL cells. The use of anti-CD20 based immunotherapy seems also to be a promising approach in *ATM* mutated patients, but this needs to be carefully tested in well characterized patients. The most anxiously awaited current drug, just entering the clinic, is ibrutinib targeting the signaling from B-cell receptor, which is critical for leukemia cells´ survival. Because also this drug has already been associated with primary or acquired resistance, further innovative treatment approaches are highly desirable. These may include e.g. targeting of DNA-damage response pathway with selective inhibitors acting on the principle of synthetic lethality or reactivation of mutated p53 protein to wild-type conformation. In any case, CLL still remains the disease which is sometimes probably better to let be as it is (if possible from the point of view of clinical symptoms) rather than to use an unpredictable and resistance-selecting therapy.

Strong nucleosomes: from chromatin to chromosome structure

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The strong nucleosomes (SNs) are discovered in genome sequences, which display visible strong periodicity in the nucleosome DNA. First, they confirm earlier established nucleosome positioning motif (RRRRRYYYYY)n, universal for plants, animals and yeasts. The SNs are accompanied by flanking sequences, strongly periodic as well, suggesting existence of columnar chromatin structure, also indicated by earlier studies. Both SNs and columns colocalize with centromeres. The SNs and columns also concentrate in matrix attachment regions and chromosome pairing centers. Since detection of SNs and columns is a simple computational procedure, the mapping of the SNs and columns becomes a new versatile tool of high potential for studying chromosome structure.

Childhood Leukaemia Investigation; Ontogeny of childhood acute lymphoblastic leukaemia

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The cause of acute lymphoblastic leukaemia, the predominant subtype of childhood malignancy, remains unknown. We have learned that major subtypes of this disorder originate in utero and evolve into the overt disease in a series of genetic events. The natural history of the leukaemic clone development involves the first prenatal hit, survival of the preleukaemic clone for years between its origin and the second hit impact leading to the overt leukaemia and possibly beyond. The evidence collected from classical observations, animal models and modern genomic data uncover the nature of the first and second steps of the leukaemogenesis and the fate of the preleukaemic clone.

Analgesics as rewards and the relation to ongoing pain: positive and negative reinforcement mechanisms

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Preclinical pain research focuses on evoked pain in animals. This is defined by the occurrence/increase of specific nocifensive behaviors, induced by thermal, tactile or electric stimuli. However, pain does not only hurt, but it is also unpleasant and aversive, i.e. is has an affective component. Furthermore, pain can occur spontaneously (i.e. non-evoked), which is a major problem clinically, but cannot be easily measured in standard pain models.

The main drawbacks of classical animal readouts of pain are that only a simple behavioral response to stimulation is monitored, these models have poor face validity for chronic pain states, and they have poor clinical predictivity. Thus, what is needed are outcome measures that reflect the complexity of clinical symptoms; they should measure spontaneous or ongoing pain and the readouts should involve higher brain centers and reflect the complexity of the pain phenotype in humans.

One approach that has been introduced recently makes use of the fact that spontaneous pain produces a tonic aversive state providing motivation that shapes behavior (in other words, "taking pain away feels good"). Thus, analgesic drugs that are not rewarding in the absence of pain become rewarding in the presence of chronic pain. Likewise, reward can be demonstrated at sites in the nervous system that are not a part of the reward circuit such as spinal cord or RVM. This reward can be assessed by demonstrating conditioned place preference (CPP) selectively in animals with ongoing pain. These principles have been demonstrated for a number of different pain states in the meantime. However, unfortunately, a number of groups have also failed to reproduce these findings. Thus, it is not yet unequivocally established to what extent this approach is valid, predictive and generalizable.

An alternative approach is to focus on the affective dimension of pain. Because pain is aversive, a discrete painful stimulus will produce a conditioned place aversion (CPA). Interestingly, many analgesic drugs, in particular opioids, have a greater potency regarding the reduction of the affective component of pain (CPA) as compared to the sensory component (e.g. in the paw pressure test). An additional interesting finding is that opioids have a reduced rewarding potential in animals with pain, as compared to animals without pain.

In summary, the new approaches to model the complexity of painful states in animals outlined here could contribute to a much needed improvement of predictive validity and translatability of preclinical pain models and thus to a more successful pharmaceutical development of analgesic drugs.

The future of structural materials: Teachings from nature

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Nature is replete with structures of various kinds and is potentially teaching materials scientists valuable lessons in terms of structure sophistication and ensuing mechanical property optimization. In this lecture I focus on a number of related questions: How does nature generate materials and structures with superior mechanical properties (or: what are nature's 'tricks')? Can biological materials, structures, and principles be easily adapted and applied to potential future synthetic structures and applications? Can lessons learned from hierarchical (from the nano-scale to the macro-scale) biological materials be easily applicable to the design of new engineering materials? An interesting case concerns the ubiquity of composite materials in nature and we will examine possible correlations between composite structure and mechanics by means of our current experimental work with turtle carapaces, dentin, enamel, antler, and more.

Recombination - genetic instability versus genome maintenance

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Recombination processes are key to repairing DNA breaks and thus genome stability of living cells. Repair can however also lead to genome reorganisation and a striking example of this is seen in the recombination of deprotected chromosome ends, initiating chromosomal breakage-fusion-bridge cycles which lead to severe genomic instability and potentially cell death or cancer. Loss of telomeric cap structures thus causes the ends of linear eukaryotic chromosomes to recombine and fuse with dramatic consequences to the genome, the cell and the organism. But what would be the fate of these deprotected chromosome ends if they are unable to recombine? Would they instead be eroded by nucleases, also leading to loss of genes and cell death? Or something else?

To answer these questions, we have analysed the involvement of multiple end-joining recombination pathways in telomere fusions and the consequences of this on genomic instability and growth of the flowering plant, *Arabidopsis thaliana*. Strikingly, the absence of the multiple end-joining pathways eliminates chromosome fusion and restores normal growth and development to cst mutant plants. It is thus the chromosomal fusions, *per se*, which are the underlying cause of the severe developmental defects. Further analyses show that this rescue is mediated by telomerase-dependent telomere extension, revealing a competition between telomerase and end-joining recombination proteins for access to deprotected telomeres.

Amiard, S., Olivier, M., Allain, E., Choi, K., Smith-Unna, R., Henderson, I., White, C., and M.E. Gallego. (2014). Telomere stability and development of ctc1 mutants are rescued by inhibition of EJ recombination pathways in a telomerase-dependent manner. **Nucleic Acids Research:** in press.

4D imaging of cancer cell invasion in vitro and in vivo

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Tumor cell migration through 3D tissue depends on a physicochemical balance between tissue constraints and deformability of cell and nucleus, respectively, and is further governed by integrin- and actomyosin-mediated traction and contact-dependent ECM degradation mediated by matrix metalloproteinases (MMPs). We here dissect the relative contributions of these parameters under conditions of space confinement. Using MMP-degradable collagen lattices or non-degradable substrates of varying porosity, we quantitatively identify the limits of cell migration by physical arrest. MMP-independent migration declined as linear function of pore size and deformation of the nucleus, with arrest reached at 10% of nuclear cross-sections. Residual migration under space restriction strongly depended upon MMP-dependent ECM cleavage by enlarging matrix pore diameters, and integrin- and actomyosin-dependent force generation, which jointly propelled the nucleus. To examine the effect of overall nuclear deformability on migration rates through spatially confined substrates, lamin A/C was either transiently knocked down or stably overexpressed yielding reduced and enhanced stiffness values of the nucleus, respectively, as detected by atomic force microscopy. Whereas lamin knockdown significantly enhanced migration rates through both collagen lattices and transwell membranes of 15-20 μm^2 pore cross sections, equal migration efficacy and thus 'physical rescue' was reached at pore sizes of 50-60 μm^2, approximating undeformed nuclear cross sections. Vice versa, tumor cells overexpressing lamin A/C migrated at highly reduced speed through dense lattices which was, again, rescued in substrates with pore sizes matching the nucleus. Thus, the efficacy and limits of interstitial cell migration depend upon scaffold porosity and deformation of the nucleus, with pericellular collagenolysis and mechanocoupling as modulators. – Whereas the aforementioned tumor migration mechanisms were established in vitro, there are principally valid as well for invasion mechanism in tissues in vivo which provide spatial confinement, together with guidance, for migrating cells. During this talk examples of dynamic tumor cell invasion patterns along available space in the living mouse tissue will be provided.

Potential of quantitative interferometry in personalised cancer treatment

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In western civilisation, two out of five people die of cancer and the main cause of death in the cancer patients is invasion and metastasis. While molecular signatures of malignant cancer cells are complex, their behaviour is well defined by increased growth and motility. Both of these features can be rapidly and accurately measured by interferometry with tissue culture cells. Moreover, this approach can be used for pre-testing chemotherapeutical drugs with biopsy cells. We have been using a double beam transmitted-light interference Leitz microscope (Horn design) equipped with twin objective lenses and a stepper motor operating an optical wedge compensator in phase shifting mode. Using Micromanager, we developed new image acquisition/ hardware control and have also developed novel image processing algorithms to produce accurate distributions of dry mass inside tissue culture cells, calibrated in pg/μm^2. Recently we evaluated different approaches to phase unwrapping and applied cross-correlation analysis to cell protrusion and retraction. The technique has been applied to cells of murine and human origin subjected to treatments with chemotherapeutic agents and significant differences in growth and motility were readily detectable. We have also established a clear correlation between metastatic potential in vivo and motility in vitro using a sarcoma model in inbred rats.

POSTERS

1. Mechanism of hydrogen subtraction from methylphenylsilane during plasma induced fragmentation process **A. Alsheikh**
2. Self-administration of the CB_1 receptor agonist WIN55, 212-2 is enhanced in depressive-like rats **P. Amchová**
3. Mutagenesis during plant response to UV-B radiation. **K. J. Angelis**
4. Microstructural changes in gray matter of alpha synuclein overexpressing transgenic mouse model of parkinson's disease: a diffusion weighted imaging study **A. Arab**
5. Detection of anti-HCMV T cell response of potential recipients and donors in cellular immunotherapy of leukemic patients after transplantation of hematopoietic stem cells **K. Babiarová**
6. Tailoring the optical plasmonic resonances by the shape of metallic dipole-walls **J. Babocký**
7. Holographic microscopy coupled with fluorescence detection as a tool for analysis of aggressive metastatic prostate cancer cell death **J. Balvan**
8. Investigation of the ammonia sensors based on carbon nanotubes **A. G. Bannov**
9. Dealing with noise in psychophysiological interaction analysis **M. Bartoň**
10. Dielectrophoresis for Manipulation of Droplets in Microfluidic Integrated System **E. Y. Basova**
11. Microgels for multiplex and direct detection of miRNA **E. Battista**
12. Electromechanical Label-Free Detection of Prostate Cancer Biomarkers **Š. Belický**
13. The role of auxin transport in plant stress adaptation **A. Bielach**
14. Fabrication of Microfluidic Chips for Liquids Manipulation and Optical Spectroscopy **J. Borovský**
15. Detection of foodborne pathogens based on immunomagnetic separation and electrochemical magneto-genosensing **D. Brandao**
16. Whole genome sequencing of multidrug-resistant *Klebsiella pneumoniae* strains **E. Brhelová**
17. Kinetics Study of D, L-lactide and Glycolide Copolymerization on PEG Initiator by Novel Gas Chromatography Procedure **J. Brtníková**
18. Morphogenesis and viscoelastic properties of dental dimethacrylate networks **Z. Bystřický**
19. Biosensor for AIDS diagnostics and monitoring **S.Carinelli**
20. Nano-scale derivatization followed by capillary electrophoresis with fluorescence detection **A. Celá**
21. Rheological Study of PLGA-PEG-PLGA/hydroxyapatite core-shell nano-particles in thermosensitive copolymer matrix **I. Chamradová**
22. Structural insights into the aberrant splicing of CFTR exon 9. **M. Y. Chou**
23. Self-assembled, 1, 2-thiomannobiosiode containing glycoconjugates as potent ligand of *Burkholderia cenocepacia* lectins **M. Csávas**

24. Titanium oxide based anode materials for li-ion batteries **O. Čech**
25. Decoration of flexible metal-organic coordination networks by reactive Ni atoms at surfaces **J. Čechal**
26. Thermal performance analyzing of hollow microspheres coatings for building energy efficiency **M. Čekon**
27. Identification of microRNAs involved in DNA damage response in malignant B cells and their biological and clinical relevance **K. Černá**
28. Dynamic Phase Differences Method for the Assessment of Cellular Dynamic Processes **J. Čollaková**
29. SPR Strategy in Prostate Cancer Glycobiomarker Research **P. Damborský**
30. Image-based Automated Detection of First-episode Schizophrenia: The Importance of Spatial Resolution of Morphological Features and Ensemble Learning **P. Dluhoš**
31. Dissemination of beta-lactam and quinolone resistance genes mediated by IncX plasmids in *Enterobacteriaceae* of diverse sources and origins **H. Dobiášová**
32. The Analytical Centrifuge LUMiSizer as a Useful Tool for the Characterization of Nanoparticles **L. Doskočil**
33. The Effect of Crocin and Fluoxetine on CYP2C6 in vivo Metabolic Activity and Its Protein Levels in Rat Liver Microsomes **G. Dovrtělová**
34. Dynamics of Telomeres and rDNA in *Arabidopsis* Chaperone Mutants **M. Dvořáčková**
35. Interference of surface plasmon polaritons on gold films in the optical near-field **P. Dvořák**
36. Effect of naturally occurring lesions in the human telomere DNA on its quadruplex structure and stability **Z. Dvořáková**
37. Self-pulsing and chaos in coupled ring resonators with non-instantaneous Kerr-nonlinear response **Y. Ekşioğlu**
38. Numerical Simulation of Thermoelectric Effect Spectroscopy and Thermal Stimulated Current methods **H. Elhadidy**
39. ROS-Auxin interplay in regulation of photosynthesis under stress **E. Elrefaay**
40. Physicochemical and Spectroscopic Characterization of Humic and Fulvic acids Isolated from South-Bohemian Peat **V. Enev**
41. Piezoelectric Immunosensor for Detection of Aerosolized Microorganisms **Z. Farka**
42. Telomere dynamics in the *Physcomitrella patens* model plant **M. Fojtová**
43. Explore the coevolution mechanism through Host-Parasite RNA experimental evolution **T. Furubayashi**
44. Tin Dioxide – Multiwalled Carbon Nanotubes Based Gas Sensor for Isobutene Detection **I. Gablech**
45. Influence of underlying network structure on accuracy of DCM estimation **M. Gajdoš**
46. The Case of Breast Cancer Resistance Protein in the GL261 Glioma Cells **M. Hampl**
47. MARs contain strong nucleosomes **J. Hapala**
48. Application of benzofurazane and nitrophenyl redox labels in electrochemical analysis of DNA nucleotide sequences **L. Havran**
49. Thin Chalcogenide Films - Promising Materials for Optical Storage **M. Hejdová**
50. A new electrochemical DNA binding competition assay using oligonucleotide substrates tail-labeled with two different redox tags **M. Hermanová**
51. What determines chromosome breakpoints in crucifer species? **P. Hloušková**

52. Using of microsatellite markers for analysis of functionally important genetic diversity in horses **E. Horecká**
53. Study of *Ly49* genes diversity in the horse using microsatellites **Č. Horecký**
54. Interactions between receiver domain of cytokinin receptor CKI1RD and AHP protein from Arabidopsis thaliana **D. Hrebík**
55. The Investigation of Mechanical Properties the Synthetic Mineral Alloys in Conditions of Static Loading **A. M. Ignatova**
56. The development of Theory of mind from middle childhood into adolescence **M. Jáni**
57. Modeling of the N-Acetylglucosaminyltransferase V (GnT-V) **P. Janoš**
58. Synthesis of carbon nanosheets using microwave plasma torch at atmospheric pressure **O. Jašek**
59. Reactivation of Mutated p53 in Chronic Lymphocytic Leukemia Cells Using Prima-1^{MET} **Z. Jašková**
60. Study of Copper Substrate Pretreatment During CVD Growth of Graphene **P. Jelínek**
61. Structural basis for the dual role of Rtt103p in transcription termination and RNA processing **T. K. Kabzinski**
62. Light Scattering Techniques Applied for the Study of Aging of Biopolymers and Biocolloids **M. Kalina**
63. The role of surface stress in phase diagrams of Ni –Sn–Sb nanoalloys **T. Káňa**
64. Photocatalytic water splitting by Ln-doped TiO2 (Ln = Pr, Dy, Sm) **V. Kašpárek**
65. Porous aromatic organosilicates by non-hydrolitic sol-gel route **M. Kejík**
66. A Longitudinal Diffusion Kurtosis Imaging Study in TNWT-61 Transgenic Mouse Model of Parkinson´s Disease: Comparison with Conventional Diffusion Tensor Imaging **A. Khairnar**
67. Improvements in the method for measuring enzyme kinetics of β-glucosidases **P. Klimeš**
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1. Mechanism of hydrogen subtraction from methylphenylsilane during plasma induced fragmentation process

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Silicon atoms support binding of organic, polymers and biomaterials to inorganic substrates including glass, or ceramics. In that case the bio- or organic phase must be attached to inorganic surface in controlled way. Plasma enhanced chemical vapor deposition or plasma assisted chemical vapor deposition allow us using methylphenylsilane (MPS) as a compatibilization agent. The plasma deposition is accompanied by fragmentation of molecule. The composition of fragments is important parameter for reactivity of inorganic surface. The contribution will be focused to subtraction of two hydrogen atoms during fragmentation process. Two hydrogen atoms are usually dissociated in two consecutive steps. We note that fragmentation of some selected hydrogens from MPS molecule is not in accord with the simple two-steps mechanism. It seems that subtraction of two hydrogen atoms (2H) is even quicker than the subtraction of one single hydrogen atom only. The effect was observed in mass spectrum of MPS. Subtraction of 2H in the other cases follows standard two-steps mechanism. We propose a method which can predict the expected mechanism of 2H subtraction. We found that energy profile of mechanism calculated by density functional theory (DFT) is different for the cases, where the subtraction of 2H is irregular and for the cases where it runs in standard way.

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2. Self-administration of the CB_1 receptor agonist WIN55, 212-2 is enhanced in depressive-like rats

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Depression has been associated with drug consumption, including heavy or problematic cannabis use. Self-medication theory, a possible explanation for the drug abuse in depressed patients, has been hypothesized on the basis of the chemical and molecular changes in several neurotransmitter systems, including the dopaminergic one, which is a major component of the brain reward system. In addition, cannabinoid self-administration was shown to be associated to an increased dopamine (DA) transmission in the shell of the *nucleus accumbens* (NAc). According to an animal model of depression and substance use disorder comorbidity, we combined the olfactory bulbectomy model of depression with intravenous drug self-administration procedure to verify whether depressive-like rats displayed higher voluntary intake of the CB1 receptor agonist WIN55, 212-2 (WIN, 12.5 µg/kg/infusion).To this aim, olfactory-bulbectomized (OBX) and sham-operated (SHAM) Lister Hooded rats were allowed to self-administer WIN by lever-pressing under a continuous (fixed ratio 1, FR-1) schedule of reinforcement in 2h daily sessions. We used the in vivo microdialysis technique to test whether OBX and SHAM rats displayed similar increase in DA levels within the NAc shell in response to a challenge of WIN at a dose (0.3 mg/kg) mimicking daily mean amount of the drug typically self-administer by trained rats. Data showed that both OBX and SHAM rats developed stable WIN intake; yet, responses in OBX were constantly higher than in SHAM rats soon after the first week of training. Moreover, OBX rats took significantly longer to extinguish the drug-seeking behaviour after vehicle substitution. DA levels in the NAc of OBX rats did not increase in response to a WIN challenge, as in SHAM rats, indicating a dopaminergic dysfunction in bulbectomized rats. Therefore, a correlation between our microdialysis data and behavioural findings may be suggested as OBX rats may need to self-administer higher amounts of the cannabimimetic drug to achieve enhanced dopamine levels compared to the control subjects. Altogether, our findings suggest that a depressive state may alter cannabinoid CB1 receptor agonist-induced brain reward function.

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3. Mutagenesis during plant response to UV-B radiation

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To test an idea that induced mutagenesis due to unrepaired DNA lesions, here the UV photoproducts, is underlying mechanism of UV impact on plant phenotype we used moss Physcomitrella patens protonema culture having 50 % of apical cells and thus mimicking actively growing tissue, the most vulnerable stage for the induction of mutations. After UV irradiation we estimated mutation rate and selected, isolated and analyzed by sequencing numerous clones mutated in adenosine phosphotrasferase gene (APT) of various repair deficient background (pplig4, ppku70, pprad50, ppmre11). In parallel we also measured repair kinetics and removal of Py^Py dimers by comet assay combined with UV dimer specific T4 endonuclease V. We show that UV induces massive, sequence specific, error prone bypass repair that is responsible for high mutation rate owing to relatively slow, though error-free, removal of photoproducts by nucleotide excision repair (NER).

4. Microstructural changes in gray matter of alpha synuclein overexpressing transgenic mouse model of parkinson's disease: a diffusion weighted imaging study

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Purpose: Compelling evidence suggests that accumulation and aggregation of α-synuclein contribute to the pathogenesis of Parkinson's disease. The aim of this study was to evaluate changes in grey matter microstructure induced by α-synuclein aggregation in *TNWT-61* mice by MRI diffusion weighted imaging (both diffusion kurtosis – DKI and tensor imaging – DTI) and correlate the results with behavioural data. We hypothesized that the presence of α-synuclein aggregates in the transgenic mouse model would create more water diffusion barriers resulting in higher kurtosis and no changes in diffusion metrics.

Methods: *Thy1aSyn* mice and wild type littermates at the age of 9 months underwent behavioural studies to detect the motor impairment and 9.4 Tesla DKI and DTI MRI scanning to detect structural changes in the brains in vivo. Kurtosis and diffusion metrics were obtained for multiple regions (substantia nigra, striatum, hippocampus, sensorimotor cortex and thalamus).

Results: *TNWT-61* mice showed significant progressive impairment of motor coordination compared to their wild type littermates. Values of mean, radial and axial diffusion kurtosis were significantly elevated in the *TNWT-61* group as compared to the wild type control group. For the diffusion parameters (mean, radial and axial diffusivity) no inter-groups differences were observed in any of the analysed regions.

Conclusion: The current study provides evidence that DKI is sensitive for *in vivo* detection of the microstructural changes in basal ganglia induced by α-synuclein accumulation. Thus DKI has the potential to improve the clinical diagnosis of Parkinson's disease compared to conventional diffusion-tensor imaging.

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5. Detection of anti-HCMV T cell response of potential recipients and donors in cellular immunotherapy of leukemic patients after transplantation of hematopoietic stem cells

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Human cytomegalovirus (HCMV) reactivation represents a great complication after hematopoietic stem cell transplantation (HSCT). The presented study has two major goals: 1) the identification of patients after HSCT, which are at high risk of developing HCMV disease; 2) the identification of the most promising antigens for *in vitro* stimulation of antiviral T cells for anti-HCMV immune therapy.

To find patients without sufficient HCMV specific cell immunity, we were monitoring the reconstitution of the anti-HCMV specific immune response on the 40th, 90th, 180th and 365th day after HSCT and day *n, n+30, n+60* and *n+90* after HCMV reactivation. Peripheral blood mononuclear cells (PBMC) were isolated from whole blood of patients after HSCT, stained with antibodies against human CD3, CD4, CD8, CD56 and CD14 and analyzed by flow cytometry, to assess the state of the immune system. Furthermore, cell mediated immunity was determined by ELISPOT- IFNgamma using the 15mer peptide pools of pp65, IE-1, US3, UL36, US29 and UL55 antigens, a set of various peptides carrying the CTL epitopes of HCMV or lysate of fibroblasts infected with the AD169 strain of HCMV. Currently, we optimize the method for intracellular cytokine staining after the stimulation of these hPBMC with the same antigens as in the ELISPOT assay.

In this study, there were included thirty patients after HSCT. Twenty-two of them experienced one or more HCMV reactivations. The anti-HCMV T cell response was detected in 24 patients. By testing the anti-CD3 IFNgamma response of PBMC from all patients we found that the reconstitution of T cells to anti-CD3 stimulation was completed at day 180 after HSCT. It seems that antigens US3, UL55 and UL36 can be favourably included in testing of T cell response against HCMV, in addition of pp65 and IE1. Although the response against HCMV lysate was missing in 70% recipients, it was very important for protection. It turned out that patients with GVHD treated with cyclosporine A and with a graft from HCMV seronegative donor had an increased risk of HCMV reactivation.

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Key words: HCMV immunity, HSCT, T cell response

6. Tailoring the optical plasmonic resonances by the shape of metallic dipole-walls

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Optical antennas are promising devices for enhancement of optical fields surrounding them. These effects are often utilized in photovoltaics, where this enhancement increases the efficiency of energy conversion process. Biosensing is another promising application as the resonant properties are influenced by refraction index of surrounding environment.

One of the key prerequisites for successful applications is possibility to precisely tune the resonant frequency of antennas. This can be quite complicated at frequencies from visible part of electromagnetic spectrum as the size of plasmonic antennas must be very small. In our work we present new approach of fabrication of plasmonic resonant structures by creating „walls“ surrounding conventional antennas, that exhibit additional vertical resonances. As these resonances are based on vertical dimensions, we can simply tune them by changing various technological parameters of electron beam lithography (EBL) and ion beam deposition (IBD).

We are using dark field spectroscopy setup, that enables us to excite and measure these vertical resonances in our structures. Our measurements are supported by boundary elements method based numerical model. This model predicts strong dependence of position and strength of this vertical resonance mode on height and sharpness of these walls, which can be easily tuned by parameters of the fabrication process.

7. Holographic microscopy coupled with fluorescence detection as a tool for analysis of aggressive metastatic prostate cancer cell death

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The identification of specific type of cell death following the cell injury seems to have great value to the dose-response and toxicological studies. Many changes typical for predominant types of cell death (oncosis/necrosis and apoptosis) are detectable by flow-cytometry. Flow-cytometric analysis of cell death using annexinV/propidium iodide assay was compared with multimodal holographic microscopy (MHM) and light microscopy. The aim was to highlight limitations of flow-cytometric analysis and point out to the advantages of MHM. MHM combines holographic imaging with well-known fluorescent imaging. This unique combination enables user-friendly verification of observed processes using one instrument.

It was clearly shown that annexinV+ /PI– phenotype is not specific for early apoptotic cells, as previously thought, and morphological criteria are necessary to assess the cell death type precisely.

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8. Investigation of the ammonia sensors based on carbon nanotubes

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The ammonia gas sensor based on carbon nanotubes were investigated. Iron catalyst was deposited by PECVD on Si/SiO_2 substrate. $Fe(CO)_5$ was used as catalyst precursor. Carbon nanotubes were deposited on the substrate with iron catalyst using CVD from C_2H_2 at the temperature range 600-700°C. Au electrodes were deposited on the sensors. Carbon nanotubes were investigated by scanning electron microscopy and Raman spectroscopy. The sensor response was determined in a range of 100 ppm – 500 ppm of NH_3 at room temperature and 200°C. It was determined that sensor resistance was strongly linked with response. The samples with higher resistance possessed higher response. The influence of the carbon nanotube synthesis conditions (deposition temperature and deposition time) on sensor response was determined.

9. Dealing with noise in psychophysiological interaction analysis

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Background

In some fields of fMRI data analysis, it is apparent that a correct methodology is crucial to achieving meaningful results. This paper provides a first quantitative evaluation of the effects of different preprocessing and noise filtration strategies on the psychophysiological interactions (PPI) – method for analysis of fMRI data [1], where noise management is not yet established.

Materials and Methods

To assess these effects, both real fMRI data and simulated fMRI data were used. Two regions of interest (ROIs) were chosen for the PPI analysis on the basis of their engagement during the task. PPI terms were computed and used in a general linear model (GLM); group-level analyses followed. This first-level PPI analysis pipeline was performed for 32 different preprocessing and analysis settings, which included either data filtration with RETROICOR [2] or no such filtration; different filtration of the ROI "seed" signal with a nuisance data-driven time series; and the involvement of these data-driven time series in the subsequent PPI GLM analysis [3]. The extent of the statistically significant results was quantified at the group level using simple descriptive statistics.

Conclusion

We conclude that different approaches for dealing with noise in PPI analysis yield appreciably different results. We definitely recommend the usage of RETROICOR. Filtering the ROI signal with data-driven signals and adding these signals to the GLM for assessing the PPI effects is apparently influential, but it is not clear whether their usage improves results in all cases.

References

[1] Friston, K.J., Buechel, C., Fink, G.R., Morris, J., Rolls, E., Dolan, R.J. (1997) Psychophysiological and modulatory interactions in neuroimaging. Neuroimage, 6:218-29.
[2] Glover, G.H., Li, T.Q., Ress, D. (2000) Image-based method for retrospective correction of physiological motion effects in fMRI: RETROICOR. Magnetic Resonance in Medicine, 44:162-167.
[3] Weissenbacher, A., Kasess, C., Gerstl, F., Lanzenberger, R., Moser, E., Windischberger, C. (2009) Correlations and anticorrelations in resting-state functional connectivity MRI: a quantitative comparison of preprocessing strategies. Neuroimage, 47:1408-16.

10. Dielectrophoresis for Manipulation of Droplets in Microfluidic Integrated System

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Droplets based microfluidic systems provide numerous benefits in biological and chemical research. Cell-containing droplets can be applied as a model for high-throughput chemical reactions, drug screening, toxicity testing as well as single-cell encapsulation. Microreactors of this type can be manipulated and applied in bio-testing. In this work we present a platform for droplet generation and manipulation by using dielectrophoresis (DEP) force. Integrated microfluidic device with a DEP chip generates surfactant stabilized droplets using an inert oil (decaline) as the continuous phase. The control circuit enables us to monitor in situ the change of motion direction a droplet. The emulsion was collected onto the array and covered with an ITO-glass using parafilm "M" as a spacer. We applied AC voltage 25V at frequency ranging from 50Hz to 1MHz across the electrodes. Under these conditions was observed positive DEP of water droplets. Droplets were attracted to the edges of electrodes, where the gradient of the electric field is the highest. The manipulation flexibility can be further increased by using higher fields, thinner electrodes or different layout of the electrode array. Applying this described platform for the future work involves single cell encapsulation and detection by optical and mass spectrometric means.

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11. Microgels for multiplex and direct detection of miRNA

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Blood borne oligonucleotides fragments contain useful clinical information whose detection and monitoring represent the new frontier in liquid biopsy as they can transform the current diagnosis procedure. For instance, recent studies have identified a new class of circulating biomarkers such as s miRNAs, and demonstrated that changes in their concentration are closely associated with the development of cancer and other pathologies. However, direct detection of miRNAs in body fluids is particularly challenging and demands high sensitivity -concentration range between atto to femtomolar- specificity, and multiplexing. Furthermore, cost effectiveness, simplicity of procedures for handling, workflow and reading must be carefully considered in a context of point-of-care testing for large screening.

Here we report on engineered multifunctional microgels and innovative probe design for a direct and multiplex detection of relevant clinical miRNAs in serum. Polyethyleneglycol-based microgels have a core-shell architecture with two spectrally encoded fluorescent dyes for multiplex analyses and are endowed with fluorescent probes for miRNA detection. Encoding and detection fluorescence signals are distinguishable by not overlapping emission spectra. Tuneable fluorescence probe conjugation and corresponding emission confinement on single microgel allows for enhanced target detection. Such suspension array has indeed high selectivity and sensitivity with a detection limit of 10-15 M and a dynamic range from 10-9 to 10-15 M, with higher accuracy than qRT-PCR.

We believe that sensitivity in the fM concentration range, signal background minimization, multiplexed capability and direct measurement in serum of such microgels will translate into diagnostic benefits if compared to other biosensing technologies such as direct microarray and qRT-PCR. The robustness, flexibility and versatility of microgel assay open up new roots toward liquid biopsy in the context of point-of-care testing through an easy and fast detection of sensitive diagnostic biomarkers directly in serum.

12. Electromechanical Label-Free Detection of Prostate Cancer Biomarkers

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Cancer is the leading cause of death worldwide and prostate cancer (PCa) is the most commonly diagnosed type of cancer and the third leading cause (after lung and large bowel cancer) for all cancer-related deaths amongst males in the European Union (EU-27). PCa is a malignant disease and is frequently asymptomatic or its symptoms are similar to benign prostate hyperplasia (prostate enlargement) and are therefore underestimated. As with every other type of cancer, also in PCa, early diagnosis tremendously improves the survivability rate. Prostate specific antigen (PSA) level test is currently a gold standard for PCa diagnosis, however it lacks both sensitivity and specificity. Therefore new methods of PCa detection are required.

Glycans are chains of carbohydrates that are attached to proteins or lipids, forming glycoproteins or glycolipids. Glycosylation is very common posttranslational modification and aberrant glycosylation patterns are characteristic for tumorigenesis, therefore glycan analysis of biomarkers can give us more reliable methods for disease diagnosis.

Lectins are proteins that are known for their ability to specifically bind carbohydrates. This means that lectins are able to recognize glycan structures on the surface of glycoproteins what makes them an ideal candidate for designing label-free, lectin based analytical methods.

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13. The role of auxin transport in plant stress adaptation

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Plants are continuously exposed to a wide and diverse range of abiotic stress conditions which can lead to overproduction of reactive oxygen species (ROS). This results in the disruption of cellular and molecular processes. Modulation of plant development in response to the environment plays a major role in plant stress adaptation which is governed by changes in plant hormones homeostasis.

Auxin is a main regulators of plant growth and development and recent studies show its emerging role in plant response to stress. However, molecular mechanism of ROS-auxin crosstalk remains scarce and in most studies has been identified as a side observation. Polar auxin transport mediated by PIN FORMED (PIN) transporters has been shown as a main modulator of plant development. Additionally, it has been proposed that some PIN transporters (PIN5, PIN6 and PIN8) participate in cellular auxin homeostasis.

Expression analysis of different promoter-GUS fusion lines grown under stress conditions reveals that PINs gene expression is differentially regulated by different stressors. Moreover, shoot phenotype of stressed -knock-out and -constitutively active overexpression *Arabidopsis* lines shows that *pin6* and *35S::PIN5* are more tolerant to the applied stress. Photosynthetic pigments' (chlorophyll a, chlorophyll b and carotenoids) concentration is not altered in PIN mutants when comparing to the wild type. Interestingly, stress induced and auxin regulated anthocyanin's biosynthesis is decreased in *pin6* mutant, which is more resistant to oxidative stress when comparing to the control plants. Our preliminary results indicate that PIN5 and PIN6 modulation of cellular auxin homeostasis could play important role during stress adaptation.

14. Fabrication of Microfluidic Chips for Liquids Manipulation and Optical Spectroscopy

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Presented poster describes improved micromachining process for microfluidic chips manufacture. Soda-lime microscope cover slips were used as a wafer for creating channel patterns by standard soft-lithography method and wet-etching process was used to prepare micro-sized rectangular channels. New technology for implanting electrodes to microfluidic chip was developed as well. This technology is compatible with thermal assisted direct bonding process for covering the micromachined channels and electrodes. Produced microfluidic chips are suitable for liquid / microdroplets manipulation and for optical spectroscopy methods (Raman spectroscopy, fluorescence) in the range (400 – 2000) nm. Manufactured chips were successfully tested with n-Decane / deionized water combination for droplet formation inside the chip. A „smart" passive structure allowing to stop the droplets in defined place for measurement was also invented and implemented. Finally, the microfluidic chips were successfully tested for measurements of the Raman shift on carbon nanotubes.

15. Detection of foodborne pathogens based on immunomagnetic separation and electrochemical magneto-genosensing

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The increasing incidence of foodborne illnesses caused by pathogenic bacteria represents a serious public health concern, resulting in stricter legislation for the food safety control. Therefore, it becomes important the development of rapid methods, especially with a multiplex capability for the detection and/or identification of more than one pathogen in a single platform, reducing assay times and costs [1].

Electrochemical genosensors can be a cheaper alternative, since it is not necessary the acquisition of expensive signal transduction equipment. Moreover, the combination of magnetic particles with traditional PCR assays offers an attractive technology with improved specificity and sensitivity [2].

In this work the detection of *Salmonella enterica* in whole milk, as well as the simultaneous detection of *Salmonella enterica*, *Escherichia coli* O157:H7 and *Listeria monocytogenes* was achieved by electrochemical magneto genosensing approach. Commercial magnetic particles were modified with antibodies specific for *S. enterica* to capture and pre-concentrate different concentration of *Salmonella* from artificially contaminated milk samples. Afterwards, the bacteria were lysed and a PCR was performed using a pair of primers tagged with fluorescein. Hence, it was shown that this approach was able to detect as low as 1 CFU/mL of *Salmonella* in milk between 3-4 h, by using anti-fluorescein-HRP, as electrochemical reporter.

A triple-tagging multiplex PCR was performed [3] using a set of primers for the amplification of TS (375 bp), *hlyA* (234 bp) and *eaeA* (151 bp), being one of the primer for each set tagged with fluorescein, biotin and digoxigenin coding for S. enterica, *L. monocytogenes* and *E. coli* O157:H7, respectively. Finally, simultaneous detection based on electrochemical magneto-genosensing bacteria was achieved by using silica magnetic particles and three different enzyme marker, specific for each pathogen. The features of this approach are discussed and compared with conventional microbiological culture and gel electrophoresis detection, concluding that this strategy is able to clearly distinguish among the pathogenic bacteria tested and that it can be considered as a rapid alternative to the time consuming classical methodology.

[1] Gehring, A. G.; Tu S., Annu. Rev. Anal. Chem., **2011**, 4, 151–172.
[2] Liébana, S.; Lermo, A.; Campoy, S.; Barbé, J.; Alegret, S.; Pividori, M. I., Anal Chem, **2009**, 81, 5812–5820.
[3] Kawasaki, S.; Horikoshi, N.; Okada, Y.;Takeshita, K.; Sameshima, T.; Kawamoto, S., J Food Prot, **2005**, 68, 551-556.

16. Whole genome sequencing of multidrug-resistant *Klebsiella pneumoniae* strains

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Fast-growing modern Next-Generation Sequencing (NGS) technologies gradually supplant conventional sequencing methods in both basic and applied research. The main objective of this study was to investigate major basic molecular characteristics such as sequence type, resistance genes and number of plasmids using whole genome sequencing (WGS). In our study we applied the whole genome shotgun sequencing approach to sequence 11 multidrug-resistant strains of *Klebsiella pneumoniae* (~ 5, 3 Mb).

Bacterial DNA was isolated from overnight culture of extended-spectrum beta-lactamase producing *Klebsiella* pneumoniae strains. DNA library was prepared with New England BioLabs and Kapa Biosystems kits. Sequencing was done with MiSeq Reagent Kit 300v2. Bacterial DNA sequencing and data generation was carried out on Illumina MiSeq sequencing platforms. Bioinformatic processing and evaluation of data was largely done through interactive online tools. Basic rapid strain typing such as detection of resistance genes and Multi-Locus Sequence Typing (MLST) identification was done by web-based method provided by Center for Genomic Epidemiology, among additional tools we've used to analyze NGS data were CLC Genomics Workbench 6.0.4, MEGA 6, and other software.

Average read obtained as the result of high-throughput DNA sequencing was 250 nucleotide bases long and 99.97% accurate and reliable according to average PHRED score. We determined an appropriate sequence type (ST) by MLST, identified antibiotic resistance genes of 8 antimicrobial groups and some small plasmids present in our analyzed bacterial strains. We have also discovered a new MLST type 1646, which was approved by specialists from Institut Pasteur and subsequently submitted to Institut Pasteur MLST Database.

Due to large amounts of data provided by whole genome sequencing it is possible to identify ST and resistance genes, type plasmids and analyze many other genetically determined characteristics (virulence factors etc.). Therefore we can state that WGS is a powerful modern technology, which introduces a new comprehensive approach to solving both basic research and ordinary practical tasks.

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17. Kinetics Study of D, L-lactide and Glycolide Copolymerization on PEG Initiator by Novel Gas Chromatography Procedure

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Biodegradable thermosensitive ABA triblock copolymer, where A stands for hydrophobic poly[(lactic acid)-co-(glycolic acid)] and B for hydrophilic poly(ethylene glycol) (PLGA-PEG-PLGA) is used in medicine as injectable drug delivery carrier. However, due to the similar sublimation temperature of both D, L-lactide and glycolide monomers, it is difficult to use the gas chromatography (GC) for their separation and conversion evaluation. In this work, GC procedure has been optimized in terms of choosing right type of column, GC standard, set-up certain initial and maximal column temperature together with suitable heating rate, injection temperature, the type of carrier gas and flow rate. Finally, novel procedure of GC has been developed to study the kinetics of D, L-lactide and glycolide copolymerization in order to set-up optimal copolymerization conditions. Ring opening polymerization (ROP) proceeded in a bulk under nitrogen atmosphere at 130°C for 3 hours. Conversion of monomers into PLGA-PEG-PLGA copolymer was determined either by common gravimetric method or by developed GC method with a flame ionization detector using anisole as an internal standard. Both methods showed linear increase in conversion up to 1.5 h followed by the plateau. However, monomers' conversion evaluated using the gravimetric method exhibited 80 % in 3 hrs while the value obtained by gas chromatography showed 95 %. The 15 % difference was caused probably by the loss of copolymer within the gravimetric method, where the synthesized copolymer has to be purified from the original mixture prior the weighting. Preferably, the GC method does not need any purification prior the measurement, the method is accurate and fast.

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18. Morphogenesis and viscoelastic properties of dental dimethacrylate networks

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The main goal of the work was to study the morphogenesis of dimethacrylate networks. Connections between the structural features of distinct monomer species, network formation kinetics and viscoelastic properties were highlighted. In the study, the most commonly employed monomers in the nowadays dental practice were used. This includes 2, 2-bis[4-(2-hydroxy-3-methacryloxyprop-1-oxy) phenyl] propane (bis-GMA), bisphenol A ethoxylate dimethacrylate (EPBDMA), 1, 6-bis(methacryloxy-2-ethoxycarbonylamino)-2, 4, 4-trimethylhexane (UDMA) as base monomers and triethyleneglycol dimethacrylate (TEGDMA) as a viscosity reducer.

Tetrafunctional network morphogenesis was studied regarding the structural differences and a varying molar ratio of the comonomers. Photopolymerization kinetic data provided the base for understanding the structure formation process achieving the network with the defined morphology. An attempt to quantify the relationship between the supramolecular structure and complex viscoelastic moduli had been made. Reaction kinetics was studying using differential photocalorimetry (DPC), double bond conversion was determined by infrared spectroscopy (FTIR). Basic viscoelastic parameters were measured by dynamic-mechanical analysis (DMA) and interpreted using the known models. The kinetics of thermal decomposition was investigated using thermogravimetric analysis (TGA).

Monomer reaction potential and viscosity are derived from its molecular structure. The rigidity and the potential for hydrogen bonding of Bis-GMA significantly decrease polymerization rate (R_p) and functional groups conversion ($P_{C=C}$). Absence of the –OH functionalities (EPBDMA) and of the rigid core structure (UDMA) when compared to Bis-GMA results in the increase of both R_p and $P_{C=C}$. Dilution by flexible TEGDMA leads to the shift of reaction diffusion controlled kinetics to higher conversions and thus, to the increase of the overall conversion. However, the presence of flexible backbone monomers promotes the origination of structural heterogeneities associated with the primary cyclization reactions, microgel domains formation and ineffective crosslinking as observed by broadening of relaxation times spectrum (loss tangent peak) and by two-step degradation process. This is associated with the coexistence of loosely crosslinked and more densely crosslinked regions in the network and ultimately results in the reduction of mechanical properties.

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19. Biosensor for AIDS diagnostics and monitoring

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The impact of HIV infection and clinical disease on global health continues affecting, particularly in resource-limited countries. According to the WHO reports, an estimated 35.5 million people are currently living with HIV and despite of advances in the treatment of HIV-infected individuals only 34% (9.7 million) of people considered eligible received ART (antiretroviral treatment).[1]

Initiating ART early is vital for successful treatment. The median CD4 count when ART is initiated is 350 cells μL^{-1}, and about 1 in 4 people in low-income settings initiate ART late, with CD4 counts <100 cells μL^{-1}. Access to CD4 testing remains limited, with less than 20% of the people who test HIV-positive getting a CD4 count in some settings. Point-of-care CD4 testing can significantly speed up the initiation of ART and improve retention among people who are eligible for ART.[2] Therefore, substantial efforts have been taken to build low-cost and reliable point-of-care (POC) HIV diagnostic and monitoring solutions that effectively make early diagnosis and treatments available to even the most resource-constrained communities.

This work addresses for the first time a biosensor with electrochemical/optical readout for HIV clinical diagnosis based on the enumeration of $CD4^+$ T lymphocytes. CD4 cells are preconcentrated by a one-step immunomagnetic separation and labeled with an enzymatic reporter. The results showed a good correlation between the analytical signal and the $CD4^+$ T lymphocytes counted with flow citometry. The limit of detection obtained with the optical readout strategy was 100 cells µL-1 while for the electrochemical strategy was 140 cells μL^{-1}. Recovery assays were also performed in order to evaluate the accuracy of these methods showing excellent values from 90 to 110%. Due to the high sensitivity, the CD4 counting electrochemical magneto immunosensor offers outstanding analytical performances as well as advantages compared with the gold standard flow citometry strategy. These approaches are highly suitable as alternative diagnostic tools at the community and primary care level, especially in developing countries.

1] UN Joint Programme on HIV/AIDS. Global Report: UNAIDS Report on the Global AIDS Epidemic 2013. Joint United Nations Programme on HIV/AIDS (UNAIDS). 2013.
[2] UNAIDS/UNICEF. Global update on HIV treatment 2013: results, impact and opportunities. 2013. WHO report, in partnership with UNICEF and UNAIDS.

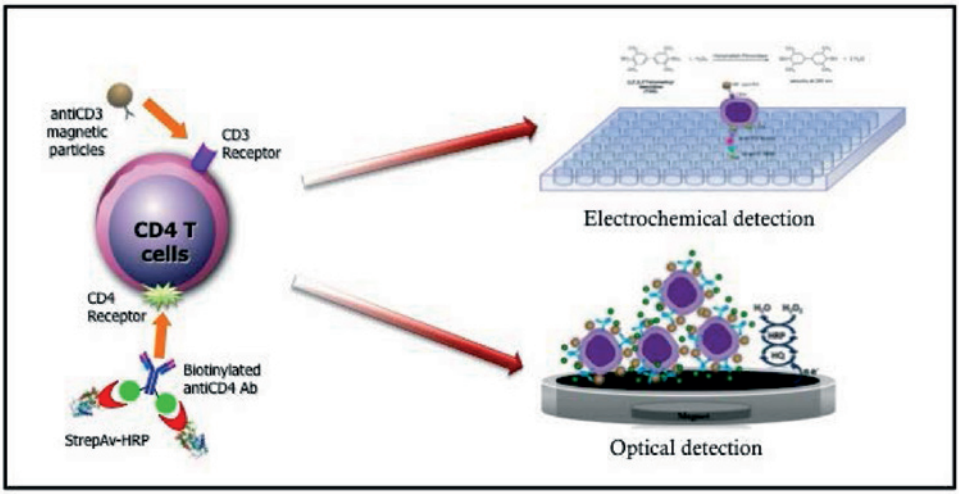

Figure 1. Schematic representation of the magneto-ELISA and electrochemical biosensing strategy.

20. Nano-scale derivatization followed by capillary electrophoresis with fluorescence detection

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Derivatization reaction allows modifying structure of originally non-fluorescent compounds in order to detect original compounds by fluorescence detection. Such compounds could be amino acids (AA), which play very important role in living organisms. Derivatization reagents are in general expensive and derivatization procedure is often time-consuming, laborious, and the products are instable. As a result, precision of quantification is compromised. Capillary electrophoresis is the analytical technique where separations occur in narrow capillaries. Capillary can be also used as a reaction chamber of nano-scale dimensions allowing automation of derivatization reaction minimizing manual steps, consumption of samples and reagents, and the precision could be higher in comparison with conventional approaches. Method development of in-capillary derivatization reaction followed directly by capillary electrophoresis coupled with light-emitting diode induced fluorescence detection of AA as well as optimization of separation conditions for analysis the reaction products are the subjects of this contribution. Derivatization reagent naphthalene-2, 3-dicarboxaldehyde was used to target the amino group of AA in presence of cyanide anion. Reaction completed in 5 minutes allowing quite precise quantification of AA derivatives with significantly lower yields of side-products.

21. Rheological Study of PLGA-PEG-PLGA/hydroxyapatite core-shell nano-particles in thermosensitive copolymer matrix

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The main objective of present work is preparation of core-shell (CS) nanoparticles and rheological study of CS particles in thermosensitive copolymer matrix. The shell consists also of well-defined "smart" hydrogel based on hydrophilic poly(ethylene glycol) (PEG) and hydrophobic poly(lactic acid)—*co*—poly(glycolic acid) (PLA/PGA) copolymer (PLGA—PEG—PLGA), which gels at body temperature and core is composed from inorganic bioactive nano-sized hydroxyapatite (n-HAp).

The original copolymer solution exhibited two phase transitions (sol-gel and gel-suspension) depending on the temperature. The maximum of gel stiffness (G´max) was about 40 Pa at 39.5 °C. CS/copolymer composite containing either 5 or 10 wt. % of CS exhibited still two phase transitions and the G'_{max} of samples with either 5 or 10 wt%. of CS increase similarly up to 150 Pa in comparison with the original copolymer matrix without CS nanoparticles.

After adding up to 10 wt% of CS to the polymer solution stiffness of the gel increased almost 4 times (from 40 kPa up to 150 kPa) and the G'_{max} was moved to higher temperatures (from 40 °C to 41, 5°) maintaining two-phase transition while 20 wt% of CS nanoparticles in copolymer solution exhibited gel-like structure. Investigated PLGA—PEG—PLGA/CS nanocomposite could be a potential resorbable system for bone regeneration or injectable drug delivery carries.

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22. Structural insights into the aberrant splicing of CFTR exon 9

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Skipping the splicing of exon 9 of cystic fibrosis transmembrane conductance regulator (CFTR) pre-mRNA leading to the translation of a non-functional chloride channel is associated with severe forms of cystic fibrosis (CF). This splicing event depends primarily on the composition of the polymorphic $(TG)_mT_n$ locus between the branch point and the 3´ splice site (3´ss) of exon 9. The (UG)-rich pre-mRNA sequence was reported to be recognized by TDP43 (43 kDa TAR DNA binding protein), and this, in concert with other splicing factors (e.g. hnRNPs), prevents the recognition of the 3´ss of exon 9 by the canonical splicing factors and generates the CFTR mRNA without exon 9.

We examined the binding of the RRMs of TDP43(102-269) and the RRMs of hnRNPA1(1-196) with CFTR 3´ss RNAs. The SAXS and NMR data indicate two copies of TDP43(102-269) proteins and one copy of hnRNPA1 bind to the (UG)12U5 CFTR 3´ss RNA suggesting that these distinct interactions favour CFTR exon 9 skipping.

23. Self-assembled, 1, 2-thiomannobiosiode containing glycoconjugates as potent ligand of *Burkholderia cenocepacia* lectins

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Burkholderia cenocepacia is a Gram-negative bacterium causing a number of diseases in plants but this micro-organism also infects immunocompromised humans, especially cystic fibrosis (CF) patients. Crucial role in the infection could be played by bacterial lectins. Lectins are sugar-binding proteins of non-immune origin occurring in all organisms and executing various functions. Microbial lectins can recognize carbohydrates on host cells (glycoproteins or glycolipides) and mediate adhesion to host cells or mucosal surfaces. Adhesion is considered to be an important step in infection development as it protects pathogen from natural cleansing mechanisms of the host. Our work is focused on investigation of binding properties of lectins from B. cenocepacia. Lectins vary also in fine binding specificity but all of them recognize D-mannose. As blocking of lectins with 1, 2-thiomannobioside containing, multivalent glycoconjugates, as potent inhibitors (**1** and **2**), could decrease adhesion of pathogens to host cells, these proteins are potential candidates for an anti-adhesion therapy. Therefore, binding specificity of lectins was determined by surface plasmon resonance and isothermal titration calorimetry.

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24. Titanium oxide based anode materials for li-ion batteries

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Chemical and thermal stability are the key bottlenecks of conventional graphite anodes used in the li-ion batteries today. Graphitic materials are the most common anode active materials mostly due to their high specific capacity and relatively low cost. On the other hand, drawbacks like low stability of solid electrolyte interface layer protecting it from spontaneous reaction with electrolyte and the risk of thermal runaway are more and more evident with rising of interest in electro mobility and EV.

One of the most promising successors in the field of lithium anode active materials is the group of ceramic materials based on titanium oxide. Their thermal and chemical stability is incomparable with graphitic materials – they are almost inert and due to higher operation voltage they do not suffer from irreversible capacity loss as a result of SEI layer formation.

This work deals with structural and electrochemical characterisation of spinel $Li_4Ti_5O_{12}$ and TiO_2 nanostructured anode active materials for lithium-ion batteries. Electrochemical characterization by cyclic voltammetry, galvanostatic cycling, electrochemical impedance spectroscopy and rate capability was made and physical characterization by XRD and SEM is provided.

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25. Decoration of flexible metal-organic coordination networks by reactive Ni atoms at surfaces

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The formation of extended two dimensional metal-organic coordination networks showing high adaptability to surface step edges and structural defects is revealed by scanning tunneling microscopy. Independent from the chosen substrate (Au, Ag) and its symmetry the networks grows continuously over multiple surface terraces through mutual in-phase structure adaptation of network domains at step edges and on terraces. This is mainly ascribed to the high degree of conformational flexibility of the butadiynyl functionality of the ligand.

The butadiyne functionality is further employed to binding Ni atoms in non-oxidative way on the silver substrate: the metal-organic template steers the growth of the Ni clusters within the first substrate layer. The Ni decorated network is stable up to 450 K. Further, the decorated NW can be utilized for binding additional molecular ligands into the NW cavities.

26. Thermal performance analyzing of hollow microspheres coatings for building energy efficiency

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Currently, the major emphasis of building energy efficiency is focused on improving the thermal properties of building envelope (EPBD II.). In research and development of reflective coating is considered potential for reducing thermal loads in buildings. On contrary, within basic requirements to standards for the thermal protection of buildings the radiating heat flow represents in general a negligible proportion of the resulting energy flow. Primarily, the main focus is based on conventional thermal insulation systems of buildings, but at the same time research trends has led to the progressive development and building envelope innovation applying recent nanotechnology findings. Impacts the optical properties of building elements are often neglected in the design of the building envelopes, but these are significantly involved in the heat transfer phenomena for dynamic conditions.
The starting point for analyzing the performance of various coatings in correlation of the building energy efficiency for Central Europe conditions was based upon the fact that currently research field and building market offers coatings with all sorts of positive features predominantly declared by their producers. They maintain additionally that their products cannot be analyzed by conventional methods of building physics; therefore, their thermal aspects can only be determined by specific methodical approaches. On the whole, besides other important issues, their properties considerably depend on dynamic changes.
The main objective of this study resides in investigating the optical properties and thermodynamic aspects of coatings that are based upon hollow ceramic microspheres (HCM), as compared predominantly with the standard façade coatings. Both laboratory and full scale tests were conducted in order to analyze non-steady state heat transfer monitoring through samples. Similarly all external radiation impacts on highly insolated and weakly isolated walls were involved in experimental surveys. Testing periods represented clear sky conditions in a mild climate zone.
The results of spectral analysis demonstrate that within the infrared region, however, both in the near and the longwave one, compared coatings can be assessed as having almost identical reflective properties. As regards in a visible region, both have very negligible differences. On the full scale tests level, the final results allow formulating a conclusion that the coatings based upon ceramic microspheres can contribute to positive energetic effect due to reducing cooling loads at the low level as in comparison of the standard façade coatings of an identical colour shade, which in other words does not represent significant benefit in current standards of building envelopes for building energy efficiency of mild climate zone, as it was demonstrated on highly insolated and weakly isolated vertical components.

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27. Identification of microRNAs involved in DNA damage response in malignant B cells and their biological and clinical relevance

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We and others have shown that expression of miRNAs influences the biology of B cell malignancies (Calin *et al.*, 2005; Mraz *et al.*, 2009, 2012, 2013, 2014). The aim of this study was to identify miRNAs involved in the apoptotic response of malignant B cells. Purified primary B cells of chronic lymphocytic leukemia (CLL) patients (pts.) were treated in vitro with fludarabine (F). Five paired (n=10) samples (with and without F) were analyzed using 2 NGS platforms-SOLiD (ABI) and HiSeq (Illumina). Obtained sequences were mapped against miRBase using 3 tools (CLC Gen Workbench, SHRiMP2, miRanalyzer) and miRNA changes were analyzed by a pair-wise comparison with edgeR and baySeq packages. The overlap of 2 NGS approaches analyzed by all above mentioned methods identified 6 miRNAs significantly changed with DNA damage. Hi-Seq validation on 5 additional independent paired samples (n=10) confirmed the changed expression of all 6 identified miRNAs (3 down-, 3 up-regulated). We further screened the expression of three up-regulated miRNAs (miR-34a, miR-1246, miR-1248) in a cohort of patients (n=40) treated with F, cyclophosphamide and rituximab (FCR) regimen in vivo. Pts. samples were collected before the therapy and 48 hrs after FCR administration. We observed significant induction of all upper mentioned miRNAs ($p<0.0001$; $p=0.04$; $p=0.12$ respectively). The most constantly up-regulated was miR-34a, which was previously shown to be regulated by p53 (He *et al.*, 2005). Interestingly, low miR-34a basal levels before the therapy intervention were able to predict shorter progression free survival (PFS) (median PFS 19.9 vs. 26.4 mth; $p=0.05$; HR: 2.29 [CI 0.97-5.36]). Importantly, the miR-34a level was able to predict the PFS in the group of wt-*TP53* samples (20.2 mth vs. 27.3 mth; $p=0.02$; HR: 3.04 [CI 1.12-8.29]). Different levels of miR-34a were also observed in patients with different final response to the therapy (complete remission vs. other; $p=0.05$).

We further determined the expression of miR-34a in a large cohort of CLL pts. (n=158) using an in-house designed assay for its copy-number quantification. We defined a cut-point (number of miR-34a copies) that segregates pts. with extremely unfavorable prognosis (overall survival [OS] 15.8 mth vs. not reached; $p<0.0001$; HR: 4.05 [CI 2.17-7.57]). This cut-point serves as the strongest OS predictor even in the group of wt-*TP53* samples only (36.8 mth vs. not reached; $p=0.004$; HR: 5.00 [CI 1.48-16.96]). The molecular pathways affected by levels of miR-34a and other identified miRNAs in B cells are largely unknown. These miRNAs are currently being investigated using integrated analysis of miRNA and transcriptome profiling.

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28. Dynamic Phase Differences Method for the Assessment of Cellular Dynamic Processes

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Imaging of cellular mass transfers and therefore the traits of live cell behaviour is nowadays a rewarding task in life sciences. As a tool for revealing those dynamic processes, we present a method of dynamic phase differences (DPD). DPD fully exploits the quantitative phase images obtained by the Coherence-Controlled Holographic Microscope (CCHM) developed in Brno University of Technology. DPD method is employed for the objective evaluation of the growth, migration and motility of cells, which would not be obvious only from the quantitative phase images. The results can be directly visualised by the 2D colour image, which could lead to identification of typical patterns characterizing the behaviour of cells. The method also provides the opportunity to calculate the transferred mass of the cell over a distance and therefore enables to quantitatively measure the movements of the cell (in picograms).

29. SPR Strategy in Prostate Cancer Glycobiomarker Research

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Prostate cancer diagnostics can be effectively addressed using sensor-based approaches. Such biosensor technology enable fast and efficient determination of proteins as well as give a remarkable insight to changes in protein structure, such as aberrant glycosylation, what all together can increase performance, sensitivity and specificity of clinic assays. However, for full comprehension of aberrant protein glycosylation is fundamental to understand their biospecific interaction. Currently, surface plasmon resonance (SPR) is one of the most effective label-free biosensing techniques for both quantitative and qualitative parameters of biospecific interactions. Prostate specific antigen (PSA), is currently used as a traditional serum biomarker for early detection of prostate cancer. However, this serum glycoprotein assay is associated with significant sensitivity and specificity issues in the diagnostic „gray zone" of 2.5 to 10 ng/ml. Kinetic parameters together with affinity constants of PSA/antibody and PSA/lectin interactions were determined. Experiments with antibodies (anti-PSA) specific against different epitopes of PSA followed by lectin glycol-profiling were performed. All these data are crucial for construction of highly selective, sensitive and robust biosensor devices and their applications in clinical diagnostics.

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30. Image-based Automated Detection of First-episode Schizophrenia: The Importance of Spatial Resolution of Morphological Features and Ensemble Learning

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Machine learning methods are increasingly utilized for automated classification and diagnosis in various areas of medicine. In schizophrenia, however, the straightforward approaches fail. The aim of the presented study was to address the problem by using combination of two methods: 1) multiresolution image data representation for extracting features on multiple spatial scales and 2) classification based on ensemble learning providing desired robustness. 52 first episode-schizophrenia (FES) patients and 52 healthy controls were scanned by 1.5T MR device. The resulting T1-weighted images were spatially normalized and segmented into tissue types. In order to reduce noise and obtain succinct representation of contained information, discrete wavelet transform (DWT) was applied to the gray matter tissue segments and only coefficients surpassing chosen threshold were retained. The ensemble classification algorithm based on 1000 independent support vector machines (SVM) was trained and its performance was tested by stratified 52-fold cross validation. Combination of the DWT preprocessing and SVM ensemble classification achieved accuracy of 78% while maintaining balanced values of sensitivity and specificity. Features crucial for the correct classification were extracted and projected backwards onto corresponding brain areas to visualize brain structures important for classification - dorsolateral prefrontal cortex, medial prefrontal cortex, anterior cingulate, orbitofrontal areas, mediotemporal structures, temporal neocortex, caudate head, mediodorsal part of thalamus, and cerebellum.

31. Dissemination of beta-lactam and quinolone resistance genes mediated by IncX plasmids in *Enterobacteriaceae* of diverse sources and origins

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IncX plasmids were recently recognized as a diverse group of self-transferrable plasmids that could significantly contribute to the spread of plasmid-mediated quinolone resistance, extended spectrum beta-lactamase and carbapenemase genes in *Enterobacteriaceae*. The aim of the study was to compare molecular characteristics of IncX plasmids harbouring these resistance genes from Enterobacteriaceae of various sources and origin.

A collection 1800 *Enterobacteriaceae* isolates resistant to cefotaxime or with reduced susceptibility to ciprofloxacin from animals, humans and the environment obtained in Europe, North and South America, Africa, Australia were tested for IncX1-IncX4 plasmids using PCR. Antibiotic resistance genes were screened using PCR and sequencing. Clonality of *Enterobacteriaceae* isolates and size of IncX plasmids were determined using pulsed-field gel electrophoresis. Self-transferability of IncX plasmids was tested using conjugation and transformation. Restriction analysis and hybridization with labelled probes targeting antibiotic resistance and *taxC* genes were performed to assess the relatedness of these plasmids.

At least one subgroup of IncX plasmids (30-80 kb) was detected in 189 *Enterobacteriaceae* isolates (10.5%, n=1800) resistant to cefotaxime or with reduced susceptibility to ciprofloxacin. The predominant subgroup IncX1 was identified in 107 isolates, IncX2, IncX4 and IncX3 in 47, 28 and 16 isolates, respectively. In non-related *Enterobacteriaceae* isolates from different sources, the predominant types of IncX plasmids were IncX1 plasmids harbouring *qnrS1* separately or in combination with bla_{TEM-1} (52 plasmids) and IncX2 plasmids harbouring *qnrS1* separately or in combination with *tet*(A) (26 plasmids). IncX3 plasmids carried bla_{SHV-12} (2 plasmids) and *qnrS1* (1). In this study, further characterisation of IncX4 plasmids was not possible due to transfer of multiple plasmids along with IncX4 plasmids from *Enterobacteriaceae* isolates.
Our results showed that IncX plasmids play an important role in dissemination of plasmid-mediated quinolone resistance genes in *Enterobacteriaceae* originating from various sources and country of origin. Further transmission of IncX plasmids harbouring quinolone resistance genes might represent a public health concern.

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32. The Analytical Centrifuge LUMiSizer as a Useful Tool for the Characterization of Nanoparticles

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The analytical centrifuge LUMiSizer allows to speed-up the separation of dispersions by application of a centrifugal force and by raising the temperature (acceleration of diffusion limited processes, decreasing the viscosity). The LUMiSizer employs the STEP-Technology (**S**pace and **T**ime resolved **E**xtinction **P**rofiles), which allows measuring the intensity of the transmitted light as function of time and position over the entire sample height (in the cell) simultaneously. The data (so called transmission profiles) are displayed as a function of the transmittances in the time and the radial position (e.g. the distance from the center of the rotation). The transmission profiles contain the information on stability and on the kinetics of the separation processes and facilitate particle characterization. By means of available analysis modes, the separation and the particle size can be quantify and analysed in detail. At the same time up to 12 different samples can be analysed simultaneously at constant or variable centrifugal force up to 4000 rpm.

LUMiSizer allows easily detect/distinguish the aggregated/flocculated/dispersed particles in the dispersion medium. It is one of the candidates to fast and sensitively characterize the affinity of the particle to the liquids. This technique may also be used to determine the (nano)particle hydrodynamic density, particle size distribution etc.

The separation process of our nanoparticle dispersion is characterized by the very polydisperse sedimentation (no sharp front) of particles with relatively narrow particle size distribution of fine particles which move with different speed. Polydisperse sedimentation is characteristic for colloidal stable dispersions (stable against particle aggregation). Prior to separation, particle concentration is equally distributed along the complete sample length, i.e. transmission is constant at a low level (first transmission profile with the lowest transmission). As sedimentation proceeds the concentration of particles decreases in the region of meniscus and transmission increases. The last profile (highest transmission) indicates that the smallest particles have not yet fully separated out from the supernatant within the applied centrifugation time. Only the very small sediment is observed due to a dense packing of particles characteristic for colloidal stable systems with the repulsive particle-particle interaction.

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33. The Effect of Crocin and Fluoxetine on CYP2C6 in vivo Metabolic Activity and Its Protein Levels in Rat Liver Microsomes

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The aim of our study was to determine the effect of crocin and fluoxetine on the metabolic activity of selected cytochrome P450 (CYP) enzymes, one of the most important enzymatic systems for xenobiotic biotransformation. Presented results describe the influence of tested substances on the CYP2C6 enzyme metabolic activity, its protein levels in rat liver microsomes and total CYP microsomal content.
Crocin is one of the main active substances of Crocus sativus L. and saffron (dried petals of Crocus flowers). It is being intensively studied in the last few years because of its biological effects which might be therapeutically exploitable. Besides its antidepressant effects, also its antiproliferative, antioxidative, hypnosedative and other activities have been tested at either preclinical or clinical level.
Fluoxetine (selective serotonin reuptake inhibitor) has been established antidepressant in clinical practice for a long time. It is a substrate of human CYP2D6 and it also inhibits its own metabolism (CYP2D6) and moderately also CYP2C19, CYP2C9 and slightly CYP3A3/4.

In this study, we focused on the rat enzyme CYP2C6 which can be regarded as an orthologue of human enzyme CYP2C9. Thirty Wistar albino male rats were used in the experiment and they were randomly allocated into three groups. The control group was intraperitoneally treated with saline (1 ml/kg), the second group was i.p. injected with crocin (100 mg/kg) and the third group with fluoxetine (10 mg/kg) during 9 consecutive days. On the Day 10, diclofenac, the specific marker of CYP2C6, was administered i.p. in the Min 0 and blood samples were drawn after 20, 60, 100 and 180 min. The samples were analysed using HPLC and the levels of diclofenac and its CYP2C6 specific metabolite 4-hydroxydiclofenac were determined. Molar ratios of diclofenac to 4-hydroxydiclofenac (metabolic ratios) were then calculated. Liver microsomes were isolated from individual animal by differential ultracentrifugation. The total protein content and level of CYP2C6 protein was determined from this liver microsomal fraction.

Our results showed that systemic administration of crocin in rats had no effect on CYP2C6 activity but significantly decreased the number of total protein. On the other hand, fluoxetine had inhibitory effect on CYP2C6 after 180 min and significantly increased the CYP2C6 protein level in the liver microsomes. These results are expected to be correlated with outcomes of the ongoing in vitro studies.

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34. Dynamics of Telomeres and rDNA in *Arabidopsis* Chaperone Mutants

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We have recently found that Chromatin assembly factor, CAF1, participates in maintenance of telomeres as well as rDNA in Arabidopsis, (Mozgova et al, 2010). Precise distribution of euchromatin and heterochromatin in the nucleus ensures that all vital processes occur in correct spatio-temporal order. Aside, rDNA represents a good model to study both chromatin subdomains where its actively transcribed part present in the nucleolus is highly decondensed while transcriptionally inactive copies form heterochromatic loci outside of the nucleolus. CAF1 is a histone H3/H4 chaperone required for cell proliferation and development, involved in processes such as DNA replication, repair, silencing of certain genome loci and recombination. We have looked here at another H3/H4 chaperone Asf1 as well as H2A, H2B chaperones and chaperone related proteins to dissect the effect of replication and DNA repair in dynamics of rDNA and telomeres.

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35. Interference of surface plasmon polaritons on gold films in the optical near-field

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Our work deals with a study of surface plasmon polaritons (SPPs) on interference structures. To get direct information about electromagnetic field distribution it is necessary to study these structures in the near-field ($d<<\lambda$). For the detection of SPPs a scanning near-field optical microscope (SNOM) was utilized in the transmission mode. He-Ne laser (λ=633nm) was used as a source of light for illumination of the structures. The signal from the sample was collected by an optical fibre with etched and metalized tip.

SPPs are excited at the interference structures and propagate between them. The SPPs interfere and as a result a standing evanescent wave is formed. This constructive and destructive interference can be detected in the near-field. Interference structures were prepared by focused ion beam (FIB) into a 200 nm-thick gold film deposited on a quartz substrate with 3 nm of titanium as an adhesive layer by ion beam sputtering deposition. Three types of structures were designed - parallel and non-parallel grooves and, additionally, square slits in the Au layer. The width of the grooves ($\approx$ 100nm) was far below the diffraction limit.

We introduce several ways how to design and control the appearance of the resulting electromagnetic near-field intensity distribution: proper geometrical design of interference structures, changing the excitation laser polarization and application of inhomogeneous illumination. We submit the experimental results of the SPP interference pattern modification in comparison with analytical and numerical models.

[1] DVORAK, P.; NEUMAN, T.; BRINEK, L.; SAMORIL, T.; KALOUSEK, R.; DUB, P.; VARGA, P.; SIKOLA, T. *Control and Near- Field Detection of Surface Plasmon Interference Patterns*. NANO LETTERS, (2013), vol. **13**, no. 6, p. 2558-2563.

36. Effect of naturally occurring lesions in the human telomere DNA on its quadruplex structure and stability

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DNA in living organisms is continuously exposed to attacks of various environmental factors. The effect of these agents is often associated with formation of free radicals, which attack and damage DNA (sugar and base modifications, single- and double-strand breaks, DNA-protein crosslinks). Base damage may arise anywhere in the chromosomal DNA, including telomeres. Repetitive TTAGGG sequences constituting human telomere DNA can fold into four-stranded structures called quadruplexes. These structures are capable of hindering the function of telomerase. Telomerase activity is low with healthy cells while most of the cancer cells show a high activity of this enzyme. Therefore, the telomere DNA quadruplex has become a promising target for developing drugs with anticancer effect.

Using circular dichroism, gel electrophoresis, and UV absorption spectroscopy we have studied effects of naturally occurring damages to telomeric DNA on its ability to form quadruplex [1, 2, 3]. The results obtained show that the impact of guanine lesions is more influenced by the position of the damage within the quadruplex core than by its type. The most sensitive sites are guanines in the middle tetrad of the three-tetrad telomere quadruplex.

The effect of lesions of TTA loops between the guanine blocks has been found to depend on both the position and the type of the damage: The loss of the adenine base causes a change in quadruplex folding along with a slight destabilization of the structure, whereas the oxidation of adenine for the 8-oxoadenine rather increases the quadruplex stability . Surprisingly, substitution of 5-hydroxymethyluracil for thymine affects neither the topology nor the stability of the quadruplex.

Our results show that lesions of telomeric DNA affect the structure and stability of their quadruplexes. In view of the importance of the quadruplexes in the process of cell aging and carcinogenesis, the unrepaired damages in these genome regions may have serious biological consequences.

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References

1. Skolakova P., Bednarova K., Vorlickova M., Sagi J., *Quadruplexes of human telomere $dG_3(TTAG_3)_3$* sequences containing guanine abasic sites. Biochem. Biophys. Res. Commun., **399** (2010) 203-208.

2. Tomasko M., Vorlickova M., Sagi J., Substitution of adenine for guanine in the quadruplex-forming human telomere DNA sequence $G_3(T_2AG_3)_3$. Biochimie, **91** (2009) 171-179.

3. Vorlickova M., Tomasko M., Sagi A., Bednarova K., Sagi J., 8-oxoguanine in a quadruplex of the human telomere DNA sequence. FEBS J., **279** (2012) 29-39.

37. Self-pulsing and chaos in coupled ring resonators with non-instantaneous Kerr-nonlinear response

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We present dynamical analysis of nonlinear photonic structures consisting of two coupled ring resonators. We consider non-instantaneous Kerr response and the effect of loss. We demonstrate transitions from stable states to periodic (self-pulsing) and chaotic states. We identify parameters that can significantly affect onset of self-pulsing and chaos. We show the effects of loss and finite relaxation time on the period of self-pulsing states.

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38. Numerical Simulation of Thermoelectric Effect Spectroscopy and Thermal Stimulated Current methods

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Thermoelectric Effect Spectroscopy (TEES) and thermally stimulated current (TSC) are important methods used for characterization of parameters of deep levels in semiconductors (energy, capture-cross section and type of trap). We present a model based on numerical solution of kinetic equation, when trapping, emission and recombination are described by Shockley-Read-Hall model. The model calculates the thermally stimulated current as a function of temperature and heating rate. A set of simulations with different input parameters of deep levels was performed. The glow curves were then evaluated by heatimg rate method and the results were compared with the input parameters of the calculation. We observed, that if N levels are present in the system, N-1 peaks are observed in the spectrum. The formula including retrapping to the level was derived and the results of evaluation of capture-cross section were compared with the standard formula which neglects retrapping. Deviation of the established capture cross-section from the input value was demonstrated for the strong retrapping. It was concluded that the trap capture cross-section might be incorrect due to invalid premises of models, which neglect retrapping.

39. ROS-Auxin interplay in regulation of photosynthesis under stress

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Environmental cues represent the major hardships to crop productivity worldwide. Plant cells integrate photosynthetic, developmental and environmental stress signals through the modulation of reactive oxygen species (ROS) and hormone network signals. Particularly, ROS and auxin pathways are the main players that impact on plant stress adaptation responses.1 Owing to the vital importance of photosynthetic processes for plant growth and survival, a complex signaling network operates to control photosynthesis in response to biotic and abiotic stress which directly affects ROS and auxin homeostasis. Notwithstanding the large number of evidence showing interplay between ROS and auxin on photosynthesis, molecular insight into the nature of ROS-auxin crosstalk remains scarce. Therefore future studies are necessary to shed light on crosstalk between chloroplast and auxin network. Previously, it was demonstrated that the expression of a cyanobacterial flavodoxin (Fld) in chloroplasts prevents ROS accumulation under a wide range of environmental challenges, protecting thus photosynthesis from photo-inhibition.2 Therefore, Arabidopsis transgenic plants *(pfld)* can be used as an innovative tool to dissect the auxin-plastid ROS crosstalk regulatory network involved in photosynthesis regulation under stress by genetic screen.

As photosynthesis is one of the most relevant traits in plant breeding programs, the identification of genes involved in stress-induced photosynthesis regulation will bring novel approaches to optimize crop performance in changing environmental field conditions.

References

1. Tognetti et al., (2011).). Plant Cell & Environment. 35:321-333.
2. Zurbriggen et al., (2008a). Trends Biotechnol. 26: 531-537.

40. Physicochemical and Spectroscopic Characterization of Humic and Fulvic acids Isolated from South-Bohemian Peat

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The aim of this work was study chemical composition and structure of different HS. Object of our study were two samples HS which were isolated from South-Bohemian peat from the quarry Branná near Třeboň, Czech Republic. Isolation of HS was performed according to the procedure recommended by the IHSS. All samples of HS were characterized by elemental analysis (EA), ultraviolet-visible spectroscopy (UV/Vis), infrared spectroscopy (FTIR) and steady-state fluorescence spectroscopy. Absorption coefficients (E_{ET}/E_{Bz}, E_{250}/E_{365} and E_{465}/E_{665}) of HS were calculated from the absorbance values. Infrared spectroscopy is a useful technique in characterization of structure, functional groups and formation modes of HS. For the fluorescence experiments the final concentration of the HS was adjusted to 10 mg·dm^{-3}. The pH-value of the samples was adjusted to seven using a standard phosphate buffer. Fluorescence mono-dimensional spectra and total luminescence spectra (TLS) of peat HS were obtained using steady-state fluorescence spectroscopy. All fluorescence spectra were performed on a Horiba Scientific Fluorolog. The fluorescence intensity (I_F) values (in CPS) of samples were corrected using method of Lakowicz. Fluorescence coefficients (Milori index and HIX) of HS were calculated from the area of the emission spectra. Biological/autochthonous index (BIX) and fluorescence index (FI) of HS were calculated from the ratio of different emission intensities. Our results showed that the chemical properties of the HS from South-Bohemian peat used in these experiments were well described using seven complementary indexes derived from the ultraviolet-visible and fluorescence spectra (E_{ET}/E_{Bz}, E_{250}/E_{365}, E_{465}/E_{665}, FI, BIX, Milori index and HIX). Peat HA was characterized high molecular weight, low molecular heterogeneity, high degree of aromatic polycondensation, high level of conjugated fluorophores, and high humification degree. Fulvic acid was characterized and higher content O-containing functional groups (e.g. hydroxyl, carbonyl, ester, especially carboxyl groups on aromatic ring), lower molecular mass, simple structural components of wide molecular heterogeneity, lower degree of aromatic polycondensation, and lower humification degree.

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41. Piezoelectric Immunosensor for Detection of Aerosolized Microorganisms

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Detection of microorganisms has a crucial role in many fields. Even though the traditional microbiological methods are robust and provide low limits of detection, the analysis is usually time consuming. In some cases (e.g. monitoring of airborne pathogens), the fast detection is essential. Rapid and specific analysis can be provided by biosensors. In this work, the piezoelectric immunosensor for detection of aerosolized microorganisms was developed. Nonpathogenic *E. coli* K-12 was chosen as a biological warfare agents' surrogate. The biosensing layer was prepared by immobilization of specific antibodies using bifunctional crosslinker Sulfo-SMCC. To allow safe work with aerosolized microorganisms, bioaerosol chamber was constructed. The dissemination was done using a piezoelectric aerosol generator and the samples were collected by a wetted-wall cyclone on-line coupled with the piezoelectric immunosensor. The concentration 1.45×10^4 $CFU\cdot L^{-1}$ of *E. coli* K-12 in air was successfully detected in 16 min. The whole measurement cycle including sensor surface regeneration and filling the chamber with clean air was completed in 40 min. Reference measurements were done using commercial particle counter and by cultivation method. The specificity was also tested on real air samples. The achieved results indicate that the developed sensor can be used for screening of microorganisms in the air.

42. Telomere dynamics in the *Physcomitrella patens* model plant

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Moss species *Physcomitrella patens* is an important model organism for elucidation of the higher plants evolution because of the availability of a complete genome sequence and the ability of gene targeting due to the efficient system of DNA repair by homologous recombination. Telomeres are complex nucleoprotein structures protecting ends of linear eukaryotic chromosomes. Interestingly, many proteins involved in detection, signaling and repair of double-strand DNA breaks associate and form functional complexes with telomeres contributing to the maintenance of proper telomere structure and function. Our research is focused on the comparative analysis of telomere maintenance in common model plant *Arabidopsis thaliana* with low level of homologous recombination and *P. patens* with highly efficient homologous recombination; new data on respective crosstalk between these pathways and on their involvements in DNA damage processing and telomere maintenance are expected.

We analyzed telomerase transcription and activity and telomere lengths during *P. patens* culture growth and in mutant strains deficient in essential repair factors *(pprad50, ppmre11, ppnbs1, ppku70)*. The moss protonemas grow via apical cells division and with the increased mass of moss the number of dividing apical cells decreases. We observed slight but reproducible increase of telomerase activity and transcription in the first day after the sub-culturing when the level of apical cells was very high. In following time intervals, telomerase activity and transcription were comparable to the standard 7-day-old culture. Length of telomeres was maintained during the *P. patens* culturing which is compatible with the results obtained using higher plant models, where telomeres are preserved in all tissues.

In *P. patens* mutants, telomerase activity and transcription assayed by quantitative approaches did not significantly differ from values obtained for wild type culture. Interestingly, telomeres in mutants were more or less uniformly shortened. Based on these data, malfunction of proteins involved in repair and recombination pathways influenced telomere homeostasis by different ways compared to the common higher plant model *A. thaliana*.

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43. Explore the coevolution mechanism through Host-Parasite RNA experimental evolution

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A host-parasite relationship is universally seen in nature, in which host organisms are unilaterally exploited by parasitic organisms or viruses. Parasites are basically harmful existence for hosts in short time scale, but in long time scale, the existence of parasites is said to have greatly contributed to the evolution of their hosts, in that they might have accelerated host evolution through "evolutionary arms-race" or helped expanding the diversity of their hosts. Although there are a lot of hypotheses about host-parasite coevolution, there have been few experimental verifications due to the lack of a good experimental model. Here, we show an experimental approach to explore the mechanism of host-parasite coevolution through an evolvable artificial host-parasite system constructed in our laboratory. In this system, both of hosts and parasites are RNA and replicated by the replication enzyme encoded in the sequence of host RNA, and they have the ability to evolve by replication errors. Through the coevolution of host-parasite RNA in tiny water droplets dispersed in oil over many generations, we have observed that there are different phases of host-parasite population dynamics. We will further explore these host-parasite coevolution processes in molecular level, through sequence analyses and kinetic assays.

44. Tin Dioxide – Multiwalled Carbon Nanotubes Based Gas Sensor for Isobutene Detection

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The aim of this work is design, fabrication and characterization of semiconductive gas sensor with active layer based on tin dioxide and multiwalled carbon nanotubes for isobutene detection. Combination of LTCC technology and thick-film technology was chosen for sensor fabrication due to the need of high operating temperature. These technologies allow the sensor to be heated up to 500 °C. Gold/titanium interdigitated electrodes on silicon substrate were chosen to achieve higher sensitivity. Titanium layer has high temperature resistance and provides very good adhesion to silicon substrate and top gold layer that improves conductivity of these finger electrodes. Several important parameters of active layer influencing sensor properties were investigated. Influence of suitable volume of multiwalled carbon nanotubes in tin dioxide active layer, active layer thickness and operating temperature to sensing properties were studied. It was found that the active layer with the largest amount of multiwalled carbon nanotubes and operating temperature of 350 °C has the best sensitivity. The thinnest active layer has the fastest response and recovery time.

45. Influence of underlying network structure on accuracy of DCM estimation

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Dynamic causal modeling (DCM) is a method used for estimation of effective connectivity in neuroscience. The aim of this work is to test how structure of underlying network of effective connectivity in BOLD (blood oxygen level dependence) data affects accuracy of Variational Bayesian inference under Laplace assumptions using simulated BOLD signals with several levels of signal to noise ratio (SNR) and sampling period (TR). To test this influence we used 30870 realizations of Monte Carlo simulations. We created simulated BOLD signals of the length 800 s, generated through 20 types of simple network with 3 nodes and one exogenous input. We computed model specific mean of structural errors for every combination of TR and SNR. After this procedure we computed correlations on model specific mean of structural errors. This correlation matrix was used to compute agglomerative hierarchical cluster tree using ward method. According this tree we identified five families of models, four involved in compact clusters, fifth with longer distance to all other members of families. We realized that family 1 is under stronger influence of SNR than TR. Higher SNR and lower TR leads to better results. Family 2 has similar type of pattern, but values are globally one error worse. In family 3 is the influence of SNR and TR balanced. Family 4 shows similar pattern to family 3, influence of TR and SNR is balanced, but with lower SNR and higher TR are errors increasing slower than in family 3. Using simulated data, we observed expected effect of better DCM estimation with higher SNR and lower TR. In the model family 1 and 2, every model has direct connection between input and both nodes, which is not present in other families. This could cause the similarity of families 1 and 2. The family 2 contains models with more connections than most models in the family 1. This could cause more errors, especially in BOLD signals with lower SNR and higher TR. In the family 3 and 4, there is any case in which the input region is connected simultaneously to both adjacent nodes. This may cause similarity between these two families. Distances between members of the family 5 are too long; therefore looking for similarities is not as relevant as in other families.

46. The Case of Breast Cancer Resistance Protein in the GL261 Glioma Cells

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Glioblastoma multiforme is the most malignant brain tumor. Standard therapy consists of surgical removal of tumor followed by concomitant chemotherapy. However, patients suffer with the high recurrence of disease caused by inability to target the cancer stem cells (CSC) (for review see Šlampa et al. 2010). To study CSC, the mouse models were developed and even stable cell lines are available. One of them is line GL261, which features resemble the human type the most (Newcomb and Zagzag 2009). To get population of cells enriched with CSC the so called "side population" (SP) strategy is often used. SP cells, unlike the major cell population, has higher expression of transporting proteins from ABC family, usually Breast Cancer Resistance Protein (BCRP/ABC G2) and thus more effectively effluxes the fluorescent substrate of BCRP (e.g. Bunting et al. 2002). We aimed to use this method to obtain population with increased incidence of CSC to study the cell cycle-associated expression of regulatory proteins. However, literature provides opposite information regarding the ability to get SP, thus we had to perform our own study (Rappa et al. 2008, Wu et al. 2008). Level of BCRP mRNA in GL261 cell was similar to C2C12 mouse myoblast cell line, well known model for SP method having the high expression of BCRP (Adamski et al. 2007). Western blot analysis confirmed that C2C12 and GL261 have similar level of protein expression. Nevertheless, the analysis of BCRP function using its fluorescent substrates DCFH, Bodipy-prazosin, and pheophorbide revealed that the BCRP protein in GL261 cells is not working properly. This conclusion was confirmed also by different spatial distribution of protein in GL261 and C2C12 cells. Unsurprisingly, we were thus not able to obtain reliable assessment of SP in GL261 cells.

Adamski D., Mayol J-F., Platet N., et al., 2007, Effects of Hoechst 33342 on C2C12 and PC12 cell differentiation, FEBS Letters, 581(16): 3076–3080.
Bunting K.D., 2002, ABC transporters as phenotypic markers and functional regulators of stem cells. Stem Cells, 20(1):11-20.
Newcomb and Zagzag, 2009, The Murine GL261 Glioma Experimental Model to Assess Novel Brain Tumor Treatments, CNS Cancer, Cancer Drug Discovery and Development: 227-241.
Šlampa, P., Burkoň, P., Komínek, L., 2010, Glioblastoma – treatment overview, Postgraduální medicína, vol. 7.
Rappa G., Mercapide J., et al., 2008, Growth of cancer cell lines under stem cell-like conditions has the potential to unveil therapeutic targets, Exp Cell Res, 314 (10): 2110-2122.
Wu A., Oh S., Wiesner S.M., 2008, Persistence of CD133+ cells in human and mouse glioma cell lines: detailed characterization of GL261 glioma cells with cancer stem cell-like properties. Stem Cells Dev, 17(1):173-84.

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47. MARs contain strong nucleosomes

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Introduction

Recent studies showed that arrays of strong nucleosomes (i.e. DNA sequences with high affinity to histone octamer) are present in the centromeres of mouse X chromosome, *A. thaliana* chromosomes and meitoic chromosomes of *C. elegans*. These studies indicate a functional role of large-scale chromatin architecture.
Another point of interest in this area is nuclear matrix or scaffold. Chromatin fiber loops were shown to bind to the nuclear matrix and the matrix is suspected to influence the gene regulation. The attachment of the chromatin to the matrix is intervened by specific loci in the DNA sequence, called matrix attachment regions (MARs).

Methods

A database of 1358 MARs in *Arabidopsis thaliana* chromosome 4 has been published recently (Pascuzzi et al., 2014). We examined the affinity of these sequences to histone octamers with a sequence probe described in (Tripathi et al., 2014).

Results

Contrary to the findings in (Pascuzzi et al., 2014), we have found nucleosomes in the MARs data. Moreover, we identified 21 sequences with nucleosome positioning score above the threshold used in (Salih et al., 2013) for strong nucleosomes. The density of strong nucleosomes in MARs is as high as in centromere regions of *A. thaliana* genome.

References

Pascuzzi PE, Flores-Vergara MA, Lee TJ, Sosinski B, Vaughn MW, Hanley-Bowdoin L, Thompson WF, Allen GC (2014). In Vivo Mapping of Arabidopsis Scaffold/Matrix Attachment Regions Reveals Link to Nucleosome-Disfavoring Poly (dA: dT) Tracts. *Plant Cell*, 26(1).

Salih BF, Teif VB, Tripathi V, Trifonov EN (2013). Strong nucleosomes of A. thaliana concentrate in centromere regions. *J Biomol Struct Dyn*, published online 27 Nov 2013.

Tripathi V, Salih BF, Trifonov EN (2014). Universal full length nucleosome mapping sequence probe. *J Biomol Struct Dyn*, published online 10 Mar 2014.

48. Application of benzofurazane and nitrophenyl redox labels in electrochemical analysis of DNA nucleotide sequences

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DNA electrochemical biosensors are rapidly developing field in nowadays. Besides intrinsic DNA electroactivity connected mainly with reduction or oxidation of bases residue [1] utilization of different redox DNA chemical labels found use in this field [2]. Enzymatic incorporation of chemically modified deoxynucleoside triphosphates (dNTP) by DNA polymerases is one from methods for preparing of DNA bearing redox labels [3]. Chemically modified dNTPs can be synthesized by simple cross-coupling reaction in aqueous media. In this contribution will be presented application of completely new redox DNA label benzofurazane for electrochemical analysis of DNA [4]. PEX incorporation of the benzofurazane-modified dNTPs by DNA polymerases has been developed for the construction of labeled oligonucleotide probes. The combination of two reducible labels benzofurazane and nitrophenyl has proved to be excellent for ratiometric analysis of DNA nucleotide sequences and is suitable for bioanalytical applications.

References

[1] E. Palecek, M. Bartosik, Chem. Rev. 112 (2012) 3427
[2] M. Fojta, L. Havran, H. Pivonkova, P. Horakova, and M. Hocek Curr. Org. Chem., 15 (2011) 2936
[3] M. Hocek, M. Fojta, Org. Biomol. Chem., 6 (2008) 2233
[4] J. Balintova, M. Plucnara, P. Vidlakova, R. Pohl, L. Havran, M. Fojta, and M. Hocek, Chem. Eur. J., 19 (2013) 12720

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49. Thin Chalcogenide Films - Promising Materials for Optical Storage

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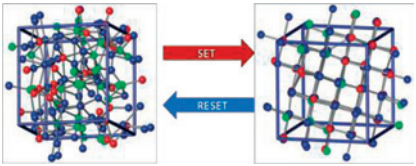
Phase-change memory (PCM) depends upon the ability of the active material to reversibly switch, upon the application of a current pulse, between a high-resistance "RESET" state, corresponding to the amorphous phase of the phase-change material, and a low-resistance crystalline "SET" state. Detailed knowledge of the structure and properties of thin films is the first step for possible application. Ge-Se-Te thin films seems to be promising material.

Thin films (~1 μm) Ge_{30} $Se_{70-x}Te_x$ (x = 0, 10, 20) were prepared from bulk glasses. The glass transition and crystallization of bulks and films decrease with increasing Te content. Comparing to bulks, both characteristics temperatures are lower for the thin films. Optical gap also decreases with Te content. Structure was studied using Raman spectroscopy too. Te-Te-Te vibrations were not observed, so it appears that Te is gradually built into the glassy matrix forming mixed $GeSe_{(4-x/2)}Te_{x/2}$ and $GeTe_{4/2}$ tetrahedra. The main task for the future is to solve the problem of the structural model for the glassy network.



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50. A new electrochemical DNA binding competition assay using oligonucleotide substrates tail-labeled with two different redox tags

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We present a new approach for detection of protein binding to various DNA substrates. Terminal deoxynucleotidyl transferase (TdT) can create DNA tails as it attaches nucleotides at the 3´OH terminus of DNA using dNTPs as substrates [1]. Previously it was shown that DNA tail-labeled using TdT can be used for immunoprecipitation binding experiments with tumor suppressor protein p53 and that sequence-specific DNA binding can be distinguished from non-specific DNA binding [2]. However, application of a single DNA label did not enable to conduct competition assays for a direct determining which DNA substrate is chosen by the protein from a mixture. Therefore, a new methodology using labeling by two different modified deoxynucleotide triphosphates has been developed. Two types of single-stranded oligonucleotides (containing or lacking p53 binding site; PGM1 or noCON) were tail-labeled with either nitrophenyl or benzofurazan modified dATPs using TdT. For the p53-DNA binding a previously introduced magnetic beads immunoprecipitation assay was used [3]. The recovered tail-labeled oligonucleotides were analyzed using cyclic voltammetry at a hanging mercury drop electrode (HMDE). Cyclic voltammogram of oligonucleotides released from the magnetic beads showed two peaks typical for DNA, an anodic G peak at potential -0.25 V and a cathodic CA peak at -1.5 V. Additional peaks could be observed, depending on the label used: a cathodic peak typical for NO_2 at -0.5 V [2] for oligonucleotides tail-labelled with $dA^{NO2}TP$ or a cathodic peak typical for benzofurazan at -0.85 V [4] for oligonucleotides tail-labeled with $dA^{BF}TP$. Both peaks were well developed when the respective oligonucleotides were used for the binding experiments. When the oligonucleotides were used for the competition binding experiment, the intensity of the peaks decreased as the labeled DNA substrates mutually competed for the protein. The decrease of the signal was more significant for the peak gained for the noCON substrate (e.g. peak BF, where noCON substrate was labeled with BF and PGM1 with NO_2) whereas the decrease of the peak gained for the PGM1 was only weak, which corresponds to the binding specificity of the p53 protein. This approach enables distinguishing between sequence-specific and non-specific p53-DNA binding when using $dA^{NO2}TP$ and $dA^{BF}TP$ tail-labeled oligonucleotides as DNA substrates. However, more experiments need to be conducted in order to allow quantification of the gained results and exact determination of the level of sequence-specific and non-specific p53-DNA binding.

[1] Michelson, A. M., Orkin S.H., *J. Biol. Chem*. 1982, *257*, 14773-14782.
[2] Horáková, P., Macíčková-Cahová, H., Pivoňková, H., Špaček, J., Havran, L., Hocek, M., Fojta, M., *Org. Biomol. Chem*. 2011, *9*, 1366-1371.
[3] Pivoňková, H., Šebest, P., Pečinka, P., Tichá, O., Němcová, K., Brázdová, M., Brázdová-Jagelská, E., Brázda, V., Fojta, M., *Biochem. Biophys. Res. Commun*. 2010, *393*, 894-899.
[4] Balintová, J., Plucnara, M., Vidláková, P., Pohl, R., Havran, L., Fojta, M., Hocek, M., *Chem. Eur. J*. 2013, *19*, 12720-12731.

51. What determines chromosome breakpoints in crucifer species?

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Genomes of crucifer species (Brassicaceae) show extensive inter-species chromosome collinearity as shown by comparative chromosome painting studies and comparative genomic sequencing. In several crucifer lineages, ancestral genomes have been altered by large-scale chromosome rearrangements which reshuffled genomic blocks and independently decreased chromosome numbers (e.g., n = 8 7-5; n = 21 10). Here we aimed to analyse whether specific sequence motifs or other genomic characteristics can be associated with chromosome breakpoints. In multi-species comparison, we compared the arrangement of genomic blocks and chromosome breakpoints sequences among the sequenced genomes of *Arabidopsis lyrata* (n = 8), *Arabidopsis thaliana* (n = 5), *Brassica rapa* (n = 10), *Schrenkiella parvula* (syn. *Thellungiella parvula*; n = 7) and *Camelina sativa* (n = 20).

Bioinformatic analyses involved two steps: (i) localization of chromosome breakpoint sequences along the sequenced genomes, and (ii) sequence analysis of the identified breakpoint sequences as well as their flanking regions, and the whole genomes. Genome-wide inter-species collinearity was detected by SynOrths, the *A. thaliana* genome was used as the reference as the best characterized crucifer genome. Nucmer, a part of the MUMmer software, was used to compare breakpoint sequences among the analyzed genomes. *De novo* repeat family identification was done in all five species using the RepeatModeler and RepeatMasker. Non-overlapping 10 kb windows along the genomes were used to examine the genome-wide distribution of repeats along the genomes, except for telomeric and cetromeric regions.

Our analyses suggest that chromosome breakpoint regions in Brassicaceae species generally exhibit a lower GC content (Mann-Whitney U test, $p < 0.05$) and are enriched for tandem repeats (Mann-Whitney U test, $p < 0.05$).

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52. Using of microsatellite markers for analysis of functionally important genetic diversity in horses

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Major histocompatibility complex (MHC) is immunologically important region in genome of vertebrates. Genes in MHC encode proteins, which are involved in innate and adaptive immune response. Genetic changes in this region can have effect on immune reaction in animals. But complex documentation of genetic variation in MHC absents in most domestic animals, including horses. MHC in horses is located on chromo-some 20, region 27 403 526 – 33 865 081 bp. The aim of this study was utilisation of described microsatellite markers to analyse the genetic diversity of selected populations of horses. This study extend the original panel of five microsatellites (Klumplerová et al., 2013) about next five microsatellites for better and uniform coverage of whole MHC region.

53. Study of *Ly49* genes diversity in the horse using microsatellites

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Domestic mammals represent suitable models for evolutionary biology in general. The genes of the immune response represent a functionally important region of vertebrate genome with demonstrated intense effect of selection. Natural killer cells (NK) are related to antigen recognition process through their highly variable receptors. The genetic variability and different expression of genes for receptors underlies functional variability of individual NK cells. As like in mouse model, the Ly49 receptors on horse NK cells are believed to bind to MHC class I molecules of target cells. The six Ly49 genes form a gene family located on horse chromosome 6 between 38 200 Kbp – 38 500 Kbp. Identification and genotyping of microsatellites located in Ly49 region using this methodological work may contribute to the estimate of its genetic diversity.

The whole genome sequences of six horses in the areas of Ly49 gene family and adjacent parts (35 – 43 Mbp) were used to select markers in silico. For such markers, primers were designed and optimized to allow multiplex amplification and paralleled detection of alleles. Nine markers that showed the highest variability in the test panel of animals were selected. This set of markers was subsequently tested on nine populations of various horse breeds from different parts of the world using fluorescent fragment analysis on genetic analyzer ABI PRISM 3500. The numbers of alleles found in microsatellite markers were identified in populations of fifteen horse breeds from different parts of the world, where 400 individuals were included. Genetic diversity of the Ly49 region will be estimated based on allele frequencies of the selected microsatellites.

This work extends the number of genetic markers for analysis of Ly49 NK cell receptors genetic variability. We enriched the set of usable markers with 9 microsatellites. The combined genotyping of SNPs and microsatellites may help to define haplotypes of Ly49 genes. The family Equidae consisting of a single genus, Equus with different free-living and domesticated species exposed to a variety of pathogens in different habitats is a suitable model for analyzing diversity and evolution of immunity-related genes. Haplotypes may be more informative for describing the genetic variability in this functionally significant and important part of the immune system.

54. Interactions between receiver domain of cytokinin receptor CKI1RD and AHP protein from Arabidopsis thaliana

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In our project, we studied signaling pathway, called multistep phosphorelay signaling system in the plant *Arabidopsis thaliana*. The multistep phosphorelay signaling system has a great influence on many aspects of growth and development of plants. This signaling system is based on phosphate transfer between the cytoplasmatic membrane and nucleus. In the plant *Arabidopsis thaliana*, histidine kinase is phosphorylated upon signal recognition, and forwards the phosphate group through histidine phosphotransfer proteins to a response regulator protein located in nucleus, where the response take place. The input signal can be light, osmotic changes or hormones. The interactions between the two proteins were studied by nuclear magnetic resonance. The first protein was $CKI1_{RD}$ (Cytokinin independent 1 - receiver domain) and the second was protein from the AHP (*Arabidopsis* histidine phosphotransfer protein) family, both involved in the multistep phosphorelay. The proteins were expressed in *E.coli* and the protein $CKI1_{RD}$ was labeled by stable isotope ^{15}N.

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55. The Investigation of Mechanical Properties the Synthetic Mineral Alloys in Conditions of Static Loading

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The synthetic mineral alloys - are materials produced by melting from silicate raw, them structure contain the crystalline and amorphous phases, which have a different size and a different shapes, this materials often have low tensile strength and high compressive strength (220-250 MPa). High compressive strength and low price of producing give opportunity to use the synthetic mineral alloys as construction material, for example, for building include underground construction, but it is fairly fragile material and for using them in construction, very important investigate mechanical properties the synthetic mineral alloys in conditions of static loading.
There are many different types of the synthetic mineral alloys, in this research the object of study is alloys based on the system SiO_2-Al_2O_3-FeO.
The method of test was uniaxial compression, recording acoustic emission and determination of the exfoliation microparticles from the surface of samples in the process of loading. Experiments on uniaxial compression was made by Zwick - Z250. In the process of loading the samples is deforming, the accumulation of early stress and generation of microcracks, all this phenomenon can be determined through acoustic emission (AE). Register acoustic activity in the process of loading secured with AES device - USB – 8. In the tests, we obtained graphical diagrams characterizing load growth and activity of the AE during the tests. The exfoliation from the surface of samples in the process of loading we determinate by our self method which assumes sedimentation microparticles from the surface of samples in the process of loading in the water and fallowing investigation of the solute with that particles. The result of the investigation of the solute is fraction composition of particles and this data can use for assessment deformation in early stage.

The investigation showed that the processes of deformation and fracture of the synthetic mineral alloys in conditions of static loading beginning from changes in the amorphous phase. It is followed by a period of accumulation of local structural stresses. After that followed the relaxation of the formation and rapid growth of cracks. Determined that, the nature of the acoustic oscillations depends on the structure of the amorphous component, more than her, the faster the destruction and the less manifest in different stages of the process. Since the structure of the material is not homogeneous and the destruction of their uneven. The beginning of destruction begins in the exfoliation from the surface and us method give opportunity to determination qualitative assessment of destruction in first beginning stage it mean that we can do practical experiments for determination recommendation for work of products and construction in building construction.

56. The development of Theory of mind from middle childhood into adolescence

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Theory of mind (ToM) is the ability to imagine and understand our own mental states and the mental states of others (e.g. desires, beliefs or thoughts). Preschool children can master 2nd order of intentionality while the peak in adults is around 5th order of intentionality. ToM continues to develop due to integration with other cognitive processes such as memory and executive functioning. Rapid development of ToM is expected to occur during school years as the interaction with peers increases dramatically in this age. The aim of this study was to investigate this assumption. Three stories with complex social scenarios, developed for this purpose, were presented to middle childhood group (age 9-10, N = 29) and adolescent group (age 13-14, N = 43). After each story, participants were asked to fill out questions about mental states of others (experimental condition measuring orders of intentionality) or about simple facts (control condition). The average order of intentionality achieved by adolescents was found to be significantly higher (m = 4.58, sd = 0.48) than that of a younger group (m = 3.92, sd = 0.79). The adolescent group performed better also in control condition (m = 13.4, sd = 0.9) than younger group (m = 12.66, sd = 1.2). When analyzed separately, only one story followed the same pattern. Significant differences between age groups in other two stories were found only in ToM condition while memory performance was comparable across age groups. However, ToM performance showed rather continuous improvement with age, which is reflected in rich social life even in middle childhood. In addition, the relationship between ToM and memory was found significant only in middle childhood group (r_s = .60; p = .001) and not in the adolescent group (r_s = .16; p = .311), suggesting that the development of ToM might be still dependent on other cognitive processes (such as memory or reading literacy) in this age.

57. Modeling of the N-Acetylglucosaminyltransferase V (GnT-V)

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Glycosyltransferases are group of enzymes, that catalyze the formation of glycosidic linkages by transferring sugar moiety from activated donor substrates to a variety of different acceptor substrates. As such they are responsible for synthesis of complex glycan structures, that have been shown to play crucial roles in cell–cell, cell–matrix and cell–pathogen interactions and thus impact huge range of biological processes.

The subject of this study is N-Acetylglucosaminyltransferase V (GnT-V), a glycosyltranferase involved in biosynthesis of asparagine-linked oligosaccharides. Specifically it catalyzes the transfer of GlcNAc moiety from UDP–GlcNAc to the 6 position of the α-1-6 linked Man residue to the core N-glycans to form branched tri- and tetra-antenna-like oligosaccharides. GnT-V has been linked to cancer growth and metastasis, which makes it an interesting target for drug development. However the lack of structural information on this enzyme is holding these efforts back. In our work, we attempt to devise structural model of GnT-V by means of homology modeling with aims to use it to guide future experiments, to elucidate the catalytic mechanism and to identify amino acids crucial for its function.

58. Synthesis of carbon nanosheets using microwave plasma torch at atmospheric pressure

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Graphene and graphene related materials are rapidly progressing field in many areas of basic and applied research. In our work we used microwave plasma torch (2.45 GHz, 140-300 W) at atmospheric pressure to synthesize carbon nanosheets consisting of 1 or several graphene layers with rectangular shape and typical size of hundreds of nanometers. The synthesis is based on the decomposition of organic precursor (ethanol, toluene 0.02-0.06 g/min) which is introduced by carried gas (Ar, 700-2800 sccm), in given, center or outer, channel of our nozzle. The torch is ignited in argon (250-1000 sccm) flowing through the central channel. The plasma torch system is enclosed in quartz tube (8 cm diameter, 20 cm length) with flanges. The synthesized nanosheets are collected on the silicon substrate located in the sample holder below the top flange or from the tube wall. The deposition process (discharge power, gas flows, substrate temperature) is investigated with aim to obtain maximum amount of single layer graphene. Such prepared samples are analyzed by scanning and transmission electron microscopy, Raman spectroscopy and energy-dispersive X-ray spectroscopy (EDX).

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59. Reactivation of Mutated p53 in Chronic Lymphocytic Leukemia Cells Using Prima-1^{MET}

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In chronic lymphocytic leukemia (CLL), *TP53* mutations are connected with strong resistance to current therapies; therefore, alternative treatment approaches are highly desirable.

We tested possibility of subtle molecular manipulation of accumulated mutated p53 in CLL cells using small molecule PRIMA-1^{MET} presumably leading to restoration of wild-type p53 conformation. The reactivation was tested at p53 protein level by WB and at p53 downstream pathway by qRT-PCR. We first assessed CLL cells metabolic activity after 48h PRIMA-1^{MET} treatment to determine adequate concentrations for the reactivation. While higher concentrations (›10μM) led to severe damage of CLL cells, the lower ones (up to 4μM) provided concentration-dependent curve of viability in all studied samples (n=5). Thus, the 2μM and 4μM concentrations were further applied for p53 reactivation testing at molecular level. Among 7 p53 mutated samples, 4 showed clearly diminished or completely absent p53 protein level after 4μM PRIMA-1^{MET} treatment and 2 manifested partially reduced level. The best response was observed for mutations Y205C and C275R, where the p53 protein level was completely eliminated even at 2μM concentration. The p53 downstream genes expression was heterogeneous among the samples, with the induction of *p21* and *GADD45* being much more prominent compared to *PUMA* and *BAX*. The *p21* and *GADD45* expression was especially strong in 2 samples with complete p53 protein elimination (Y205C, C275R) and also in samples with C277F and S127F mutation.

Our results demonstrate that PRIMA-1^{MET} can effectively manipulate mutated p53 protein in CLL cells and could be tested in this disease for therapeutic application to design a rational and effective therapy.

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60. Study of Copper Substrate Pretreatment During CVD Growth of Graphene

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„Pretreatment of copper substrates is an important step in graphene production by thermal CVD process. In our work we investigated behaviour of copper catalyst during controled heating and annealing under various conditions. We used two types of substrates, first group of substrates are twotypes of 25 micrometer thin copper foil - Alfa.

Aesar (item No. 13382) and MTI (EQ-bccf-25u). We used this foil in „as is" and cleaned by acetone and IPA. The second group of substrates is formed by a silicon wafer (10x15 mm) with 100 nm thermal silicon oxide overlayer with thermally evaporated copper in form of 8 mm diameter disc(thickness 300 nm and 500 nm). Experiments were carried in evacuated laboratory furnace. We modified annealing temperature (700, 800, 900 and 1000 C), substrate heating rate and an atmosphere in the furnace (vaccum, hydrogen and argon or their mixture). The copper substrates annealed under these conditions were analyzed by optical and scanning electron microscopy. The analysis result showed different copper recrystalisation, especially grain size above 800 C and disintegration of evaporated films, copper dewetting on SiO2, depending on the annealing atmosphere. Graphene growth is tested on selected substrates."

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61. Structural basis for the dual role of Rtt103p in transcription termination and RNA processing

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In *Saccharomyces cerevisiae*, Rtt103p is a part of the complex that recognizes RNA polymerase II (RNA pol II) C-terminal domain (CTD) phosphorylated at Serine 2 (pSer2), thus bridging 5'-3' exoribonuclease, Rat1/Rai1, to RNA pol II during transcription of mRNAs. Rat1/Rai complex is believed to terminate the transcription process by itself or with the help of Pcf11 protein. In contrast to mRNAs, transcription termination of non-coding RNAs is performed by the Nrd1-Nab3-Sen1 (NNS) complex, and is followed by 3'-5' RNA degradation/processing by the nuclear exosome.

In our previous work, we have shown that transcription termination of non-coding RNAs transcribed by RNA pol II is physically coupled to RNA degradation/processing. The coupling is achieved by to the CTD-interacting domain (CID) of Nrd1p that recognizes a CTD-mimic (Nrd1p-interactin motif, NIM) within the TRAMP (Trf4-Air2-Mtr4) complex, a cofactor of the nuclear exosome. Here, we show that CID of Rtt103p also recognizes the NIM, and another region of Trf4p specific only to Rtt103p, the RIM (Rtt103-interacting motif). Specifically, we report the structure of Rtt103p-CID bound to Trf4p-NIM and initial structural studies of the complex between Rtt103p-CID and Trf4p-RIM. We show that Rtt103p-CID utilizes the same pocket to bind both CTD pSer2 and Trf4p-NIM. Overall, our results unveil a novel role of the Rtt103p-CID in not only transcription termination but also in RNA processing by the nuclear exosome.

62. Light Scattering Techniques Applied for the Study of Aging of Biopolymers and Biocolloids

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Biopolymers and biocolloids as natural product are often subjected to aggregation and degradation. Both these processes are extremely important and must be taken into account in the case of industrial application of these natural substances. Aggregated or degraded material used as a raw material for industrial production can significantly change the final yield and properties of product. This contribution is discussing the application of light scattering techniques for observation of stability of these compounds in time and for study of influence of different parameters (UV light, temperature, pH, ionic strength) on the stability. In experimental work proteins (bovine serum albumin, cystein) and polysaccharides (chitosan, glucose, sucrose, carboxymethylcellulose and sodium alginate) were used as model biopolymers. Biocolloids were represented by humic acids. Stability and degradation of proteins and polysaccharides was studied using zeta potential (method of electrophoretic light scattering) and molecular weight determination (combination of size exclusion chromatography with multiangle light scattering detection). Aging of humic acids was investigated by means of zeta potential differences in time and time development of particle size (method of dynamic light scattering). Results showed that light scattering techniques can be used as easy and quick methods for purposes of detection of the aggregation or degradation of biomaterials. These methods can be useful either in the case of checking of stored stock solutions, for continual controlling of raw materials for industrial production and also for detection of any deviations of final products.

63. The role of surface stress in phase diagrams of Ni –Sn–Sb nanoalloys

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Making an assessment of phase diagrams for Ni–Sn–Sb nanoalloys is motivated by their technological application as lead-free solders. For calculations of binary phase diagrams of the Ni–Sn–Sb system in the bulk phase, one mostly uses the *CALPHAD* method [1]. However, determining phase diagrams of Ni–Sn–Sb nanoalloys requires the knowledge of the additional part G^{surf} of the Gibbs free energy that corresponds to the surface stress in nanoalloys. The total Gibbs energy of a nanoalloy can be written as

$$G^{nanoalloy} = G^{bulk} + G^{surf} = G^{bulk} + 2\,C\,\sigma\,V_m / r, \qquad (1)$$

where σ stands for surface stress, V_m for molar volume, r is the nanoparticle radius and C is a constant depending on the shape of nanoparticles. We usually assume spherical nanoparticles characterized by their radius r.

In order to estimate the surface stress of Ni, Sn, Sb and their most common intermetallics, we performed *ab initio* calculations in the frame of density functional theory (DFT) as implemented in the code WIEN2k [2]. The calculated values then serve as an input to the *CALPHAD* method. In comparison to the bulk Ni–Sn–Sb system, the melting point of the nanoalloys and the temperature of their eutectic reactions decrease with the decreasing radius r.

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[1] N. Saunders, A. P. Miodownik, *CALPHAD (A Comprehensive Guide)*, Pergamon Press, Oxford, 1998.
[2] P. Blaha, K. Schwarz, G. K. H. Madsen, D. Kvasnicka and J. Luitz, *WIEN2k, An Augmented Plane Wave + Local Orbitals Program for Calculating Crystal Properties* (Karlheinz Schwarz, Techn. Universität Wien, Austria), 2001.

64. Photocatalytic water splitting by Ln-doped TiO_2 (Ln = Pr, Dy, Sm)

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Ln-doped TiO_2 nanoparticles (Ln = Pr, Dy, Sm) were prepared by the sol-gel low-temperature reaction of titanium tetraisopropoxide with aqueous solutions of organic acid and lanthanide salts. Reaction was carried out with 0.3%, 0.5% and 0.7% lanthanide ions in acidic solution at 100 °C for 10 hours. XRD analysis revealed the content of crystalline phase of anatase and brookite. Prepared nanoparticles contained crystallites ranging in size from 2.0 to 5.0 nm. Specific surface area of Ln-doped TiO_2 ranged from 210-250 m^2/g. Differential photocatalytic activity of titania nanoparticles was evaluated at photocatalytic water splitting (of 20% aqueous solution of methanol) in batch quartz reactor. The hydrogen content in the gas phase was measured by mass spectrometry. Co-catalyst, 0.5% of Pt, was deposited on the surface of titania by photochemical method. It was found that the higher photocatalytic activity had sample with 0.7% of Dy and its photoactivity was increased with the content of dysprosium ions in biphasic catalyst.

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65. Porous aromatic organosilicates by non-hydrolitic sol-gel routes

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A series of porous hybrid organosilicate materials was prepared via non-hydrolytic sol-gel reactions of silicon(IV) acetate with rigid aromatic building blocks bearing phenolic hydroxyl functional groups with focus on readily available compounds that were previously utilized for synthesis of materials such as the porous aromatic frameworks (PAFs) and covalent organic frameworks (COFs). Upon elimination of acetic acid byproduct an organosilicate gel is formed in polar ether solvent. Drying in vacuo offers powdered xerogels. The synthesis procedure was optimised in order to reach maximum degree of condensation (as monitored by gravimmetry). All of the prepared materials were found to be amorphous, they do however possess large specific surface areas exceeding 800 m^2 g-1 and generally exhibit high thermal stability (up to 400 °C under inert gas). The relationship between size and symmetry of employed organic linker and the surface properties as well as the degree of condensation and thermal behavior were studied. Additionally the chemical properties and stability of these materials were studied in reactions with a variety of organic, inorganic and organometallic reagents (water, alcohols, phenol, chlorosilanes, AlR_3, $AlCl_3$, n-BuLi, ...). A method for post-synthetic replacement of the highly coordinating residual acetate functional groups was also found. Products were characterized by elemental analyses, solid-state ^{13}C, ^{27}Al, and ^{29}Si NMR, IR spectroscopy, surface area analysis, thermal analysis TG/DSC, and XRD measurements.

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66. A Longitudinal Diffusion Kurtosis Imaging Study in TNWT-61 Transgenic Mouse Model of Parkinson´s Disease: Comparison with Conventional Diffusion Tensor Imaging

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Purpose: Compelling evidence suggests that accumulation and aggregation of α-synuclein contribute to the pathogenesis of Parkinson's disease. The aim of this study was to evaluate age dependent changes in grey matter microstructure induced by α-synuclein aggregation in the transgenic human α-synuclein overexpressing mouse model (TNWT-61) by MRI diffusion weighted imaging (kurtosis – DKI and tensor imaging – DTI). We hypothesized that the presence of α-synuclein aggregates in the transgenic mouse model would create more water diffusion barriers resulting in higher kurtosis.

Methods: TNWT-61 mice and wild type (WT) littermates (9 and 14 months old) underwent DKI and DTI scanning using 9.4 T Bruker system in vivo. Kurtosis and diffusion metrics were obtained for substantia nigra, striatum, hippocampus, sensorimotor cortex and thalamus.

Results: TNWT-61 9 month old mice showed a significant increase in mean and radial kurtosis as compared to WT littermates. Interestingly in the 14 month old TNWT-61 group similar kurtosis values were obtained as in the 9 month old animals. However WT showed an age dependent increase in kurtosis metrics and this effect abolished the difference between the transgenic and WT animals in 14 month old mice. There were no mean, radial and axial diffusivity differences observed in any of the groups.

Conclusion: The current study provides evidence that DKI has the potential to improve the clinical diagnosis of PD compared to conventional DTI at the earlier stage of pathology. DKI is also highly sensitive for age dependent microstructural changes in the basal ganglia.

67. Improvements in the method for measuring enzyme kinetics of β-glucosidases

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The maize β-glucosidase Zm-p60.1 is important for the regulation of plant development through its role in the targeted release of free cytokinins from cytokinin-O-glucosides, their inactive storage forms. Enzyme kinetics studies using these scarce substrates close to physiological concentrations are difficult. We developed a glucose assay using a system comprising three enzymes β-glucosidase, glucose oxidase and horseradish peroxidase, with the substrate N-acetyl-3, 7-dihydroxyphenoxazine. The assay is suited for automated procedures, and potential applications include its use in studying the physiological role(s) of enzymes that cleave scarce glucoside substrates and development of high-throughput assays for new β-glucosidases testing.

Enzyme kinetics of β-glucosidase Zm-p60.1 was measured by this improved method on natural substrate trans-zeatin-O-β-D-glucopyranoside. During development of this method we reached a partial miniaturization. Volume of reaction mixture was lowered as well as amount of sample which is desirable because natural substrates are very expensive.

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68. Graphene as a promising material for preparation of an ultrasensitive lectin biosensor

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Nanotechnology is dealing with materials, which have at least one dimension less than 100 nm. At nanoscale, matter often exhibits impressive properties in comparison to their macroscopic counterparts. Therefore, nanomaterials have attracted attention in the field of biosensing where they are widely used for improving their operational features. The term "graphene" represents a one-atom-thick planar sheet of sp2-bonded carbon atoms arranged in a honeycomb crystal lattice. This structure of carbon was discovered in 2004 and due to its exceptional properties (e.g. very good room-temperature electron mobility, high thermal conductivity, extremely high sensing surface, easy modification, biocompatibility) it is nowadays commonly used in designing of various types of biosensors. Generally, lectin biosensor consists of three basic elements: lectin, a transducer and a detector. Such a device can be used for distinguishing normal from aberrant glycosylation patterns or for searching for potential new biomarkers of many diseases. Lectin biosensor utilising high surface area of graphene introduces the possibility for immobilisation of larger amount of lectin and thus improve the sensitivity of prepared biosensor. Although there are many bioassays reported, which employ lectins as a biorecognition element, the concept combining lectins and graphene-based materials is still rare.

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69. Function of cyclin-dependent kinase 12 in mouse development

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Gene expression is a highly organized and tightly controlled process involved in a broad spectrum of biological processes, ultimately giving cells the ability to take control of their growth, cell division, apoptosis, differentiation and developmental potential. Regulation of gene expression is orchestrated primarily at the level of transcription of specific mRNAs by RNA polymerase II (RNAPII) and its activity is primarily regulated at the level of posttranslational modifications, among them phosphorylation is the most prevalent. We have demonstrated that the cyclin-dependent kinase 12 (CDK12) phosphorylates RNAPII and thereby regulates expression of several DDR genes, such as BRCA1, RAD51, ATR, FANCI and FANCD2 proteins. Even though down regulation of the CDK12 led to sensitivity to DNA damaging agents and genomic instability, yet, the loss of CDK12 in mouse might have different reasons. In order to examine function of CDK12 in mouse development, we employed TALEN (transcription activator-like effector nucleases) to generate mouse with deletion of several nucleotides in exon 4 of CDK12 gene leading to expression of truncated CDK12 form in all cells. Mice with wild type allele were born at regular rates and appear normal yet, CDK12 complete knock-out mice (CDK12 CKO) were not born at all. Detailed analyses revealed lethality of CDK12 CKO mice around 5.5 days post conception resembling severe developmental defects. At the moment, we are dissecting functional consequences of CDK12 loss-of-function during the preimplantational stage.

In summary, genetic depletion of CDK12 leads to early embryonic lethality in mice most likely due to increased aberrant development and genetic instability.

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70. The formation of barrier-type anodic films on aluminium: a tool for estimating the quality of thin metal layers

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Porous anodic alumina (PAA) grown on Al in certain electrolytes has received numerous studied due to its unique self-organized nanostructured porous-cellular nature and diverse applications as protective and decorating layers, templates, functional coatings for electronics and optics. However, the formation of well-ordered PAA on thin Al layers remains a challenge due to the thickness-related limitation for growth of low-aspect ratio nanopores and physical imperfections in the films. Thus, preparation of uniform continuous defect-free Al layers is a must for further development of high-quality PAA films and related nanomaterials.

In this work we have begun a systematic study of electrochemical responses during the formation of barrier-type anodic films on thin Al layers prepared by various vacuum deposition techniques striving to reveal and exploit the relation between the morphology, structure and physical properties of thin aluminium films and their anodizing behavior.

A citric acid solution of pH 3 and a sodium borate buffer of pH 7.4 were selected as formation electrolytes due to the substantial difference in the pH values and hence the dissolution ability towards Al and A_2O_3. The initial films were a 100-μm thick pure Al foil, used as a standard, and 500-nm thick Al films prepared by magnetron sputter-deposition, ion beam assisted deposition and thermal evaporation onto Si wafers.

The findings revealed that the voltage-time and current-time responses during galvanostatic and potentiodynamic polarization of the deposited aluminium, unlike the case of the foil, depend upon the electrolyte composition and are certainly influenced by the film formation approach, the individual film characteristics such as morphology, structure, grain size, configuration and packaging density, the features of air-formed oxide films and the degree of metal oxidation, hydroxylation and contamination by foreign elements such as carbon or nitrogen. Thus, by analyzing the anodizing behavior during barrier-type oxide growth one may decide whether or not the film quality is sufficient for further PAA fabrication.

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71. One-dimensional nanostructures for detection of biomolecules

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In our contribution, we will present the latest results on the growth of one-dimensional nanostructures. We are capable to grow oxides (sylica), semiconductors (germanium) and semiconducting oxides (titania) having a nanowire (one-dimensional) geometry. The growth modes of these nanostructures will be discussed based on real-time in-situ scanning electron microscopy observations. Additionally, several concepts how these nanostructured materials can be utilized for detection of biomolecules, with special emphasis on DNA strands, will be presented.

72. Possibilities of imaging through scattering media by means of a coherence-controlled holographic microscope

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Coherence-controlled holographic microscope (CCHM) makes possible observation and evaluation of amplitude as well as phase of objects without labeling them. It has therefore good potential for biological applications. The microscope is designed for usage of incoherent illumination which leads to the coherence-gating effect. Due to it, the samples can be observed also through some scattering media. In the contribution, the possibilities of imaging through the scattering media will be discussed from the theoretical point of view. Numerical simulations and some experimental examples will be demonstrated.

73. Proton conductivity study of two aluminum metal-organic framework compound using impedance spectroscopy

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Nowadays a new class of coordination polymers called metal organic framework (MOF) have attracted considerable attention for their proton conductivity, due to their potential applications in fuel cell as a proton exchange membrane (PEM) [1, 2]. We have studied proton conduction of such two aluminum based MOFs using impedance spectroscopy (IS). The first MOF was synthesized using aluminum salt and biphenyl-4, 4'-dicarboxylic acid (H_2bpdc) under solvothermal condition (further denoted as DUT-5)[3]. The second MOF (Al-SBPDC) is a sulfone-functionalized analog of DUT-5 synthesized under the same condition using ligand functionalization. It was found that these MOFs form 3D porous frameworks containing 1D inorganic chains. The high porosity of these compounds facilitates uptake of water in them so they act as proton conductors.

We studied proton conductivity of these MOFs as a function of temperature and relative humidity (RH). We evaluated the obtained data both by a traditional equivalent electrical circuit (EEC) approach, and by a new approach called random-walk (RW) approach [4, 5]. From the comparison of conductivity values evaluated by the EEC and RW approaches we found that the *RW* approach was applicable for the evaluation of the IS data of our materials and provided additional parameters like the number of charge carrier (*N*) and the diffusion coefficient (*D*). In both MOFs the conductivity increases with respect to temperature and RH due to an increase of the number of charge carriers (*N*) and the diffusion coefficient (*D*). DUT-5 has higher conductivity than Al-SBPDC, which implies that the sulfone functionalization reduces the conductivity of this type of MOF. By evaluating activation energy it was found that in DUT-5 the proton conduction was through Grotthuss mechanism while in Al-SBPDC a vehicle mechanism of conductivity is more probable. This change in the conduction mechanism was probably due to high electro negativity of the sulfone group.

References

1. R. Wang, X.-Y. Dong, H. Xu, R.-B. Pei, M.-L. Ma, S.-Q. Zang, H.-W. Hou, T.C.W. Mak, Chem. Commun. 50 (2014) 9153-9156.

2. S. S. Nagarkar, S. M. Unni, A. Sharma, S. Kurungot, and S. K. Ghosh, Angew. Chem. Int. Ed. 53 (2014) 2638 –2642.

3. I. Senkovska, F. Hoffmann, M. Froba, J. Getzschmann, W. Bohlmann, S. Kaskel, Micropor. Mesopor. Mat. 122 (2009) 93.

4. D.S. Patil, K. Shimakawa, V. Zima, J. Macak, T. Wagner, J. Appl. Phys. 113 (2013) 143705.

5. V. Zima, D. S. Patil, D. S. Raja, T. Chang, C. Lin, K. Shimakawa , T. Wagner J. Solid State Chem. 217 (2014) 150 –158.

74. Proteomic Core Facility at CEITEC-MU

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The Masaryk University CEITEC Proteomic Core Facility provides academic community and other subjects with access to advanced proteomic technologies based on shared resources and highly trained staff. The expensive instrumentation and specialized know-how enable the Core Facility respond promptly to demands of the research community and contribute to the effective utilization of resources as well. The Core Facility is part of Czech National Affiliated Centre of INSTRUCT. Czech and international researchers from universities and research institutes interested in accessing the core facility can benefit from support of CEITEC – open access project funded by the Ministry of Education, Youth and Sports of the Czech Republic.

A centralized core of expertise means that non-experts who wish to incorporate proteomics into their research may approachthe facility for expert advice in terms of discussing the merits/flaws of proteomics for their research, planning experiments, budgeting costs, possible outcomes and technical details.

75. Production of scFv antibodies against viral N protein of Porcine reproductive and respiratory syndrome virus

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Porcine reproductive and respiratory virus (PRRSv) is a member of the *Arteriviridae* family (order Nidovirales, genus Arterivirus). It is an enveloped virus with (+)ssRNA genome. The natural host of PRRS virus is a pig. PRRSv infection results in abortions or respiratory disease.

The studies of the virus life cycle or ultrastructural studies require the use of monoclonal antibodies, which could be obtained by classical way using hybridoma cells or alternatively by selection of recombinant scFv antibodies. Recombinant antibodies are useful tools in virus diagnostics and imaging; moreover they can be produced faster and cheaper compared to classical hybridoma antibodies.

In our experiments, we have selected scFv antibodies against PRRSv recombinant ORF7 protein from non-immune Tomlinson library. Through several rounds of selection we have isolated three individual phage colonies and subsequently characterized them by sequencing.

All three individual clones bound to PRRSv N protein in ELISA and immunoblot.

76. Determination of stress distribution and fracture parameters of orthotropic bi-material notches

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A bi-material notch composed of two orthotropic parts leads to stress singularity different from the crack or the single material notch case. The stress field around a bi-material notch inherently covers combined normal and shear modes of loading. Theory of anisotropic elasticity - the Lekhnitskii-Eshelby-Stroh formalism is employed as an elegant method to express the stress and displacement fields. The stress distribution at the notch tip is characterised by the exponents of singular terms and the generalised stress intensity factors. The exponents of singularity and corresponding eigenvectors are solution of the eigenvalue problem resulting from the prescribed boundary conditions. The generalised stress intensity factors are calculated by usage of the two state conservation integrals so called Psi-integrals. Based on knowledge of these parameters the stress distribution is known. In order to determine the conditions of crack initiation in notches of this kind, the strain energy density factor is evaluated. The minimum value of mean strain energy density factor is determined to predict crack initiation direction. Then the critical value of generalised stress intensity factor corresponding to the direction of assumed crack initiation is used to establish the notch stability criterion.

77. Protein dynamics on mercury surface

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The adsorption of proteins on surfaces is one of the main interest in the field of biocompatibility, biosensing, biomaterials development, etc. Experimental techniques like potentiometry and voltammetry give us only an partial information about the conformation of proteins, thus we have used all-atom computer simulations to describe details of the adsorption of proteins.

The adsorption of the oligopeptides on the surface with and without applied electric field was studied through molecular dynamic using Amber ff99SBildn force-field. We chose liquid mercury surface since it is widely used in electrochemical experiments. The surface was modeled as a rigid solid for simplicity. The main aim was to describe the orientation of the protein, to see the influence of the electric field on the conformation, and to quantify the strength of the protein - surface interactions.

We gratefully acknowledge support from the Czech Science Foundation (projects P208/12/0622 and 13-08651S).

78. Mapping of Plasmonic Model in Gold Nanoparticles by Means of Electron Energy Loss Spectroscopy

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In metallic nanoparticles, collective oscillations of free electron plasma strongly couple to the electromagnetic field forming the excitations called localised surafce plasmons (LSP). A characteristic feature of LSP is a strong enhancement of electromagnetic field in surrounding dielectric together with its confinement on the subwavelength scale, which in turn influences the optical properties in visible and near infrared spectral region. For example, a plasmonic particle attached to an optical emitter can be used to enhance the absorption of the exciting radiation, amplify or quench the spontaneous emission rate of the emitter, directing the emitted light or as a coupler for a waveguide in integrated optical circuits.

Electron energy loss spectroscopy (EELS) is a method allowing to study both the spatial and spectral distribution of LSP resonances. The electron beam with narrow kinetic energy distribution passes through or close to the metallic nanoparticle and induces LSP excitations, whose electric field in turn scatters the electrons inelastically. Scanning the sample and recording the energy loss spectrum at each position allows to reconstruct the LSP properties.

In our contribution we present EELS study of gold crescent-shape nanoparticles. These structures exhibit particularly large field enhancement near their sharp features, support two non-degenerate dipolar (i.e., optically active) LSP resonances and are widely tunable. Depending on the volume and shape, we resolved upto four LSP resonances in every metallic particle in energy range 0.8 – 2.4 eV. The low energy response was concentrated along the tips of the structure while the edges supported higher energy resonances. The boundary element method calculations helped to identify the character of the resonances and showed that some of the experimental peaks belong to two nearly degenerate resonances and that the highest energy feature is a multi-resonance assembly. The two lowest resonances are of importance due to their dipolar character (with mutually perpendicular polarization). Remarkably, they are both concentrated near the tips of the crescent, spectrally well resolved and their energies can be tuned between 0.8 – 1.5 eV and 1.2 – 2.0 eV, respectively. As the lower spectral range covers the telecom wavelengths 1.3 and 1.55 μm, we envisage the use of such nanostructures in infrared communication technology.

79. Differences in dynamics of cell activity in 3D collagen gel as visualized with coherence-controlled holographic microscope

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Keywords: cancer cell, 3D collagen gel, holographic microscopy

An ability to form metastasis is considered basis of malignancy of solid tumours. It is derived from invasive behaviour of tumour cells. Invasiveness of tumour cells can be investigated in cellular systems in vitro, which then serve as tools in searching for the anti-invasive agents [1]. Preformed 3D collagen gel is used for assessing static cell morphology and/or depth of neoplastic cell burrowed into. The ordinary evaluation is done just by measured refocusing using Hoffman modulation contrast. Thus, detailed knowledge about the dynamics of the process of interaction of cells differing in malignancy with such a 3D environment is still missing.
Coherence-controlled holographic microscope (CCHM) [2, 3] was tested for visualization of cells in 3D collagen gel. It was found that both the cell activity and reaction of the collagen fibres can be registered. CCHM enables visualization of cells in the simulated extracellular matrix more precisely because it does not suffer from halo artefact. Moreover the quality of imaging is not substantially impaired by the presence of the matrix owing to low coherence of illumination. CCHM could focus into depth of collagen gel. The maximal depth of focus depends on the objective used. Then with time-lapsed recording the dynamics of interaction of cells, differing in malignancy, with the mimicked 3D environment can be followed up.
In the pilot experiments it was observed that the metastasizing rat sarcoma A3 cells manifested a type of cell behaviour that resembled amoeboid motility employed in pushing the extracellular matrix away from cell body for making a cavity around the cell. Human breast carcinoma MCF7 cells on the other hand produced prolonged processes which apparently got attached to the collagen fibrils in the gel and exerted traction.
In conclusion we proposed the CCHM as a suitable tool for studies of cells in simulated extracellular 3D matrixes *in vitro*. Owing to high contrast quantitative phase images and time-lapse recording study of dynamics of cancer cell reactions to the partially opaque surroundings is made more realistic. This situation is prone to provide new knowledge of cell motile responses to various stimuli under life-like conditions.

[1] D. Rösel et al, OncoTargets and therapy, **6** (2013), p. 767.
[2] T. Slaby et al, Optics Express, **21** (2013), p. 14747.
[3] P. Kolman and R. Chmelik, Optics Express, **18** (2010), p. 21990.
[4] The authors acknowledge funding from the Ministry of Industry and Trade of the Czech Republic, project Multimodal Holographic Microscope (FR-TI4/660); from European Regional Development Fund, CEITEC-Central European Institute of Technology (CZ.1.05/1.1.00/02.0068) and from CEITEC – open access project, ID number LM2011020.

80. Neurotransmitter changes induced by methamphetamine in olfactory bulbectomy model rat of depression, an *in vivo* microdialysis study

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Background: Depressive disorders are reported in context of enhanced vulnerability to drug addiction. As a possible explanation of this comorbidity the self-medication hypothesis assumes that symptoms related to potential monoaminergic deficits in depression may be relieved by the drug of abuse. The aim of this study was to elucidate the neurotransmitter changes in a rat model of depression by measuring levels of dopamine, serotonin and their metabolites, glutamate and GABA in the nucleus accumbens shell (NACs), which is typically involved in the drug of abuse acquisition mechanisms.

Methods: Depression was modeled by the bilateral olfactory bulbectomy (OBX) in one half of 20 Wistar rat males, the other half was sham operated. After 14 days of recovery following OBX surgery, microdialysis guides were implanted into NACs by standard technique. 48 hours later, in vivo microdialysis was performed collecting extracellular samples starting from the 60 min baseline and following 3 hours after single IP injection of methamphetamine (5 mg/kg). The method for determination of dopamine and its metabolites in microdialysate was HPLC-MS analysis.

Results: OBX animals had lower basal levels of dopamine, serotonin and their metabolites together with higher levels of glutamate and GABA in NACs. However, after methamphetamine challenge the dopamine and serotonin release was significantly stronger in the OBX rats as compared to sham operated controls. On the other hand, glutamate release was found lower and GABA not influenced.

Conclusion: The presented data provide an evidence of mesolimbic changes in the rat model of depression and addiction comorbidity which may elucidate mechanisms of results received earlier in two IV self-administration studies in which OBX rats were demonstrated to have higher drug intake in comparison to controls.

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81. Electrode-less plasma jet synthesis of core-shell iron/iron-oxide nanoparticles

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In recent years, the interest in iron and iron oxide based nanomaterials (e.g. nanoparticles, nanopowders, nanolayers, etc.) is growing due to their unique properties. The research and development is truly interdisciplinary: magnetism, catalysis, optics, sorption, biocompatibility, chemical stability, etc.

Nowadays, the iron based nanomaterials are produced commercially using well-established chemical methods, for example co-precipitation, micelles, microemulsion or hydrothermal syntheses. However, these methods often use solvents or surfactants and involve complicated or multistep procedures. As a result, there is a modern trend to replace the wet chemical steps by plasma based technology that could provide an environmentally attractive alternative. Successful preparation of iron and iron-oxide nanopowders in microwave atmospheric pressure plasma were reported in [1-3].

We report on core-shell iron and iron oxide nanoparticle prepared by plasma synthesis controlled by process gas mixture. The synthesis was carried out using an atmospheric pressure microwave electrode-less plasma jet. We focused on phase composition control and better passivation. We studied the influence of O_2, H_2 and N_2 admixtures to the Ar process gas on the properties of the prepared nanomaterial. Similarly to thermal process, the oxygen admixture preferentially produced Fe_2O_3 nanoparticles whereas hydrogen admixture resulted in particles with higher metallic content. However, in contrast to thermal process, the addition of nitrogen did not produce iron nitride, but iron/iron-oxide core-shell nanoparticles with enhanced chemical stability.

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[1] P. Synek, O. Jasek, L. Zajickova, B. David, V. Kudrle, N. Pizurova:
Mater. Lett. 65 (2011) 982
[2] L. Zajickova, P. Synek, O. Jasek, M. Elias, B. David, J. Bursik:
Appl. Surf. Sci. 255 10 (2009), 5421
[3] B. David, N. Pizurova, O. Schneeweiss, E. Santava, O. Jasek, V. Kudrle:
Journal of Physics: Conference Series 303 (2011), 012090.

82. Recombinant Lectin from insect pathogen *Photorhabdus luminisnce*: structural and functional studies

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Photorhabdus luminescence is Gram-negative bacilli, exhibit duel nature of lifecycle one, being pathogenic towards insect whereas other, symbiosis with entamo-pathogenic nematodes. The lectin gene was amplified from the genome of *P. luminescence* and cloned with polyhistidine tag. Expression and production of the lectin was optimized and further purified it using nickel affinity chromatography. The lectin was found to be tetramer of 160 kDa exhibiting 41 kDa monomers interconnected with disulphide bridge. Crystallization of the lectin was achieved after various screening kits and optimized further by hanging drop method. The crystal structure was solved at 1.8 A^0 resolution. The lectin was found to be seven-bladed β-propeller fold with possibly two different binding sites per monomer. One of the binding site was found to be fucose specific however other was also suggested to be saccharide specificity probably towards a saccharide with higher level of hydroxylation than L-fucopyranose. Lectin was found to exhibit binding ability to haemocytes from insect larvae however was not found to be lethal to *Galleria mellonella* insect larvae.

83. Elastomeric Polyurethanes Based on Poly(ethylene glycol) and Poly(caprolactone) with Potential Use in Medicine

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Polyurethane elastomers with potential in regenerative medicine were synthesized by solvent free method using poly(ethylene glycol) (PEG), poly(caprolactone) (PCL) and hexamethylene diisocyanate (HDI) catalyzed with stannous octoate. Different diol hydrophilic (PEG) to hydrophobic (PCL) content ratios of 7:3, 1:1 and 3:7 were used. Samples made of one type of diol, either hydrophilic PEG or hydrophobic PCL, were synthesized as well. Subsequently samples made of PEG/PCL in ratios 2:8 and 1:9 were synthesized also to tune the mechanical properties. Influence of the composition on the mechanical and degradable properties is compared in this contribution.

Tensile tests showed that presented PCL in the mixture affects mechanical properties rapidly, but only if PCL was presented in higher amounts (PEG/PCL = 2/8 (w/w) and higher). At this ratio the Young's modulus, stress at break and strain at break increased in one order. Increase in values is caused by semicrystalline nature of PCL presented in the macromolecular network, which was confirmed by differential scanning calorimetry.

Swelling and hydrolytic degradation tests showed that the presence of PEG (hydrophilic part) strongly influences the ability to absorb water for the PU samples, which also has impact on the rate of hydrolytic degradation. Sample made of only PEG and HDI had the highest water uptake (77.9 ± 6.3 % after one day), the values for the other specimens decreased with decreasing amount of PEG in the macrodiols' mixture. Specimen made of PCL and HDI absorbed the smallest amount of water (1.8 ± 0.1 % after one day). The same trend was observed in the rate of hydrolytic degradation, when PU sample made of PEG and HDI was completely degraded after 5 months compared with the other specimens that are stable for more than 1 year.

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84. Structural studies of mycobacterial glycosyltransferase GlfT1

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Mycobacterium tuberculosis is a gram positive bacterium causing chronic illnesses. Tuberculosis is associeted with disease persistence and prolonged treatment regime. Spread of multidrug resistant strains stipulates an urgent demand for new antituberculosis drugs. A combination of the sophisticated defence barrier and the slow metabolism of the bacterium makes it a difficult target for therapeutics. The mycobacterial cell wall is an essential, covalently linked complex of highly hydrophobic mycolic acids, peptidoglycan and arabinogalactan.

Arabinogalactan is composed of about 30 alternating 5- and 6-linked β-d-galactofuranose (Galf) units. It anchores in peptidoglycan by a dedicated linker unit, α-l-rhamnopyranosyl-(1 → 3)-N-acetyl-α-d-glucosaminyl-phosphate [Rha-(1 → 3)-GlcNAc-P]. Two to three arabinan chains containing approximately 31 d-arabinofuranose (Araf) units each, composed mostly of 5-linked α-d-Araf with branching introduced by 3, 5-α-d-Araf, are bound to the galactan. Mycobacterial enzyme GlfT1 is a membrane associated, metal dependent galactofuranosyl transferase involved in the initial steps of arabinogalactan formation . It transfers the first and the second Gal*f* units from UDP-Gal*f* to the C50-P-P-GlcNAc-Rha, creating ß-(1-->4) and ß-(1-->5) linkages. The arabinogalactan synthesis is essential for the mycobacterium survival but not present in vertebrates. This fact makes the involved glycosyltransferases an attractive drug target. The rational design of enzyme inhibitors is connected with determination of the 3D protein structure. Unfortunately, glycosyltransferases are in general notoriously difficult to express and purify.

A new MBP-GlfT1 fusion was constructed to increase solubility. A complex purification protocol was implemented to avoid proteolysis, aggregation and chaperone inpurities. The activity assay based on a different mobility of the substrate and the product in thin layer chromatography confirmed the activity of the enzyme after multiple purification steps. The methods of analytical ultracentrifugation (AUC) and dynamic light scattering (DLS) were used to test the homogenity of the protein and to determine the oligomerization status. Diffracting crystals of MBP-GlfT1 were obtained and diffraction images collected. The general shape of the protein in solution will be determined by small angle X-ray scattering (SAX).

The research leading to the results obtained financial contribution from the EU under the 7th Framework Programme by CEITEC (CZ.1.05/1.1.00/02.0068) project from European Regional Development Fund.

85. Application of capillary electrophoresis hyphenated to mass spectrometry to protein – drug interaction study

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Capillary electrophoresis coupled to mass spectrometry in frontal analysis mode (CE/FA-MS) presents an attractive technique for characterization of drug – protein interactions. These interactions have a significant impact on both pharmacokinetics (i.e. absorption, distribution, metabolism and excretion) and pharmacodynamics (pharmacological effects). The advantages of the CE/FA-MS method include low requirement on the amount of sample, fast analyses and near-physiological conditions for studied interactions. MS detection offers enhanced sensitivity in comparison to common used UV detection. This increased sensitivity can be exploited for the determination affinity constants of poorly soluble compounds.

We were focused on interaction between anti-diabetic agent glimepiride and plasma protein human serum albumin. Glimepiride is poorly soluble drug and belongs to third generation sulfonylurea used for treatment of diabetes mellitus type 2 because it acts as an insulin secretagogue.

In this study binding constants for glimepiride and human serum albumin was determined by CE/FA-MS.

86. Optimization of transport liquid substances through natural porous material

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Human skin has similar structure as a plant cuticle, which form upper respectively bottom layer on the plant surface. This porous material enables the penetration of compounds from outer to inner environment and its function is regulation of water, substances and protection against biotical effect.

Diffusion experiments of liquid substances through natural plant material were realized in agarose hydrogels, which are taken as non-reactive medium. Used methods for these types of experiments were diffusion pair (stationary diffusion) and free diffusion (non-stationary diffusion). Diffusion processes were studied at specified time intervals (after 24 hours) and were quantified by UV-VIS spectra. Diffusion parameters such as effective diffusion coefficients were obtained from experimental data. The rate of diffusion was compared by fundamental diffusion parameters.

1 hm. % humic acids were transported through plant cuticles. Humic acids are used for the agrochemical application as the additives for fertilizers, which have positive influence on quality of soil, fertility, growth and productive of plants.

These experiments enable simulation the penetration processes through the human skin and determine suitable quantity, which is able to penetrate through the skin. For the detailed characterization, the fluorescence-lifetime imaging microscopy (FLIM) was used, which was used for study the structure of plant cuticles respectively determined the distribution the lifetime of fluorescence.

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87. Porous-alumina-assisted preparation of multi-segment nanowires

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The topic deals with the method of multi-segment nanowires preparation with focus on a gold/polyaniline/gold structure. The methodology include preparation of a template by smart anodizing of aluminium leading to a porous anodic alumina (PAA) formation. This PAA layer is then chemically separate from a substrate, modified and a gold layer is deposited by PVD techniques on the bottom to play role of working electrode during electrochemical deposition of nanowires. Various multi-segment nanowires (e.g. Au/PANI/Au, Au/Ni, Cu, Cr) were prepared and deposition parameters were tuned. Beside this large-scale, cost-effective method for single or multi composite nanowire array fabrication another simple and fast method was developed for large-scale highly-ordered array of gold nanoparticles.

88. 2D assembly of gold colloids using charged particle beams

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Metallic nanoparticles possess unique physical and chemical properties and therefore can be utilized for a wide range of technological and medical applications. Although one can exploit these properties while the nanoparticles are in colloidal solutions, it is equally important to immobilize these nanoparticles onto solid surfaces, preferably with a possibility to control their precise position and orientation. We describe a simple method resulting in guided self-assembly of gold nanoparticles onto silicon substrates, which is based on modification of these substrates by electron beam and appropriate adjustment of pH of nanoparticle colloidal solution. This process results in an ordered array of nanoparticles positioned with precision better than twenty nanometres.

89. Deciphering the diploid ancestral genome of the mesohexaploid *Brassica rapa*

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The genus *Brassica* includes several important agricultural and horticultural crops. Their current genome structures were shaped by whole-genome triplication followed by extensive diploidization. The availability of several crucifer genome sequences, especially that of Chinese cabbage (*Brassica rapa*), enables study of the evolution of the mesohexaploid *Brassica* genomes from their diploid progenitors. We reconstructed three ancestral subgenomes of *B. rapa* (n = 10) by comparing its whole-genome sequence to ancestral and extant Brassicaceae genomes. All three *B. rapa* paleogenomes apparently consisted of seven chromosomes, similar to the ancestral translocation Proto-Calepineae Karyotype (tPCK; n = 7), which is the evolutionarily younger variant of the Proto-Calepineae Karyotype (n = 7). Based on comparative analysis of genome sequences or linkage maps of *Brassica oleracea, Brassica nigra*, radish (*Raphanus sativus*), and other closely related species, we propose a two-step merging of three tPCK-like genomes to form the hexaploid ancestor of the tribe Brassiceae with 42 chromosomes. Subsequent diversification of the Brassiceae was marked by extensive genome reshuffling and chromosome number reduction mediated by translocation events and followed by loss and/or inactivation of centromeres. This work was supported by the European Social Fund (CZ.1.07/2.3.00/20.0189).

90. Morphology and photosynthesis based screen to unravel mechanism of ROS-auxin crosstalk

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Plants are continuously exposed to a variety of external conditions which primarily affect photosynthesis and cell growth, compromising thus their growth, development and productivity. Reactive oxygen species (ROS) are produced as a normal product of plant cellular metabolism. Responses to various environmental stresses lead to excessive production of ROS and also mediate plant growth hormones. Recent evidence indicates that among phytohormones, auxin play crucial role in response of plant to abiotic stress (Tognetti *et al*., 2011).
Stress induced morphogenic response (SIMR) are strongly influenced by reciprocal interaction between ROS and auxin. Auxin homeostasis, induced by different mechanism including *de novo* biosynthesis, transport, metabolism and signalling (Woodwart and Bartel, 2005), is affected by increased ROS production induced by stressors (Tognetti *et al*., 2011). Although connection between auxin and photosynthesis is poorly understood, there is evidence supporting hypothesis of auxin-dependent regulation of photosynthetic apparatus in order to minimize photo-oxidative damage under stress (Tognetti *et al*., 2010).To understand the mechanism underlying ROS-auxin crosstalk, we are performing in parallel a morphological and photosystem II photochemical efficiency based screenings using *Arabidopsis* auxin related lines (knock out and over- expressors).
Morphological Screening allows us to determine the SIMR responses in adult leaf and root tissues. In addition, it helps us to identify target genes involved in ROS-auxin crosstalk under SIMR growth conditions.. The photosynthesis based screen helps us to identify the genes harboring the auxin dependent regulation of photosynthetic apparatus in response to stress. More ever, the effect of ROS derived signals on auxin pathways will be identified. The lines with both SIMR and photosynthetic effects will be tested further in high throughtput phenotyping system for drought stress. These findings will provide a basis for further explorations in other studies for improving the crop yield and stress tolerant crops.

References

1. Tognetti V. B., Van Aken O., Morreel K., Vandenbroucke K., Van de Cotte B., Declercq I., Chiwocha S., Fenske R., Prinsen E., Genty B., Inzé D. and Van Breusegem F. (2010). Perturbation of indole-3-butyric acid homeostasis by the UDP glucosyltransferase UGT74E2 modulates Arabidopsis architecture and water stress tolerance.Plant Cell 22: 2660-2679.
2. Tognetti V. B., Muhlenbock P., Van Breusegem F. (2011).Stress homeostasis - the redox and auxin perspective. Plant Cell & Environment. 35:321-333.
3. Woodward A.W., Bartel B. (2005) Auxin: regulation, action, and interaction. Annals of Botany 95:707-735.

91. Metabolomics: the tool for the improvement of pregnancy outcome in the human assisted reproduction

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Metabolomics is the scientific field studying functional biology and it is focused on the analysis of small molecules that are transformed during metabolism. Metabolomics provides information about immediate physiological state of an organism thus it is ideal for description of health state. The one of the potential employment of metabolomics could be in the human assisted reproduction. The human assisted reproduction is dealing with the infertility issue, which is a common disorder affecting around 15 % of couples in the reproductive age. Success rate of *in vitro* fertilization treatment is around 33 % per cycle which is far from ideal. A key step for improvement of the success rate of the *in vitro* fertilization is considered to be a proper selection of an embryo with the highest implantation potential. Currently employed light microscopy suffers from low precision and subjectivity thus novel approaches are sought. One of the approaches is metabolomics which can noninvasively provide information reflecting ongoing processes in an embryo thus improve precision of the embryo selection and pregnancy outcome, consequently.

92. Enzymatic sensor of biogenic amines with optical oxygen transducer

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The biogenic amines (BA) are formed by the decarboxylation of amino acids. Because of their impact on human health, they have been used as markers for assessing the freshness and quality of a wide variety of protein-containing products such as fish, meat, wine and beer where BA accumulate in the process of food spoilage and high concentration of BA is put into context with formation of cancer. In contrast to sensors with electrical transducers, optical sensors of BA might be integral components of a sealed package and a content of biogenic amines might be measured noninvasively.

We studied biosensor for BA with an optical oxygen transducer that contained a diamine oxidase from pea and ruthenium complex. Both active components were incorporated in chemically stable polymer to form sensitive films thickness of 100 - 200 µm. The sensor is based on the measurement of oxygen consumption due to oxidation of BA catalyzed by enzyme diamine oxidase. Ruthenium complex serves as an optical transducer. Its fluorescence is quenched proportionally with oxygen concentration. The sensor is composed from a lens coated with sensitive film and a bundle of optical fibers connected to a light source and detector. The fluorescence lifetime is determined by the measurement of light intensities at four points after the cessation of the excitation.

The sensitivities of putrescine (model BA) determinations, measured under saturation with air were between 3.40 and 4.37 µs.L.mmol^{-1} in repetitive experiments conducted over a period of one year. With increasing oxygen saturation DO (dissolved oxygen) from 10 % to 100 % the biosensor sensitivity decreased ten times and its dynamic range increased one order.

With aim to optimize a composition and manufacturing of the biosensor we developed a mathematical model of processes inside the sensing film that revealed qualitative relations among sensor analytical responses, characteristics of the sensitive layer and substrate concentrations (DO and putrescine) in a measured solution. The developed mathematical model revealed qualitative dependencies of the biosensor behavior (sensitivity, response time and dynamic range) on the (i) thickness of the sensitive layer, (ii) concentrations of substrates in the solution, (iii) enzyme activity in the layer and (iv) layer permeability for the substrates.

93. Development of effective QCM biosensors by cyclopropylamine plasma polymerization and antibody immobilization using cross-linking reactions

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Biosensors have been extensively developed and applied for biomedical and environmental study. Although there are many different types of biosensing techniques, all these methods require immobilization of biomolecules (DNA, antibody, enzyme) onto the sensor surface. The bio-molecule immobilization step is critical in the development of any sort of biosensor, as it can affect operational properties of the sensor. For many years self-assembled monolayers (SAMs) of alkanethiolates have been widely used for functionalization of the gold surfaces, in spite of low stability of the S-Au linkage at higher temperature or UV radiation. One of the most suitable candidates to substitute SAMs for sensing surface modification is the plasma polymerization providing thin functional films. Among various functional plasma coatings, the amine-rich films are extensively employed for numerous biomedical applications, thanks to the reactivity of the introduced primary amine groups.

In this work, cyclopropylamine plasma polymerization was employed to deposit stable amine thin films on the surface of quartz crystal microbalance (QCM) biosensors. The antibody specific to human serum albumin (anti-HSA) was attached to the QCM surface via crosslinkage obtained by intermediate reaction with glutaraldehyde. All steps of the bio-immobilization were controlled by XPS and FT-IR to characterize surface and layer(bulk) chemistry. The stability of the deposited coating during immobilization and within biosensor operation were studied by SEM imaging and ellipsometry.

We have tested a number of QCM-biosensors with two types of nanofilms prepared using two different plasma set-ups . In first group of samples (type-1), the substrate was placed at a floating potential, while in the second group (type-2), the subtrates were self-biased.

XPS and FT-IR confirmed the successful immobilization of anti-HSA antibody. After each step of bioimmobilization, the change of the QCM resonant frequency was measured. The increase of the mass after each chemical reaction was observed for the QCM-biosensors coated by the type-1 film. These samples also exhibited highest response towards the anti-HSA antigen. The QCM-biosensors coated by a type-2 films exhibited lower and irregular change of the mass and therefore they were less reliable for the measurement. The biosensors of the first type have the highest efficiency and are very promising for future use in the biosensing field.

94. Deposition of amine-rich films by cyclopropylamine plasma for increased biocompatibility of polycaprolactone nanofibers

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The electrospinning of the micro/nanofibrous materials gained a high interest due to the potential applications for biomedical devices, tissue engineering, drug delivery on the one hand and due to the low cost on the other one. However, because of the low surface free energy and poor wettability of the untreated nanofibers, this material cannot be used directly as prepared and their surface must be activated, e.g. by plasma polymerization. Amine-rich films prepared by plasma polymerization have great application potential, beside others for biomolecule immobilization and enhanced cell adhesion [1]. Among numerous precursors used for amine plasma polymerization, cyclopropylamine (CPA) is one of the most promising non-toxic monomer recently used for the deposition of sufficiently stable amine-rich thin films [2, 3].

In this work, the electrospun polycaprolactone (PCL) micro/nanofibers with the mean thickness of 1.2 ± 0.4 μm and a pore size of 8 ± 2 μm were coated by amine-rich thin films using low pressure capacitively coupled plasma polymerization of CPA in continuous wave and pulsed mode. In pulsed discharges the film exhibited 7 at.% of amine groups and only 2 % of the thickness was lost after immersion of this layer in water for 216 hours. The deposition of this coating improves the cell adhesion and proliferation, as the number of mouse myoblasts grown on the substrate surface during 1 day of cultivation increased by a factor of five after deposition of CPA plasma polymer compared to the uncoated substrate results.

References

[1] K. Siow, L. Britcher, S.Kumar, H. Grieser. Plasma Process. Polym. 2006, 3, 392-418
[2] A. Manakhov et al. Plasma Process. Polym. 2014, DOI: 10.1002/ppap.201300177
[3] A. Manakhov, D. Nečas, J. Čechal, D. Pavlinak, M. Eliáš, L. Zajíčková, Thin Solid Films (2014), DOI: 10.1016/j.tsf.2014.09.015

95. A textbook example of recent polyploidy in *Cardamine*: the true story

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Hybridization and polyploidization may lead to speciation. Although the origin of several recently formed (100 to 200 years old) allopolyploid plant species was well characterized, the role of triploid hybrids gained less attention. Triploids are viewed as bridges between diploids and tetraploids, but rarely as parental genomes of high-level polyploids. Here we explore the origin of a bitter-cress neopolyploid, *Cardamine schulzii*, considered as one of the few classical textbook examples of recently formed allopolyploid plant species. We reconstructed ~100 years history of man-induced hybridization and allopolyploidy in *Cardamine* at Urnerboden (Swiss Alps), using newly available cytogenomic and molecular approaches. We show that the triploid hybrid genome of *C.* ×*insueta* (RRA, 2n = 24) comprises structurally stable chromosome complements of two diploid (2n = 2x = 16) species, *C. amara* (AA) and *C. rivularis* (RR). Furthermore, we gained compelling evidence that the triploid hybrid hybridized at least twice with the hypotetraploid *C. pratensis* (PPPP, 2n = 2x-2 = 30). These hybridization events resulted in the origin of tri-genomic hypopentaploid (2n = 5x-2 = 38, PPRRA) and hypohexaploid (2n = 6x-2 = 46, PPPPRA) hybrids of *C.* ×*schulzii*. Our data question the existence of the neopolyploid species *C. schulzii* and suggest that the populations of *C. schulzii* in fact represent tri-genomic hybrid plants. This work demonstrates how important is to revisit familiar textbook examples using modern techniques and that semi-fertile triploid hybrids may promote hybrid speciation by mediating hybridization between diploid and tetraploid species.

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96. 3D BIOPRINTER PROJECT: #hacking the #CEITEC equipment to develop #bioprinting technology

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"Compared with non-biological printing, 3D bioprinting involves additional complexities, such as the choice of materials, cell types, growth and differentiation factors, and technical challenges related to the sensitivities of living cells and the construction of tissues. Addressing these complexities requires the integration of technologies from the fields of engineering, biomaterials science, cell biology, physics and medicine." Sean V Murphy & Anthony Atala Nature Biotechnology 32, 773–785 (2014)

Fortunately combining life sciences, advanced materials with the cutting-edge technology is the essence of the CEITEC project so we started building our bioprinter by simply hacking the equipment we already have. ☺

Our bioprinter is based on high precision liquid handling robot enclosed in the programmable thermostatic chamber. An additional independent temperature control of the environment and printing target is installed for precise regulation of the process conditions. This setup is developed for hybrid inkjet-like bioprinting with the possibility of using temperature-controlled material-handling. The printed head is equipped with eight independent channels for composite formation possibilities. UV sterilization of the interior including the robot enables work with a sensitive cell samples. The whole system is controlled on site or remotely using chamber (main) camera and robot arm (target) camera.

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97. Determination of Binding Constants of Selected Sulfonylureas Drugs

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The binding constant is commonly used to describe the strength of binding between ligand such as drug and protein. The strength of the interaction has a significant effect on biological activity of the drug. Various sulfonylureas (antidiabetic drugs) may induce hypoglycemia as a result of excesses in insulin production and release. Therefore, it is important to understand in detail the pharmacokinetics and pharmacodynamics of these drugs. The advantages of capillary electrophoresis for determination of binding constant are very low sample consumption, high resolution and also it does not require the highly purified samples, immobilization or labelling of any of the interacting species. The investigated interaction takes place in a solution, which can be simulated physiological conditions.

The main objectives of this study were to determine the binding constants of the selected drugs (tolbutamide, chlorpropamide) with HSA using capillary electrophoresis – in physiological conditions.

98. Fluorescence probes as indicators of the interactions between biopolymer and hydrophobic species supported by drying method

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Hyaluronan is a chemical, which has prevalent hydrophilic character in native form. The chain contains also hydrophobic parts, but these areas are protected in the solution by probably highly organized molecules of water. Therefore the interactions between native hydrophilic biopolymers and hydrophobic species must be somehow supported. In some works surfactants are used as a bridge between hydrophobic and hydrophilic parts. Other way is hydrophobic modification of polymer. In this work we used drying method to open up the binding sites. For purposes of this study the fluorescence probes (perylene, prodan) were used as indicators. Efficiency of drying of the system was studied by fluorescence spectroscopy. For various probes specific parameters were monitored, which provided information about polarity of environment, in which fluorescence probes molecules were soluble. In case of perylene observed parameter was the fluorescence intensity. Perylene is insoluble in water but soluble in nonpolar solvents and that is the reason why the fluorescence signal is measurable only in hydrophobic solutes. On the other side prodan is soluble in solvents with wide range of polarity. Than his fluorescence spectrum has maximum at different wavelength, which were monitored. It was observed, that drying of the systems by low temperature and pressure was effective way to support the interactions. The results showed that the perylene has no fluorescence response if we just mixed up the components in water solution. On the other side dried and rehydrated system provided significant fluorescence signal. Therefore we consider the drying of the studied system as a useful tool for supporting of the interactions between biopolymers and hydrophobic species.

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99. Effect of Crosslinking Time of Functionalized PLGA-PEG-PLGA Copolymers and Its Influence on Swelling Behaviors

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In this work, temperature-sensitive PLGA–PEG–PLGA triblock copolymer modified by itaconic anhydride (ITA) with reactive double bonds and carboxylic acid groups was synthesized by ring opening polymerization. Resulting well-defined copolymer was chemically crosslinked for 10, 20, 30, 40 and 50 minutes by blue light in presence of camphoroquinone as initiator and 2-(dimethylamino) ethyl methacrylate as catalyst. Effects of crosslinking time on swelling ratio and hydrolytical stability of prepared hydrogels in ultrapure water at ambient temperature were investigated.

It was found that within first 5 days prepared hydrogels were able to absorb large amount of water and water content increased to 2670, 3300 and 3772 % related to dry polymer for samples crosslinked 10, 20 and 30 minutes. Then the hydrogels started to be hydrolytically unstable and totally dissolved between 12th and 17th day. Crosslinking time longer than 30 minutes had not significant influence on water uptake and on swelling time.

Elimination of double bonds of original copolymer coming from ITA modification and formation of new RRC–CHR bonds after the crosslinking was determined by infrared spectroscopy (ATR-FTIR). In conclusion, the highest crosslinking efficiency (85 %) determined by proton nuclear magnetic resonance exhibited sample crosslinked for 20 min. Prolonged crosslinking time affected the change in chemical structure reducing thermal stability of ITA/PLGA–PEG–PLGA/ITA copolymers.

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100. Synthesis of core/shell quantum dots for protein detection

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The aim of this work is synthesis and characterization of core/shell quantum dots (QDs) for protein detection. Various types of CdTe/ZnS core/shell QDs were synthetized through reflux route and stabilized using different substances containing thiol groups (e.g., mercapto-propionic acid (MPA), thioglycolic acid (TGA), glutathione (GSH)). These stabilizers provide functional groups (-COOH, -NH3) by means of which they bond to the surface of QDs on one side, and on the other they are able to bond to biomolecules such as proteins or antibodies. CdTe/ZnS QDs were further covalently bonded using CDI (1, 1-Carbonyldiimidazole) or via carbodiimide surface chemistry using EDC (N-(3-Dimethylaminopropyl)-N-ethylcarbodiimide hydrochloride) and/or NHS (N-Hydroxysuccinimide) as mediators in order to increase affinity to BSA (bovine serum albumine) and IgG (imunoglobuline G). Prepared QDs – protein/antibody conjugates were characterized by fluorescence spectroscopy (Infinite M200Pro, Tecan) and capillary electrophoresis (Agilent 7100). Results showed that the highest fluorescence intensity had CdTe/ZnS QDs stabilized with MPA, than CdTe/ZnS QDs stabilized with TGA and the lowest fluorescence intensity had CdTe/ZnS QDs stabilized with GSH, at wavelength of 630, 562 and 542 nm respectivbely. Fluorescence spectroscopy of all prepared QDs showed quenching of fluorescence intensity after binding biomolecule to QDs. The most noticeable quenching was observed in the case of CdTe/ZnS QDs stabilized with MPA than CdTe/ZnS QDs stabilized with TGA and the least quenching was in the case of CdTe/ZnS QDs stabilized with GSH. In the case of QDs conjugated with BSA using EDC as mediator, the most visible expression of QDs quenching was in the following order according to the used stabilizer: GSH, MPA and TGA. Samples were also analysed using capillary electrophoresis (CE) in order to determine whether binding of protein/antibody to QDs has occurred or not. Results of CE provided migration times of QDs or QDs – conjugates. In the case of MPA-QDs-BSA migration time was 4 min 54 sec and for CdTe/ZnS QDs it was 6 min 13 sec. In the case of MPA-QDs-IgG migration time was 3 min 50 sec and for CdTe/ZnS QDs it was 6 min 13 sec. Migration time corresponds with our assumptions and different time of migration tells us that binding has occurred. Conjugates are eluted faster than the pure CdTe/ZnS QDs due to the migration direction opposite to electro-osmotic flow. This occurs due to the reduction of negative charges on the surface of QDs due to BSA/IgG binding.

101. Degradation and corrosion inhibitors for heat transfer fluids based on polyols

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The primary purpose of the use of polyols in the heat transfer fluid is to expand the temperature range in which the heat transfer medium remains in liquid phase. Fluids based on polyols, and especially glycols, are most widespread liquid non-freezing mediums for transporting heat energy in the low temperature applications. The same basic formula of water and glycol is used in many different appliances such as cars engine cooling, solar thermal systems, on large-scale in industry etc. The popularity of this mixture is mainly due to the low production costs and the long-standing development of technologies designed in accordance with this heat transfer medium.

However, thermal systems using this mixture as the heat transfer medium are almost always constructed from metallic materials which are more or less subjects to corrosion. In this regard the fundamental lack of glycol mixtures is the increase of corrosive properties compared to the original pure substances. Furthermore, the chemical and thermal degradation of the organic matrix, which is an integral part of the service of this mixture, is causing the corrosive properties to rise. It is therefore necessary to solve the problem of corrosion caused by the glycol mixtures in each heat transfer application. There are two basic approaches. The first is based on the use of the corrosion-resistant materials however these materials tend to be rather expensive or do not meet the technological requirements of the application. The second solution is to use anti-corrosion additives.

The subject of our research is to identify the principles and products of aging and degradation of the glycol-based heat transfer fluids and use this knowledge in the design of a new anti-corrosion and anti-degradation system. Imperfect knowledge of these principles generally leads to a strong overdose or an inappropriate combination of anti-corrosion additives and unnecessary burden to the environment. Our research shows that the corrosive properties of the water-glycol mixture are dramatically influenced by the degradation products, not only in the form of organic acids but also, as further described, by other degradation products which have a direct impact on the physical parameters of the operating mixture. The proposed anti-corrosion systems based on nanomaterials then represent only a part of a much more complex solution.

102. Adaptive Evolution of a genomic RNA in an artificial cell-like model

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Can early life, an assembly of molecules, evolve? One way to address this question is to construct such a simple evolvable system from non-living molecules and experimentally evolve it. In our laboratory, we have created an artificial cell-like model, which can evolve through Darwinian principles, by combining a lipid vesicle, an artificial genomic RNA encoding Qβ replicase (RNA-dependent RNA polymerase) and a reconstituted *Escherichia coli* translation system. In this model, the genomic RNA replicates through the translation of the encoded replicase.
In the previous study, we found that this system enables the RNA to evolve to be more replicable through Darwinian principles by long-term replication experiments, in which mutations were spontaneously introduced into the RNA by replication error [1]. After that, to know if the RNA would adapt to other environments after evolving in one environment, which is one of the most remarkable characteristics of living things, we performed the same kind of experiments with the evolved RNA in a severer environment where the concentration of ribosome was reduced. Through the evolution experiments, the genomic RNA accumulated mutations and highly adapted RNA, including a variant RNA which replicates with 23-fold efficiency comparing to the original one, was condensed in the new environment [2]. The evolved RNA showed the increased activity only in the new environment. Thus, although it is demonstrated only in the limited case, the adaptive ability was partially reconstituted in the simple artificial system.

[1] N. Ichihashi et al. (2013) Darwinian evolution in a translation-coupled RNA replication system within a cell-like compartment. *Nature Communications*, 4 (2494)
[2] R. Mizuuchi et al. (2014) Adaptive Evolution of an Artificial RNA Genome to a Reduced Ribosome Environment. ACS *synthetic biology*, in press

103. Influence of silicon on preparation and properties of porous Ni_3Al

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The addition of silicon improves the filtering performance of porous Ni3Al and makes pores readily controlled during sintering process. In comparison with porous Ni-10Al, it is clearly depicted that the pores become more abundant and paths get smoother with the introduction of silicon. Addition of 4% and 6%(at.%) silicon endows porous samples with good filtering precision and efficiency, respectively. The XRD patterns reveal that the phase of porous intermetallics is still pure Ni3Al, which ensures its excellent corrosive performance to strong alkali. The contents of silicon and final sintering temperature both influence on the filtering properties and flexural strength.

104. Simultaneous determination of dispersion model parameters and local thickness of thin films in imaging spectrophotometry

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Imaging spectroscopic optical techniques, such as spectroscopic ellipsometry, photometry and polarimetry, are excellent for the characterisation of non-uniform, structured and patterned thin films and biological specimens. However, the data processing present a challenge due to large data sets and large numbers of correlated fitting parameters. This contribution presents an algorithm that solves the problem of simultaneous least-squares fitting of thicknesses of the thin film in each image pixel together with shared dispersion model parameters that describe the spectral dependencies of film optical constants. The algorithm is based on a modification of the standard Levenberg--Marquardt algorithm in which the thicknesses and dispersion model parameters are fitted alternately, however, the thicknesses are updated in the dispersion model fitting steps in order to preserve an effective optical thickness in each pixel. Using experimental imaging spectroscopic reflectometry data for CNx and $SiO_xC_yH_z$ thin films, it is shown that this modifications allows the algorithm to converge rapidly and permits analysing imaging spectophometry data in a reasonable time even on a single personal computer.

105. The Effect of Different Doses of Methamphetamine on the Biotransformation of Acute Administration Alcohol in Rats

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Methamphetamine (MA) as a psychostimulant is one of the most often abused drugs in Central Europe. MA is very often used together with alcohol. The pharmacokinetics of this combination has been extensively studied in terms of MA metabolism. It was found that due to this combination p-hydroxylation and N-demethylation of MA is slowed. However, the influence of MA on alcohol biotransformation is poorly understood. The aim of this study was to clarify if various doses of chronic MA administration influence the biotransformation of alcohol administration in rats.

Wistar albino rats were randomly divided in 4 groups (A, B, C and K). Animals from the control group (K) were i.p. injected with saline (10 mg/kg/day). The animals from groups A, B and C were administered with MA. Group A was administered with dose 0.4 mg/kg/day, group B with dose 2 mg/kg/day and group C 10 mg/kg/day. After 9 days of this pre-treatment, in vivo pharmacokinetic experiment was performed. All the groups of animals were treated with 2 g/kg dose of alcohol in a 5% glucose solution administered by an intragastric probe. Blood was sampled in the 15th, 60th and 120th minute after the p.o. administration of alcohol. Then, animals from A, B and C group were administered a combination of MA (doses followed the original schedule – *i.e.* 0.4-2.0 and 10 mg/kg/day in groups A, B and C, respectively) and alcohol (2 g/kg/day) for additional 10 days. The control group K was administered with a combination of alcohol (2 g/kg/day) and saline in adequate volume. After 10 days of treatment, another *in vivo* pharmacokinetic experiment was performed. All the groups of animals were treated with an alcohol dose of 3 g/kg in a 5% glucose solution administered by an intragastric probe. Blood was sampled in the 15th, 60th and 120th minute after the p.o. administration of alcohol. Alcohol levels were measured using the GC method.

The present preclinical experiment suggests that chronic administration of various doses of MA increases the biotransformation of alcohol in the animal model. This effect does not seem to be proportionally dependent on the dose of MA. When animals were pre-treated by a combination of MA and alcohol, the blood levels of alcohol after acute administration were lower. Due to the fact that first sampling point did not cover Cmax , we cannot assume if this effect was due to impaired absorption, increase alcohol biotransformation in the liver or increase of both biotransformation and absorption.

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106. Interaction chamber for the laser-induced Breakdown spectroscopy

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Here we present an interaction chamber that has been developed for the applications of the laser spectroscopy techniques, particularly for the Laser-Induced Breakdown Spectroscopy (LIBS, [1]). The goal of the chamber development was to provide advanced possibilities for the research in the field of LIBS, such as utilizing multi-pulse LIBS or measurement under the different ambient gas conditions, and to facilitate routine measurement procedures, such as laser autofocus, automated chemical mapping [2], etc. The parameters and main benefits of the chamber are described. Some of the application examples are showed including the chemical mapping of the chalcopyrite stone for the purpose of visualizing the lead distribution on the sample surface and the LIBS of fluorine in the reduced ambient air pressure in order to increase the signal to noise (SNR) and signal to background ratio (SBR) of the detected signal.

References

[1] NOLL, R. *Laser-Induced Breakdown Spectroscopy: Fundamentals and Applications*. Springer, 2012. ISBN 3642206670.

[2] PIÑON, V., M. P. MATEO and G. NICOLAS. Laser-Induced Breakdown Spectroscopy for Chemical Mapping of Materials. *Applied Spectroscopy Reviews* [online]. 2013, vol. 48, no. 5, pp. 357–383. ISSN 0570-4928.

Acknowledgements

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107. A pragmatic approach to numerical simulations of high-frequency laboratory discharges

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Numerical modelling of high-frequency laboratory plasmas is very desirable due to their wide range of applications. This contribution presents three examples of numerical models which are strongly application-oriented. Its main aim is to demonstrate that under reasonable approximations, interesting conclusions can be drawn even from simplified models of the complex discharges.

The first model describes the gas dynamics in a radio-frequency plasma jet. The plasma is ignited inside a glass capillary in argon and blown out into ambient atmosphere. The model requires the gas temperature near the orifice as an experimental input (calculated from OH rotational spectra).

The second model presented describes the gas dynamics and the electromagnetic (EM) field in a microwave plasma torch operating in a non-uniform argon/hydrogen mixture at the atmospheric pressure. The EM field model takes electron density profiles from Stark broadening measurements as the experimental input and it is coupled iteratively to a gas dynamics model. The latter describes the gas flow, heat transfer and mixing of the $Ar/H_2/H$ mixture.

The most complex of the three models is a fluid plasma model of coaxial microwave discharge operating in hydrogen at pressures around 100 Pa. Although it it a low pressure device, most of the model assumptions would also by justifiable at high pressures.

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108. Electrochemical transistors based on inkjet printed PEDOT: PSS for biosensing

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Organic Electronics can represent the interface between biology and electronics, as it uses organic materials compatible with electronic applications and biological processes. It enables to investigate expression of a living matter and convert it to an electric signal. Biological sensors based on OECTs provide unique features for their important combination with biomedical interfaces, simple and low voltage operation regime, flexibility and sensing ability in aqueous environment [1]. In our work we focused on preparation and characterization of organic electrochemical transistors based on poly(3, 4-ethylenedioxythiophene) polystyrene sulfonate (PEDOT:PSS). The devices electrodes in planar arrangement were deposited on flexible, transparent plastic substrates poly(ethyleneterephtalate) (PET). Fabrication was realized by inkjet printing deposition utilizing Fujifilm Dimatix Material Printer DMP 2800. Printed transistors were used to evaluate adhesion and conductivity of printed layers on the substrates. We investigate the effect of the addition of ethylene glycol and also temperature at which were devices annealed on adhesion and conductivity. The influence of preparation conditions were investigated through the testing of output and transfer characteristics of the OECT as well as device stability. The unknown additives in the inks of various producers result in various wettability of the surface and resulting conductivity, which could be improved by substrate plasma treatment, post annealing and immersing in ethylene glycol. Resulting measured conductivity is in order of 100 S/cm of all tested samples. From profilometric measurements we calculated average height and roughness values of a single printed layer (130 ± 20) nm. The annealing made it smoother. Also the transfer characteristics of the OECT became steeper, but the effect is not significant. The significant effect is post treatment in ethylene glycol in case when the ink does not contain a proper additives. The conductivity increases by two orders.

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Literature

[1] Liao, C.Z. and F. Yan, Organic Semiconductors in Organic Thin-Film Transistor-Based Chemical and Biological Sensors. Polymer Reviews, 53(3), 2013. p. 352-406.

109. Occurrence of vancomycin resistant enterococci in municipal wastewater treatment plant in Brno: strain characterization and comparison with human hospital strains

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The emergence and spread of vancomycin resistant enterococci (VRE) have become an important clinical and epidemiological problem. VRE cause hospital outbreaks worldwide. Global occurrence of resistant bacteria in wastewater treatment plants (WWTPs) is an increasing concern. WWTPs are ideal locations for transfer of resistance genes and spread of resistant bacteria because of frequent interaction of bacteria and good environmental conditions. In addition, the presence of antibiotics and their metabolites in sewage may promote selection of resistant strains and also horizontal transfer of antibiotic resistance genes.

The water samples were taken weekly in outflow from WWTP in Brno over a period between March and December 2012. In total, we collected 37 wastewater samples. Samples were cultured for enterococci on Slanetz-Bartley agar (Oxoid) with 32 mg/L vancomycin and incubated for 48 h at 37°C. Individual colonies with enterococcal morphology were selected, identified and screened for *vanA* gene. The *vanA*-carrying isolates were tested for minimal inhibitory concentration of vancomycin, susceptibility to other antibiotics by disk diffusion method and occurrence of genes responsible for resistance and virulence by PCR. *Pulsed-field gel electrophoresis (PFGE) and multi-locus sequence typing (MLST)* were used to examine the genotypic diversity of vanA-containing VRE. Characteristics of isolates found were compared with VRE strains isolated from hospital infection unit patients in Brno.

VRE carrying vanA gene were found in 32 (86%, n=37) wastewater samples, from which we obtained 49 isolates - E. faecium (44) and E. gallinarum (2), E. casseliflavus (2) and E. raffinosus (1). All E. faecium isolates were multi-resistant with resistance to five and more antibiotics. A total of 35 (71%, n=49) isolates carried virulence genes hyl and/or esp. The MLST profile of E. faecium isolates showed that majority of isolates belonged to clinically important nosocomial lineages **17** - ST17 (10 isolates), ST555 (2); **18** - ST18 (9), ST262 (1), ST273 (3), ST275 (1); and **78** - ST78 (5), ST549 (2). Remaining isolates belonged to ST323 (3) and its new single locus variant ST884 (7). VRE characterized as ST17, ST18, ST262 and the new ST884 were found also in hospitalized patients from University Hospital in Brno, and some of them showed identical PFGE profile compared to isolates from WWTP in Brno.

Our results showed that clinically important enterococci with the vanA gene occur almost permanently in outflow from WWTP indicating insufficient effect of the treatment process in removing VRE and a risk of their transmission to the environment. We demonstrated the occurrence of the same strains in WWTP and in hospitalized patients.

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110. *CDK12* mutations in ovarian carcinoma cause deficient formation of the Cdk12/CycK complex underlying defective expression and function of homologous recombination DNA repair genes

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The Cdk12/CycK complex promotes gene expression by phosphorylating the C-terminal domain of RNA polymerase II. CDK12 is among only nine genes with recurrent somatic mutations in high-grade serous ovarian carcinoma. However, the influence of these mutations on the Cdk12/CycK complex and their link to cancerogenesis remain ill-defined. Here, we show that most mutations interfere with the Cdk12/CycK complex formation, rendering the kinase inactive. By examining the mutations within the Cdk12/CycK structure, we find that they likely provoke structural rearrangements detrimental to Cdk12 activation. Our mRNA expression analysis of the patient samples containing the CDK12 mutations identifies coordinated down-regulation of key DNA damage response genes. Accordingly, we observed that the mutant Cdk12 proteins fail to promote the repair of DNA double strand breaks via homologous recombination. Together, we provide the molecular basis of how mutated CDK12 ceases to function in ovarian carcinoma. We propose that CDK12 is a tumor suppressor of which the loss-of-function mutations may elicit defects in multiple DNA repair pathways, leading to genomic instability that underlies the genesis of the cancer.

111. New Antiviral Drugs Developed Against Human Rhinovirus 16

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Human rhinoviruses (HRV) are responsible for majority of common cold cases in humans. Currently there are no anti-viral drugs approved against rhinovirus infections and the available treatments are only symptomatic. Because of the number of strains of rhinoviruses (more than 110) it is unlikely that vaccination will be practical. Furthermore, RNA viruses rapidly mutate and acquire resistance to drugs that target viral enzymes, which poses serious problems in clinical context. Therefore, there is a growing interest in the development of antiviral drugs that target host factors critical for viral replication, since they are unlikely to mutate in response to therapy. We developed five new antiviral drugs to inhibit Human rhinovirus 16 (HRV16) with potential to inhibit other strains of rhinovirus. Strain HRV16 was selected because it belongs to the major receptor group of rhinoviruses, for which the cellular receptor is intercellular adhesion molecule-1 (ICAM-1).

These drugs inhibit phosphatidylinositol-4-kinase IIIβ (PI4KIIIβ) that catalyzes the synthesis of phosphatidylinositol-4-phosphate (PI4P) and this product is essential for the replication of rhinovirus.

The relative antiviral activities of five drugs against HRV16 were determined in HeLa (strain SOH) cell monolayers with a plaque inhibition assay. All compounds demonstrated activities against HRV16. 50% inhibitory concentrations for all five inhibitors ranged from approximately 0.2 to 1 micrograms/ml against HRV16.

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112. Impedance Analysis of GaGeAgSAgI Chalcogenide System by Random-Walk Approach

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Keywords: Chalcogenide glasses, Impedance study, Power-law dependence, Diffusion coefficient, Number of mobile ions

The study of ion-conducting glasses is extremely important because these glasses can be easily fabricated into complex shapes and have a wide compositional flexibility to optimize their properties and conductivities. Chalcogenide glasses (ChGs) containing LiI or AgI have emerged as promising candidates for fast ionic conductors and optoelectronic materials of unique properties [1]. In fact, ChGs containing LiI, such as LiI-Li_2S-GeS_2-Ga_2S_3, are one of the best solid state electrolytes known up to date. Li-containing electrolytes are difficult to prepare and environmentally hazardous because of their poor chemical and thermal stability. We are trying to replace Li by Ag metal, since Ag-containing chalcogenides also were studied and showed a good ionic conductivity [1, 2]. In the present work we studied the ionic conductivity behavior of GeS_2-Ga_2S_3-AgI-Ag chalcogenide system by a random-walk approach [2].

The samples of the nominal compositions $[(GeS_2)_{100-x}$-$(Ga_2S_3)_x$-$(AgI)_x]_{100-y}$-Agy were prepared by Ren et al., as mentioned in [3]. We observed that the conductivity of these glasses increases with increasing content of both AgI and Ag. The activation energy was found to decrease from 0.47 to 0.38 eV with increasing AgI content from 5% to 40%. We also discuss the correlation of diffusion coefficient and mobile ion concentration with AgI concentration.

References

[1] S. Stehlik, V. Zima, T. Wagner, J. Ren, M. Frumar, Solid State Ionics 179, 1867 (2008).

[2] D. S. Patil, K. Shimakawa, V. Zima, J. Macak, and T. Wagner, J. App. Phys. 113, 143705 (2013).

[3] J. Ren, Q. Yan, T. Wagner, V. Zima, M. Frumar, B. Frumarova and G. Chen, J. App. Phys. 114, 023701 (2013).

113. Expression and purification of intracellular domains of the ethylene receptor ETR1 from *A. thaliana* and introduction of intein technology

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Ethylene Receptor ETR1 is a membrane-bound receptor from A. thaliana involved in ethylene signaling process. ETR1 possesses all of the sequence motifs of canonical histidine kinase (HK) domains and also reveals HK activity (1, 2). HK activity of ETR1 is supposed to play rather minor role in output of ethylene signaling and may allow the cross-talk with multistep phosphorelay (MSP) via AHP proteins, potentially influencing cytokinin signaling. However, the structural aspects of ETR1 and its HK activity in the ethylene and/or MSP signaling remain unknown.

The main objective of our work was to express and purify intracellular domains (HK, HTPase and receiver) of ETR1 for functional and structural NMR studies. Domains were expressed as fusion proteins in *E. coli* cells and purified. After cleavage and subsequent purification native proteins were obtained. Receiver domain expressed together with NpuDnaE c-intein was used for an introduction of intein technology based on protein trans-splicing (3) in our laboratory. This technique helps to overcome the size limits of NMR measurements and allows to study multi-domain proteins by NMR.

1. Gamble *et al*. (1999), *Proc. Natl. Acad. Sci. USA*, 95:7825-7829
2. Moussatche & Klee. (2004), *J. Biol. Chem*., 279: 48734-48741
3. Volkmann, G., and Iwai, H. (2010), *Molecular Biosystems*, 6: 2110-2121

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114. Analysis of plasmonic slot waveguide couplers in linear and nonlinear regimes

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Plasmonic waveguide structures have found an increasing scientific interest in many areas of applications, due to their abilities to subwavelength light confinement and manipulations. Since the structures can strongly increase local fields, inherently-weak nonlinear optical effects can be significantly enhanced, and thus applied in many potential applications. Here we consider Kerr-type nonlinearity (i.e. intensity dependent refractive index, of both dielectric as well as metal components) and study nonlinear coupling between plasmonic slot waveguides (Fig. 1a).
Based on our recent study [1], understanding the physics of such devices, comparing both linear and nonlinear performance, is a very important issue. We have studied switching characteristics of the relative output power with respect to the level of nonlinearity (Fig. 1b). We discuss various physical mechanisms that limit the coupler performance and investigate possible optimization of geometrical parameters. Significant improvement of the coupler behavior is observed in certain spectral regions (Fig. 1c). However, in order to fully exploit potential of linear plasmonic couplers for creation of ultra-compact nonlinear switches, novel metallic and dielectric materials with low losses and large nonlinearities are required.

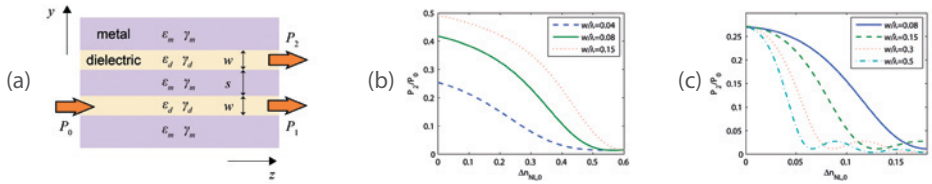

Fig. 1: (a) Two dielectric waveguides of widths *w* separated with the metallic cladding (width s), with linear (ε_m and ε_d) and nonlinear (Kerr) coefficients - γ_m and γ_d; (b) the power switching characteristics P_2/P_0 with respect to the nonlinearity level; and (c) improvement of the switching characteristics with a material of silicon nanocrystals in an amorphous silica matrix.

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References

60. J. Petráček, Applied Physics B **112**, 593, (2013).

115. Functional characterization of a minimal XPF/ERCC1 heterodimer for structural studies

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Numerous DNA repair pathways are required for genomic stability and chromosome maintenance. XPF/ERCC1 is a structure-specific endonuclease that cleaves double- to single-stranded DNA junctions that arise as intermediates during DNA repair processes. A better understanding of XPF/ERCC1 function requires structural details of DNA recognition that are currently missing. To facilitate structural studies, we have designed and characterized a minimal XPF/ERCC1 heterodimer that consists of the domains responsible for dimerization, DNA binding, and catalysis. We demonstrate that the minimal heterodimer possesses enzymatic activity in vitro, and recapitulates the findings reported on full-length XPF/ERCC1, indicating that the catalytic site is correctly formed. Comparison of affinities to different DNA substrates indicates the DNA features that account for DNA junction recognition. Preliminary NMR data strongly suggest that further structural studies are feasible. In future, we aim to elucidate the molecular basis for XPF/ERCC1 recognition and incision of DNA junctions. Such structural information will be invaluable in developing inhibitors of XPF/ERCC1 that can be administered with chemotherapeutic agents to increase their potency by inhibiting DNA repair.

116. Modification of Poly(Lactic Acid) by Radical Grafting for Application in Advanced Polymeric Materials

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In proposed study the functionalization of poly(lactic acid) (PLA) was realized by radical grafting of PLA with itaconic anhydride (IAH) which can be both derived from renewable resources. Bifunctional peroxide 2, 5-dimethyl-2, 5-bis(tert-butylperoxy)hexane (L101) was used to initiate the reaction. Reaction was achieved in laboratory mixer Brabender where reaction time was established to simulate residence time in extruder. According 6 min of residence time reaction temperature 187.4 °C was calculated using Arrhenius equation containing activation energy parameter and pre-exponential parameter of L101. Reaction time 6 min equaled to $10 \times t_{1/2}$ of peroxide where $t_{1/2}$ equals to 36 s. Calculated reaction temperature also eliminates trans-esterification which predominates above 200 °C. Various reaction time, temperature as well as monomer/initiator ratio were applied in experimental section.

The influence of reaction parameters on reaction conversion, thermal properties and material structure was investigated. Evidence of grafted IAH and relation between reaction conversion and IAH/L101 ratio were confirmed by acid-base titration, FTIR spectroscopy and 1H-NMR spectroscopy. Thermal properties as well as effect of chain branching of prepared samples were concluded as a function of monomer conversion by differential scanning calorimentry (DSC) and temperature modulated scanning calorimetry (TMDSC). End-chain grafting and chain scission occurred during reaction were successfully confirmed by size exclusion chromatography (SEC). Enhanced hydrophilic behavior of modified PLA compared to neat PLA was predicted by contact angle measuring.

Main goal of this work was to prepare PLA grafted with IAH (PLA-g-IAH) which can be potentially used as a compatibilizer in polymer blends due to reactivity of cyclic anhydride groups. Based on the results reaction mechanism was predicted as well as undesired reaction occurring during the radical reaction. Modified PLA could be used in industrial application, especially by chemical compatibilization of grafted PLA with other polymers or fillers coatings. PLA-g-IAH can be potentially used in advanced polymer blends with reduced interfacial tension, also known as "nano-blends".

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117. Label-Free Impedimetric Immunosensor Approach in Cancer Diagnosis

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Glycosylation as one of the most prevalent post- and co-translational modification of biomolecules in human body plays an important role in cell differentiation, host-pathogen interactions, tumour metastasis etc. During malignant transformation arise changes in glycosylation machinery caused by perturbed activity of glycosyltransferases and glycosidases. Altered glycosylation of cancer biomarkers may assist as suitable tool for early stage cancer diagnosis and should improve the sensitivity and specificity of analysis. Herein, we present construction, optimization and challenges of immunosensor construction based on mixed self-assembly monolayers. Prostate specific antigen (PSA) was detected by electrochemical impedance spectroscopy (EIS) as a sensitive label-free tool with limit of detection down to attomolar level. Polycrystalline gold electrode was chemically modified by mixed self-assembled monolayer and immobilization of antibody was achieved *via* EDC/NHS chemistry. We present a sandwich format analysis based on immobilization of antibody recognizing glycoprotein in the first step, followed by capture of disease-specific glycoprotein (PSA) and a final injection of lectin to glycoprofile the protein with a low sample consumption and simple miniaturization. Furthermore, a biosensor interface was examined by atomic force microscopy (AFM) showing a nanometre scale sized features of proteins investigated.

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118. Significance of Genomic Abnormalities in Evolution of Biclonal Chronic Lymphocytic Leukemia

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Objective

A subset of chronic lymphocytic leukemia (CLL) patients is characterized by expansions of multiple B-lymphocyte clones. In such cases, specific molecular features of B-cell receptor are important for clone selection and eventual predominance of a single leukemic B-lymphocyte clone (Plevova et al, Haematologica 2014). We were interested whether and in what extend also genomic makeup of individual clones might be another factor contributing to the clonal selection.

Methods

Individual leukemic clones from 10 patients with biclonal CLL were FACS-sorted; gDNA was isolated and analysed using CytoScan HD Arrays (Affymetrix). *TP53* gene mutations were identified by FASAY assay and direct sequencing. Allele-specific PCR assays targeted against immunoglobulin genes were designed to track individual leukemic clones over disease course in consecutive blood samples.

Results

Firstly, 20 individual leukemic clones were analyzed as independent entities. Deletion 13q14.2 was the most frequent copy number alteration (CNA) (10/20 clones), followed by del(17p13.1) (*TP53* locus; 4/20 clones), del(6q21q22.1) (2/20 clones) and del(5q14.2q22.3) (2/20 clones). Three or more CNAs were identified in 7/20 clones and were observed in all four clones with del(17p13.1). Mutation of *TP53* was detected in 5/20 clones (4x mut/del; 1x mut/wt). In the second step, we compared leukemic clones in pairs belonging to individual patients. CNAs of the same locus present in both co-existing clones were observed in 3/10 patients (2x del(13q14.2), 1x del(5q14.2q22.3)). Importantly, chromosomal breakpoints were not identical in any of these cases suggesting their independent origin. When we correlated genomic makeup of coexisting clones with their behavior over time, *TP53* defects and increased genomic complexity were identified as crucial factors leading to eventual prevailing of affected clones.

Conclusions

Our results indicate that apart from molecular features of B-cell receptor, specific genomic makeup is also critical for clonal selection in biclonal CLL. These data have important implications for patient management and monitoring during the disease course.

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119. Enzyme-linked electrochemical detection of SNP in human mitochondrial DNA at pencil graphite electrode

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A new strategy of using enzyme-linked assay for detection of single-nucleotide mismatch is described. Principle of technique includes selective elongation of specially designed primers by mixture of dNTPs, part of which is biotinylated, as a first step. Primers are designed so as to differ from each other by 3´-end nucleotide, which is in site of SNP. At optimal stringency of reaction synthesis of strands, resp. incorporation of biotin, take place only in case of complementarity of 3´-end nucleotide with template. Enzymatic detection using streptavidin-conjugated alkaline phosphatase and 1-naphthylphosphate as a substrate then follows. Very good resolution in signal of 1-naphthol oxidation was achieved for different primers. This assay performed at pencil graphite electrode can represent very cheap and simple disposable biosensor.

120. Synthesis of spherical gold nanoparticles for biomedical applications

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This work introduces synthesis of spherical gold nanoparticles (Au NPs) in aqueous media for application in the field of in vivo and in vitro bioimaging techniques. Au NPs were synthetized by three ecological approaches, so called green syntheses, using lemon, chitosan and onion broth. Physical and optical properties of Au NPs were characterized by techniques such as SEM, DLS and UV-VIS spectroscopy. Shape and size of Au NPs were specified by SEM technique; size of Au NPs was in range 10-150 nm and shape depended on the amount of active substance; the more of active substance, the more spherical shape was observed. Optical properties were determined by UV-VIS spectroscopy, absorbance was measured; the intensity absorption also depended on a concentration of active component, the higher concentration, the higher intensity of absorption peak was gained. DLS measurement reported a stability and zeta potential of Au NPs; chitosan Au NPS were the most stable with highly positive zeta potential, no aggregates were observed for 2 months. Lemon Au NPs were coated by PEG to increase the stability of gold colloid solution; PEGylation was successful and lemon particles were stable for 6 weeks. MTT assay with live HEK293 cells was also provided to report on cytotoxicity and biocompatibility of synthesized Au NPs; the most non-toxic particles to cells were PEGylated lemon Au NPs.

Keywords
gold nanoparticles, synthesis, characterization, imaging, PEGylation, cytotoxicity

References

[1] ZHAO, P. X. et al. State of the art in gold nanoparticle synthesis. Coordination Chemistry Reviews, 2013, vol. 257, no. 3-4, pp. 638-665. ISSN 0010-8545.
[2] NG, V. W. K. et al. Gold: a versatile tool for in vivo imaging. Journal of Materials Chemistry B, 2013, vol. 1, no. 1, pp. 9-25. ISSN 2050-750X.

121. LIBS for *in-situ* Qualitative and Quantitative Analysis of Mineral Ores

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We report on the potential of laser-induced breakdown spectroscopy (LIBS) for in-situ and real-time discrimination of geological samples. The research is focused on demonstrating the utilization of LIBS as an instrument for qualitative discrimination among copper containing mineral ores and partly for quantitative determination of copper concentration. The sample suite consists of various sedimentary rocks standards, (Ore Research and Standards, Australia) and samples collected directly at a copper mines located in East Azerbaijan region, Iran. The samples were measured as a thin film deposited on the surface of adhesive tape. Collected LIBS spectra contain the full elemental composition, i. e. the geological fingerprint, of the samples of interest. These multivariate signals are processed by the means of principal component analysis (PCA), a simple chemometric algorithm, to obtain qualitative analysis and a classification into different rock types, which is valuable geological information. Moreover, standard discrimination among various rocks is done by investigating the amount of matrix elements (Al, Ca, K, Na, and Si), i.e. plotting the so-called QAPF diagram. Clustering of LIBS data based on PCA algorithm correlates with the discrimination of rocks according to QAPF diagram. Accompanying, calibration curves were employed for quantitative analysis of the copper content. A comprehensive amount of samples could successfully be classified into individual groups; hence this unsupervised clustering *prior* to the quantitative determination of the copper content could be achieved. From this data, a strong dependence of the copper signal intensity from the ore type can be seen. However, the unambiguous classification of the samples into individual ore-types - together with custom calibration data sets for the individual minerals - could allow for an efficient suppression of this commonly known matrix effect. The approach to use individual calibration algorithms for individual rock-types, respectively, can be shown to lead to a tremendous improvement of the quantification of copper.

122. Chemical modification and characterization of hydrogel from natural polysaccharide Gum Karaya

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Gum Karaya (GK) is a natural gum exudate of *Sterculia urens* tree. GK is a partially acetylated, insoluble and anionic polysaccharide. It is obtained as calcium and magnesium salt and contains of β-D-galactose, L-rhamnose, β-D-glucuronic acid and D-galacturonic acid. The present study deals with chemical modification of GK to developed soluble material by alkali treatment for subsequent potential use in medicine (e.g. hydrogels for soft tissue regeneration of drug delivery systems). Conditions of modification reaction such as type of hydroxide, concentration of GK dispersion and time of reaction were optimized and mechanism of the reaction was proposed. Original GK and modified GK samples were studied by SEM, FTIR, TGA, DSC, ICPOES, GPC, NMR and rheological studies. The linear viscoelastic region was determined by rheological measurements. The effect of NaCl on viscosity of original GK was performed. Decreasing viscosity of GK solution with higher amount of NaCl was explained by exchange of multivalent ions by monovalent ions in the GK structure and release of the physically bonded structure resulting in higher solubility of original GK in water.

123. Simulation of processes influenced by surface diffusion

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The Monte Carlo simulation algorithm was used to describe the surface processes (desorption and diffusion). The surface-to-bulk transport and desorption processes are studied at elevated temperatures and the possible application to the Ga desorption from Si surface, and to the Au on Si oxide system is discussed. The advantage consists in utilization of the Rejection-Monte Carlo algorithm with enhanced performance by parallel simulation using the Graphics Processor Unit (GPU). This allows us to simulate these processes in experiment time scale.

124. Hypergravity influence on gliding arc in noble gases with outlook for advanced material plasma synthesis

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The innovative methods of preparation of advanced materials are nowadays intensively researched, as their usable properties are employed in increasing number of current applications. The allotropes of carbon, such as carbon nanotubes, fullerenes or graphene are promising materials with outstanding physical and chemical properties.

Plasmachemical synthesis of diamond is well established method and it is also possible to prepare other carbon structures and nanostructures using plasma. Besides the common plasma sources, some non traditional are currently tested, too. One of them is gliding arc, which has advantage in experimental simplicity and ruggedness. Recently, a gliding arc plasma synthesis of nanostructured carbon material was reported in hypergravity conditions [1, 2]. As the hypergravity affects many parameters of the plasma, especially the convective gas flow and by consequence a recirculation of growing nanoparticles, we decided to study the effects of hypergravity on the gliding arc in more detail [3, 4]. As the presence of dust (and by consequence, the nanoparticles) makes both the experimental and theoretical study difficult, for now the study is limited to case without any precursor.

The poster focuses on both time resolved and time averaged diagnostics of the gliding arc operated in four noble gases in artificial gravity from 1g to 18g with the outlook to preparation of various nanopowders.

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References

[1] J. Sperka, P. Soucek, J.J.W.A. Van Loon, et al., Mater. Res. Bull. 54 (2014) 61-65

[2] L. Potocnakova, J. Sperka, P. Soucek, et al., Proceedings of International Masaryk Conference 2013, MAGNANIMITAS 2013, 4151-4160

[3] J. Sperka, P. Soucek, J.J.W.A. Van Loon, et al., Eur. Phys. J. D 67 (2013) 261

[4] L. Potocnakova, J. Sperka, P. Zikan, et al., IEEE Trans. Plasma Sci., Special Issue on Images 2014, August 2014, Accepted for publication

125. The fate of centromeres in a mesopolyploid genome of *Stenopetalum nutans* (Brassicaceae)

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Full understanding of molecular processes determining centromere inactivation and/or loss represents a great challenge in chromosome biology. The mesotetraploid *Stenopetalum nutans* (2n = 8), an endemic Australian species with the lowest chromosome number in the family Brassicaceae, is an interesting model to investigate mechanisms leading to centromere inactivation/loss. The putative ancestor of Australian crucifers (2n = 30-32) has undergone a whole-genome duplication followed by diploidization accompanied by extensive chromosome rearrangements and chromosome number reduction towards the modern diploid-like genomes similar to that of *S. nutans* (Mandáková et al. 2010). These processes also included centromere inactivation/loss events.

The aim of our study is to characterize functional (active) centromeres including centromeric histone H3 (CenH3) and to trace the evolutionary fate of three inactive centromeres in the genome of *S. nutans*. A BAC library of *S. nutans* was constructed and subsequently screened using *Arabidopsis thaliana* BAC clones to determine BACs flanking the putative inactive centromeres. The genome of *S. nutans* was sequenced using the Illumina HighSeq technology and repetitive elements were characterized. Fluorescence *in situ* hybridization experiments with DNA probes specific for the abundant repeats elucidated their precise chromosomal distribution. In congruence with its mesotetraploid origin, two gene copies of CenH3 were identified in *S. nutans*. Comparative analyses of active and inactive centromeres in *S. nutans* and other Australian crucifer species are under way.

Mandáková T, Joly S, Krzywinski M, Mummenhoff K, Lysak MA. 2010. Fast diploidization in close mesopolyploid relatives of *Arabidopsis*. *Plant Cell* 22: 2277–2290. This work was supported by the European Social Fund (CZ.1.07/2.3.00/20.0189, CZ.1.07/2.3.00/30.0037).

126. Investigation of potential of Laser-Induced Breakdown Spectroscopy for detection of spatial deposition and µCT for volume deposition of Pt in minerals/ores

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The platinum group metals (PGMs) i.e. platinum, palladium, rhodium, iridium, osmium and ruthenium represent the key materials for automotive exhaust gas treatment. Since currently exist no adequate alternatives, the importance of these metals for automotive industry is steadily rising [1]. In order to localize new ore deposits it is important to develop suitable methods capable to detect critical metals in real time, even in extremely harsh climatic conditions of some ore deposit sites. In this paper we demonstrate that laser-induced breakdown spectroscopy (LIBS) can be applied as a suitable tool for elemental analysis, especially for 2-dimensional elemental mapping of minerals. In order to obtain the information about metals distribution in the sample volume, the µCT station has been utilized. We focused on sperrylite detection, which is a simple platinum arsenide with the chemical formula PtAs2 obtained from unique Talnakh deposit (Siberia, Russia). In our measurements it was demonstrated that LIBS is a method perfectly suitable for mineral analysis with the spatial resolution of at least 100 µm, with no need of a sample preparation and with the possibility to be performed for the distance of 6.2 m. A chemical map of the sample surface was prepared and compared with a chemical map made by reference technique – Tescan Integrated Mineral analyser (TIMA) [2]. Tomograms of each sample were prepared with the resolution of 20 µm. On the basis of the data obtained, it is then possible to reproduce spatially resolved chemical composition of ore samples.

[1] ASIMELLIS, G. et al. Platinum group metals bulk analysis in automobile catalyst recycling material by laser-induced breakdown spectroscopy. Spectrochimica Acta Part B: Atomic Spectroscopy. 2008. Vol. 63, no. 11, s. 1338–1343.
[2] KRALOVA, V. et al. TIMA - TESCAN Integrated Mineral Analyzer: New approach for rapid evaluation of critical elements ore samples. In Deposits of critical metals and related carbonatite-alkaline rock systems. 2012.

127. Electrochemical characterization of nanostructured surfaces modified by substances with thiol bound

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Nanostructured gold surfaces have been synthesized by utilizing anodic aluminium oxide (AAO) templates in combination with electrochemical galvanic deposition of gold ions [1, 2]. Topography and structure of AAO templates and gold nanowires were characterized by scanning electron microscope (SEM). Self assembled monolayer of 11-mercaptoundecanoic acid (MUA) has been achieved via two different approaches: the first deposition was from 5mM solution of absolute ethanol and the second process was chemical vapor deposition [3]. The terminal groups of self-assembled monolayer have been activated by substances EDC and NHS. In following step, streptavidin was immobilized onto activated electrode and afterwards biotin of different concentrations [4]. The avidin – biotin interaction on MUA layer deposited on gold nanostructured surface was observed by cyclic voltammetry and electrochemical impedance spectroscopy. The electrode hydrophilic/hydrophobic properties were studied by contact angle measurement.

References

[1] J.W. Cui, S.B. Adeloju, Y.C. Wu, Anal Chim Acta, 809 (2014) 134-140.
[2] Z. Wu, Y.W. Zhang, K. Du, Appl Surf Sci, 265 (2013) 149-156.
[3] C. Vericat, M.E. Vela, R.C. Salvarezza, **Phys Chem Chem Phys**, 7 (2005) 3258-3268.
[4] S.J. Ding, B.W. Chang, C.C. Wu, M.F. Lai, H.C. Chang, Electrochim Acta, 50 (2005) 3660-3666.

128. Molecular simulations of Sr-phosphonate layer with ethane-1, 2-diol

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We calculated the intercalation of various kinds of 1, 2-diols (ethane-1, 2-diol is presented) into Sr-phosphonate layers with a summary formula $Sr(C_6H_5PO_3)\cdot H_2O\cdot 0.5(HO\text{-}C_2H_4\text{-}OH)$ by molecular simulations methods. These calculations are excellent for studying various arrangements and positions of the ethane-1, 2-diol molecules in the interlayer space of the Sr-phosphonate layer to obtain a detailed view into mutual interactions between phenol rings of the Sr-phosphonate layers and ethane-1, 2-diol in the large structures. The compounds were characterized by powder X-ray diffraction and thermogravimetry and the experimental results were used to define calculations procedures and for verification of simulation results.

Parameterization of the structures was done on the base of X-ray diffraction data. Experimentally determined cell values were: a = 7.9270(6) Å; b = 8.6110(6) Å; c = 29.736(2) Å; *alpha* = *beta* = *gamma* = 90.00°. A supercell of *2a x 2b x 1c* was prepared and the space group was changed from *Pn21a* to *P1* for the calculation purposes. First calculations were made with a fixed Sr-phosphonate layer except the phenol rings so the interlayer space was optimized. Then the Sr-phosphonate layers except the phenol rings were kept rigid and the ethane-1, 2-diol molecules and the phenol rings were left without constraints. Water molecules were also included into the calculation. The model was optimized in a Compass force field and charges were calculated by a Charge equilibration approach. Nonbonding energy was calculated by an Ewald summation method with accuracy of 0.001 kcal/mole, cutoff of 0.6 nm. The calculation was run for different positions and arrangements of the ethane-1, 2-diol molecules with respect to the Sr-phosphonate layer and the phenol rings. These were partially immersed into the interlayer cavities or placed just above the Sr atoms. Various optimization conditions like different values of pressure, different values of convergence parameters were used for optimization of the structure to obtain a good agreement with the experimental results.

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129. Human embryonic stem cells require BER mediated channelling of base damage to alternative NHEJ to maintain low mutant frequency

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Genome instability presents a major obstacle in therapeutic application of human embryonic stem cells (hESCs). Point mutations and chromosomal abnormalities in culture adapted hESCs resemble cancer features eliminating therapeutic potential of these cells. Failure of DNA repair mechanisms contributes to the genome destabilization. For example our preview research showed that efficiency of base excision repair (BER) drops in hESCs in late passages, mutation frequency increases and genome stability is violated. Such alterations in BER activity are crucial not only in terms of unrepaired base damage, but also by its overlap with double strand breaks (DSBs) repair as BER mediates conversion of clustered base damage to DSBs giving rise to complementary microhomologies. Our research shows that most of such DSBs were repaired within one hour after damage, suggesting involvement of mechanism quicker than homologous recombination, such as non-homologous end joining (NHEJ). Surprisingly less DSBs were detected after ionizing radiation treatment when Ku70/80-dependent canonical NHEJ (cNHEJ) was inhibited by NU7026, suggesting involvement of microhomology driven alternative NHEJ (aNHEJ). That also corresponds to the decrease in mutation frequency, when cNHEJ is inhibited; again implicating aNHEJ is involved as a more error proof repair mechanism. Therefore we can postulate that the low mutagenic outcome of base damage repair depends on the BER-initiated and aNHEJ-completed DSBs repair in hESCs.

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130. Application of On-Line Enantioselective Capillary Electrophoretic Method: Kinetic and Inhibition Study of CYP3A4 Mediated N-Demethylation of Ketamine

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Metabolism of a chiral drug can significantly differ between application of racemate and single enantiomers. Rational drug discovery thus requires an early appraisal of this characteristic impacting on the likely success of a drug candidate in the subsequent clinical testing. Capillary electrophoresis (CE) represents a promising technique to assess the fate of drug enantiomers via in vitro assays. Besides chiral highly effective separations and high throughput, it offers the possibility of implementation of on-line methods where a fused-silica capillary is used not only as a separation column but also as a nanoscale reaction chamber integrating the incubation of an enzymatic reaction, separation of its products and their detection and quantitation into a single fully automated run.

After initial attempts [1], an improved on-line method for determination of enantioselective metabolism of anesthetic ketamine mediated by cytochrome P450 3A4 (CYP3A4) was developed. As mixing of reaction components inside the capillary based on diffusion is generic, robust and rapid, the principle of transverse diffusion of laminar flow profiles was adopted [2]. After the incubation was complete, the reaction products were separated in BGE containing highly sulfated γ-cyclodextrin used as chiral selector. The new approach was thoroughly validated for CYP3A4 mediated N-demethylation pathway of ketamine and applied to the determination of its kinetic parameters and the inhibition characteristics in presence of ketoconazole and dexmedetomidine. The results obtained were in a very good agreement with literature data assessed by different techniques. The final method thus represents a miniaturized and cost-effective tool suitable for screening of stereoselective aspects of drug candidates' metabolism in early stages of a new drug development.

[1] Kwan, H.Y., Thormann, W. Electrophoresis 33 (2012) 3299-3305.
[2] Řemínek, R., Zeisbergerová, M., Langmajerová, M., Glatz, Z. Electrophoresis 34 (2013) 2705-2711.

131. Laser Schlieren Deflectometry (LSD) - Temperature Diagnostic of Non-Thermal Microfilamentary Plasmas for Life Science

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Atmospheric pressure microdischarges are widely used for material processing [1] as well as in medicine [2]. It is well known that temperature profiles of a contracted filament affect the energetic balance and process efficiency. However, the local evolution of the neutral gas temperature in filamentary plasmas at atmospheric pressure evades the direct measurement by almost all experimental methods. Laser Schlieren Deflectometry (LSD) represents a new diagnostic technique developed especially for the characterization of the locally resolved neutral gas temperature in the RF plasma jet [3]. In this study, we investigate the effect of different analytical profiles of the refractive index which determines the neutral gas temperature in LSD. The mathematical concepts are discussed with regard to their physical relevance. The spatially and temporally resolved temperature obtained by LSD is compared with results of thermodynamic model calculations and with investigations on the visual profiles of the filament. The assumption of a Gaussian profile for the total optical emission is validated. The method allows the determination of the neutral gas temperature beyond the visual boundaries of the filament. The results indicate that the profile indeed extends substantially into this area. (Project supported within the 7th Eur. Framework Programme, No 316216, „PlasmaShape")

[1] J. Schäfer, R. Foest, A. Quade, A. Ohl, J. Meichsner, and K.-D. Weltmann, Eur. Phys. J. D 54, 211, 2009
[2] J. Lademann, H. Richter, A. Alborova, D. Humme, A. Patzelt, A. Kramer, K.-D. Weltmann, B. Hartmann, C. Ottomann, J. W. Fluhr, P. Hinz, G. Hübner, and O. Lademann, Biomed. Opt. 14(5), 054025, 2009
[3] J. Schäfer, R. Foest, S. Reuter, T. Kewitz, J. Šperka, and K.-D. Weltmann, Rev. Sci. Instrum. 83, 103506, 2012

132. A Novel Approach in Cytochrome P450 Inhibitors´ Screening: Immobilized Enzyme Microreactor for On-line Studies Using Capillary Electrophoresis

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Hepatic enzyme superfamily of cytochromes P450 (CYP) plays a key role in drug metabolism. Characterisation of the interaction between a drug candidate and CYP isoform is thus an integral part of preclinical phase of new drug development, which demands high throughput and cost effective methods. Immobilization process offers number of favourable features including prolonged enzyme stability and repeated use. Immobilized enzymatic microreactor (IMER) therefore represents a promising tool for required high throughput screening.

In this work we present an IMER integrated into capillary based on CYP 2C9 isoform, representing approximately 20 % of all CYP in human liver which is responsible for metabolizing more than 10 % of commonly used drugs. The CYP 2C9 was immobilized on magnetic microparticles Si-MAG carboxyl using the carbodiimide method and the formation of IMER was established by two permanent magnets placed in cassette for capillary electrophoresis. The enzymatic reaction was instantly followed by capillary zone electrophoresis separation so that the product could be detected and quantified with UV-DAD. Unfortunately CYP enzymes are membrane bound, which makes their immobilization rather cumbersome and displayed features are usually inferior in comparison to soluble enzymes, further optimization of incubation and separation was therefore necessary. All challenges were in the end overcome and optimized method was used to determine Michalis-Menten constant for diclofenac and to characterise the inhibition of previous reaction by sulphophenazole. All acquired data were in good correlation with literature, proving this method suitable for inhibition studies. Furthermore the consumption of enzyme and reactants is significantly lower compared to generally used methods like fluorescence microassays.

133. Identification and comparison of adenoviruses in fecal samples from wild and captive non-human primates

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DNA viruses from the family Adenoviridae infect a wide range of animal hosts, including humans and non-human primates (NHP). Adenoviruses (AdV) infecting primates are classified into seven Human adenovirus subgroups (HAdV-A – HAdV-G) or Simian adenovirus A group with the remaining species unassigned to any subgroup allocated to the genus Mastadenovirus. In an attempt to study the molecular diversity and evolution of human and NHP AdV and the possibility of cross-species transmission, we have screened a community of savanna-woodland chimpanzees (Pan troglodytes schweinfurthii) living in their natural habitat in Ugalla, Tanzania and 17 species of NHP from one Slovak and 13 Czech ZOOs. The presence of AdV in non-diarrhoeal fecal samples (113 samples from Ugalla and 153 samples from ZOOs) was detected by nested PCR using degenerated primers targeting highly conserved DNA polymerase genes. From the AdV-positive samples nearly complete hexon gene sequences (hypervariable region responsible for serotype specifity) were amplified. Phylogenetic analyses based on both DNA polymerase and hexon gene sequences proved the presence of AdV grouped into HAdV-E and HAdV-C clades in wild chimpanzees. On the other hand, phylogenetic analyses of the samples from captive NHP showed much higher heterogeneity – we revealed the presence of AdV clustering to the HAdV-B, HAdV-E, HAdV-F and SAdV-A groups and also unassigned distinct species clustering to the genus Mastadenovirus. Here we reported the first detection of AdVs in Mandrillus, Cercopithecus and Lophocebus spp. Our data showed approximately 30% prevalence of AdV DNA in non-diarrhoeal fecal samples from wild chimpanzees in Ugalla and almost 40% prevalence of AdV in samples from ZOOs. The study confirmed the presence of various AdV species in the gastrointestinal tract of both wild and captive animals and gave a new insight into the diversity of AdVs infecting NHP.

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134. Improvement of detection limits of trace elements by Laser-Induced Breakdown Spectroscopy (LIBS) with nanoparticles

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Laser-Induced Breakdown Spectroscopy (LIBS) belongs among modern methods of a chemical elemental composition analysis of materials. For the detection of individual chemical elements a spectral characterization of the radiation emitted from laser-induced plasma (LIP) plasma is used [1].

Samples composed of matrix elements may content many additives in different concentrations – so called trace elements. Low concentrations of trace elements are often under the LIBS detection limits (LODs). The typical detection limits do not reach the limits of conventional analytical methods like mass spectroscopy ICP or Raman spectroscopy, which are in the range from units to tens ppm [2].

There exist several possibilities how to improve LODs. Here, we present the trace element LODs improvement using silver nanoparticles [3]. Nanoparticles are very common, easy available and their effects are studied in different scientific fields from medicine to engineering. The nanoparticles are applied directly onto the surface of metallic materials with no further difficult preparation of the analyzed material. This means that the biggest advantages of the LIBS method stay preserved.

References

[1] Cremers D.A., Radziemski L.J.: *Handbook of laser-induced breakdown spectroscopy*. John Wiley & Sons, Ltd 2006. (ISBN-10: 0-470-092999-8)

[2] Corney A.: *Atomic and Laser Spectroscopy*. Oxford (UK) : Oxford University Press, 2006.

[3] Giacomo A. De, Gaudiuso R., Koral C., Dell´Aglio M., Pascale O. De: Anal. Chem. 85, 2013. p. 10180-10187.

135. The reactivity of cationic biopolymer studied by interactions with organic dyes

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One of the most important gaps in the knowledge of biopolymers is the study on their reactivity. Simple universal reactivity mapping tool for biopolymers is needed. Non-stationary diffusion of anionic organic dye (C.I. 20470) was realized in agarose supported matrix for purpose the study on reactivity of cationic biopolymer (chitosan). The reactivity of chitosan was studied by interactions with anionic organic dye in hydrogel matrix (agarose). The rate of interactions was determined by fundamental diffusion parameters (effective diffusion coefficients and the concentration of organic dye on the interface hydrogel – solution).
Ultraviolet visible spectroscopy was used as an analytical method for in situ determination of the concentration of organic dye at different distances from the interface hydrogel – solution. From measured UV-VIS spectra, the concentration profile can be calculated. Experimental concentration was compared with theoretical diffusion model and after this comparison; the fundamental diffusion parameters can be calculated. The influence of concentration, temperature and ionic strength on the reactivity of chitosan was studied as well. Results clearly illustrate strong immobilization effect of chitosan on the transport of anionic organic dye through the supported hydrogel. Simple diffusion techniques (non-stationary diffusion technique together with diffusion cell technique) seem to be universal reactivity mapping tool for wide range of biopolymers (e.g. alginate, hyaluronate) or biocolloids (e.g. humic acids).

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136. On the mechanical characterization of PECVD deposited diamond-like carbon thin films for biomedical applications

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Carbon-based coatings have already been recognized and accepted as biocompatible materials. The unique properties of diamond-like carbon (DLC) such as chemical inertness, high wear, and corrosion resistance, etc. [1] make it a prospective material that can be used in medical devices such as implants, stents, and lenses [1, 2]. Several recent attempts were made to modify the characteristics of a-C:H film coating by adding other chemical elements, such as oxygen, nitrogen and fluorine, into the films for each application [3, 4]. Controlled engineering of these thin films is essential and currently presents a challenge. At low thickness, material properties such as elastic modulus, hardness, adhesion, friction and electrical behavior become increasingly difficult to measure. Reliable characterization of film properties is particularly relevant for the deposition process and quality control. Highly precise force, displacement, and positioning control are requirements for continued improvement in the measurement of properties and performance of these advanced materials systems. In this work several types of DLC deposited by PECVD have been investigated in order to assess the mechanical response of such coating not only in normal atmosphere but also in underwater conditions.

References

1 A. Grill (2003). Diamond and Related Materials 12:166-70.
1 R. K. Roy, et al (2009) Vacuum 83: 1179-1183.
2 R.K. Roy, K.R. Lee (2007) J. Biomed. Mater. Res. Part B 83B: 72-84.
3 T. Hasebe, et al (2007) New Diamond and Frontier Carbon Technology 17: 263-279.
4 P. Yang, N. Huang, Y. X. Leng et al (2006), NIM B 242: 22-25.

137. Thermal evolution of silicophosphate gels: Porosity and structure

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Non-hydrolytic sol-gel reactions are viable alternatives to classical aqueous techniques in the area of synthesis of multimetallic oxides and inorganic-organic hybrid materials in the form of xerogels, nanoparticles, and thin films. We developed a non-hydrolytic sol-gel route based on acetic acid ester elimination providing silicophosphate materials and related hybrid inorganic-organic materials. The polycondensation reactions between $Si(OAc)_4$ and $OP(OSiMe_3)_3$ lead to microporous phosphosilicate xerogels with high surface areas (up to 568 $m^2\ g^{-1}$). The structure of xerogels is built exclusively from Si–O–P bonds and contains octahedrally coordinated silicon atoms, which are characteristic for crystalline silicophosphates. These xerogels start to transform into the crystalline silicophosphates at low temperatures (≈150 °C) with the simultaneous loss of surface area. Therefore the pore generating agent Pluronic P123 is added into the reaction mixture during gelation. Pluronic does not corrupt the condensation reaction and the homogeneity on atomic scale is retained within these samples. After burning out of P123 at 500 °C the samples are mesoporous with surface areas slightly above 100 $m^2\ g^{-1}$. A single crystalline phase $Si_5P_6O_{25}$ (PDF 40-457) is observed in PXRD patterns. The prepared xerogels and calcined samples are characterized by solid-state ^{13}C, ^{29}Si, ^{31}P NMR, IR, surface area analysis, TGA, SEM, and XRD.

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138. Comparison of Thermal Evaporation Process to Magnetron Sputtering for Fabrication of Titania Quantum Dots

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The titania quantum dots (QDs) are fabricated using template based non-lithographic technique. This technique is comparing to the other known methods (such as e-beam or droplet epitaxy, and lithography), cheaper, faster, and well reproducible. Nanoporous template employed in this method is created from aluminum layer. The material in the following step for creating QDs is titanium film located under the aluminum film. These films are prepared using physical vapor deposition (PVD) processes. Electrochemical properties and behavioral of these layers are key parameters for fabrication of QDs. During PVD deposition, it is very important to optimize every parameter of deposition process due to following creation of QDs. In the presented paper the comparison of two deposition methods is proposed and the deposition parameters influence on the nanofabrication of QDs is shown. In the beginning, there is the optimization of the thermal evaporation. The PVD thermal evaporation process is used due the ability of stress reducing, purity of materials, and grain structure growing. The optimizing of the evaporation is very important because of a few techniques and improvements (e.g. the substrate heating) are not allowed to be employed in order to reach the optimal titanium and aluminum layers. The PVD magnetron sputtering is one of the most applied methods for thin film deposition. In this paper, there is a discussion of nanofabrication results, namely characterization of final QDs fabricated in the process of anodization using Ti/Al bilayer deposited via the optimized thermal evaporation and the magnetron sputtering.

Keywords
Titania quantum dots, anodization, magnetron sputtering, thermal evaporation.

139. Utilization of Pyrene 1:3 Ratio Method in Study of Cationic Lipid and Hyaluronic Acid Mixtures

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Hyaluronic acid is a biodegradable, biocompatible, and viscoelastic linear polysaccharide of a wide molecular weight range (1000 to 10 000 000 Da). It is composed of alternating disaccharide units of *D*-glucuronic acid and *N*-acetyl-*D*-glucosamine with inter glycosidic linkage[1]. In the mixture with a suitable surfactant may be obtained system solubilizing hydrophobic materials useful in the targeted delivery of drugs or cosmetics.
By mixing of biocompatible lipid based on zwitterionic phosphocholine with its cationic analogue together we get composite structures with improved mechanical stability[2].
This work is based on studying aggregation behaviour of cationic liposomes in the presence of polyelectrolyte (both without modification and hydrophobized) as a foundation for further studies with zwitterionic and cationic lipid mixture. Two representatives of hydrophobized polyelectrolyte were chosen – with saturated C6 or unsaturated C18:1 acyl chain. Degree of substitution was 55 %, resp. 10 %.
CMC (critical micelle concentration) of cationic lipid and CAC (critical aggregation concentration) of cationic lipid and polyelectrolyte mixtures were measured using fluorescence spectroscopy utilizing pyrene as a fluorescence probe. Pyrene is widely used for this purpose due to its sensitivity to the surrounding environment polarity (pyrene 1:3 ratio method)[3]. Sigmoidal decrease of pyrene 1:3 ratio (intensities ratio of first and third emission band of pyrene emission spectra) is observed and CMC (or CAC) is obtained as a point of inflection.
At the beginning, CMC of cationic lipid was using fluorescence spectroscopy measured – concentration series of cationic lipid had to be prepared. Then, certain amount of polyelectrolyte stock solution was added to cationic lipid samples. This experiment was performed with different polyelectrolyte nature (hydrophobized or no modified).
In the presence of polyelectrolyte, an abrupt increase of pyrene EmPI was after CMC observed and on the wall of the vial the precipitate was formed. It has occurred at the different concentration range of cationic lipid according to the nature of polyelectrolyte.
This work was supported by project Nr. LO1211, Materials Research Centre at FCH BUT- Sustainability and Development (National Programme for Sustainability I, Ministry of Education, Youth and Sports).

Literature

1. Yu-Jin, J., Termsarasab U., Daek-Duk, K.: Hyaluronic acid in drug delivery systems. *Journal of Pharmaceutical Investigation*, 2010, vol. 40, pp. 33–43.
2. Troutier, A.-L.; Véron, L.; Delair, T.; Pchot, Ch.; Ladavière, C.: New insights into self-organization of a model lipid mixture and quantification of its adsorption on spherical polymer particles, *Langmuir*, 2005, volume 21, pp. 9901–9910
3. Aguiar, J., Carpena, P., Molina-Bolívar, J.A., Ruiz, C.C. On the determination of the critical micelle concentration by the pyrene 1:3 ratio method. *Journal of Colloid and Interface Science*, 2003, vol. 258, pp. 116–122.

140. Micro- and Nanostructures Prepared by Selective Wet Etching of Silicon, Characterization of Their Properties and Their Applications

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The wet etching belongs to very used methods for modification of material surfaces. This technique is based on application of a suitable chemical solution (acid, hydroxide, …). To provide selective (local) wet etching a mask being sufficiently resistant to the etchant should be fabricated first. Etch masks are usually prepared by lithographic methods (optical, EBL).

In our contribution, we report on fabrication of micro- and nanostructures by selective wet etching of a conductive Si(100) substrate. Two different solutions of KOH are used for this process. The masks are prepared by local Ga implantation and amorphization of the silicon substrate by Ga FIB (*Focused Ion Beam*) [1], [2], EBL (*Electron Beam Lithography*) in combination with a metal coating or FIBID (*Focused Ion Beam Induced Deposition*). The level of the surface amorphization was studied by AFM (*Atomic Force Microscopy*) and CRM (*Confocal Raman Microscopy*). The first type of the etch mask was also used as the base for fabrication of porous silicon structures [3]. The morphology of prepared structures was studied by SEM and AFM. In addition, electrical and optical properties (spectroscopic reflectivity, photoluminescence) of these micro- and nanostructures were studied as well.

References

[1] Koh, M., et al.: *Simple nanostructuring on silicon surface by means of focused beam patterning and wet etching*. Applied Surface Science, 2000, vol. 162-163, 599-603 p.
[2] Böttger, R., Bischoff, L., Schmidt, B., Krause, M.: *Characterization of Si nanowires fabricated by Ga+ FIB implantation and subsequent selective wet etching*. Journal of Micromechanics and Microengineering, 2011, vol. 21, 1-8 p.
[3] Xu, J., Steckl, A., J.: *Fabrication of visibly photoluminescent Si microstructures by focused ion beam implantation and wet etching*. Appl. Phys. Lett., 1994, vol. 65, 20812083 p.

141. Designing Novel Fluorescent Tags: A Combined Approach Using Unusual Substitution Patterns and Understanding of the Structure–Photophysical Properties Relationship

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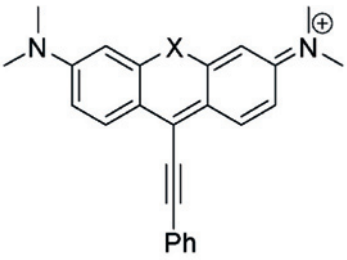
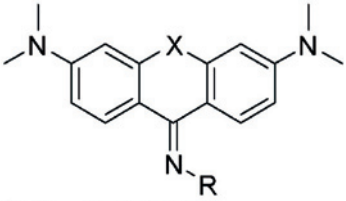
Fluorescent tags are essential tools in molecular biology, medicine and material sciences. Small synthetic organic fluorophores allows fine-tuning of their photophysical properties to yield high brightness, large Stokes shifts and emission in red or near infra-red region. In addition, their small size allows permeation to localities inaccessible for quantum dots and fluorescent proteins and brings less perturbation to the environment.
Recently we have introduced 9-phenylethynyl-pyronin analogues **1**, which upon excitation by blue or red visible light emit light in the NIR region. These dyes are also co-localizing mitochondria in living cells but not intercalating nucleic acids.



1: X = $C(CH_3)_2$; $Si(CH_3)_2$



2: X = O; $Si(CH_3)_2$
R = CO–alkyl; SO_2–alkyl

Computer-aided design also allowed us to propose, synthesize and characterize a series of 9-imido-pyronin analogues **2** with remarkable Stokes shifts up to ~200 nm in aqueous solution. We rationalized the reasons for such large Stokes shifts in order to use this approach for other families of fluorophores. We also demonstrated that a facile ligation strategy can be used to attach the 9-iminopyronin fluorophores to a model saccharide, which predestine them for biochemical and biological applications as fluorescent tags and indicators for multi-channel imaging, and for optoelectronic and dye laser applications.

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142. New perspectives of MALDI-TOF mass spectrometric profiling

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Matrix Assisted Laser Desorption-Ionization – Time of Flight Mass Spectrometry (MALDI-TOF MS) is an analytical tool developed primarily for determination of molecular weight of compounds. Due to its ability to detect also large and labile molecules, such as proteins, DNA, and polymers, the method has found applications mostly in biological and clinical research.

During the last five years, the method has entered to routine clinical laboratory diagnostics with great success. Based on direct MALDI-TOF MS analysis of whole bacterial cells, which yields characteristic protein profiles, the bacterial pathogens are identified with outstanding rapidity. However, the discriminatory power of the method has its limits, complicating its use for distinguishing between some closely related species and strains. For that purpose, we developed alternative sample preparation procedures, which improve e. g. identification of clinically relevant species of *Acinetobacter calcoaceticus-baumannii* complex, or distinguishing between individual strains of *Staphylococcus* spp.

The applicability of MALDI-TOF MS profiling is not limited only to bacteria. In our laboratory, several other modifications of the method have been developed and examined, revealing the possibility of e. g. direct analysis of spider venom, which enables the determination of the prey type, or profiling of beer samples, which can be used to identify the producer and selected brands of lager beers.

Thanks to its rapidity, simplicity, and specificity MALDI-TOF MS profiling can, prospectively, enter several analytical disciplines.

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143. Green approach for preparation of graphene oxide decorated by gold nanoparticles

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Graphene and its related derivatives due to their unique properties have great potential for various applications in many technological fields such as nanoelectronics, engineering materials, energy technology (*e.g.* fuel cell, supercapacitor, hydrogen storage), sensors, and catalysis [1]. Graphene nanosheets have been prepared by a variety of techniques, including micromechanical cleavage, chemical processing, epitaxial growth [2], and so on. One of the promising chemical route for graphene preparation is the chemical reduction of graphene oxide (GO) [3] which is a large scale and low-cost method which include noncovalent and covalent functionalization of reduced graphene oxide, respectively. The chemical reductants such as hydrazine, hydroquinone, sodium borohydride, sodium hydrogen sulfite, and hydroiodic acid have been investigated for the chemical reduction of graphene oxide [4-6], but most of them are toxic, corrosive, and highly explosive. Therefore, nontoxic or natural products that offer effective and harmless approaches to the reduction of GO are currently under the investigation.

Graphene oxide/gold nanoparticles composites were prepared by green and facile approach at room temperature using tea polyphenols as a reducing agent and chemicaly exfoliated graphite oxide and HAuCl4.3H2O as a precursors.

Scanning electron microscopy (SEM), Raman spectroscopy, FT-IR spectroscopy, UV–Vis spectroscopy, and fluorescence have been used to characterize the graphene oxide sheets decorated by gold nanoparticles. We have demonstrated that the assembling of gold nanoparticles on the graphene oxide sheets enhanced the nanocomposite properties and also prevented the particle agglomeration.

1. W. Choi, I. Lahiri, R. Seelaboyina, Y.S. Kang, Crit. Rev. Solid. State. Mater. Sci. 35 (2010) 52-71.
2. M. Inagaki, Y.A. Kim, M. Endo, J. Mater. Chem. 21 (2011) 3280-3294.
3. J. Zhang, H. Yang, G. Shen, P. Cheng, J. Zhang, S. Guo, Chem. Commun. 46 (2010) 1112-1114.
4. H. Hu, Y. Liu, Q. Wang, J. Zhao, Y. Liang, Mater. Lett. 65 (2011) 2582-2584.
5. T. Zhou, F. Chen, K. Liu, H. Deng, Q. Zhang, J. Feng, Q. Fu, Nanotechnology 22 (2011) 045704 (6pp).
6. S. Pei, J. Zhao, J. Du, W. Ren, H.M. Cheng, Carbon 48 (2010) 4466-4474.

144. Formation and electrical properties of tantalum nanotube arrays via electrodeposition from ionic liquid

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Electrochemical formation and electrical characterization of tantalum and tantalum oxide nanostructures is an important research topic for the development of emerging random access memories (RAMs) including resistive RAMs (RRAMs), which are considered as a potential alternative to the current commercially available dynamic RAMs. An enlarged (per volume or weight) surface area of the nanostructured Ta/Ta_2O_5 electrodes is also a major challenge for creating advanced metal-insulator-metal (MIM) capacitors.

Electrodeposition (ED) of tantalum at a room or medium temperature (up to 200°C) using ionic liquids (ILs) has been a challenging process over the last decade. The success of first work on ED of Ta over a planar substrate [1] inspired us to advance the process striving to create an array of well aligned spatially ordered tantalum and tantalum oxide nanostructures via electrodeposition from IL through nanopores in a thin-film alumina template prepared by anodizing of aluminium on a substrate.

The porous anodic alumina template, having ~100-nm wide and 1-μm long pores of ~10^9 cm^{-2} population density, was prepared by anodization of a layer of Al over a layer of W sputter-deposited onto an oxide-coated Si wafer. ED of Ta was carried out using IL 1-butyl-1-methylpyrrolidinium bis(trifluoromethylsulfonyl) imide, ([BMP]Tf2N) (Solvionic, 99.9%), containing 0.25M TaF_5 (Alfa Aesar, 99.9 %) and 0.25M LiF (Alfa Aesar, 99.99%).

Microscopically flat continuous Ta films (600 nm thick, well adherent to the substrate) and the free-standing arrays of Ta nanotubes filling the alumina pores were achieved via potentiostatic ED technique. Linear sweep voltammetry revealed that the reduction of tantalum in the IL proceeds in a series of steps from Ta(V) to a final insoluble Ta-containing product, which has black colour. An optimal combination of process variables, such as the formation potential, IL temperature and polarization time were determined. XPS analysis revealed the presence of C, Ta, O, Li and F at least in the outer region of the electrodeposited coatings. The coatings annealed at 800°C at 10^{-5} Pa were fluoride- and lithium-free, and their surface region comprised Ta_2O_5(~67at.%), Ta_2O, Ta and tantalum carbide (~33at.% in total). SEM study of filling the pores with tantalum metal revealed that a prolonged deposition time caused the deposit to come out from the pores and make either a cap-like or a cobweb-like coverage on the template, which finally developed to a complete uniform Ta overlayer across the alumina surface.

The *I-V* characteristics measured across the alumina-templated Ta nanotube array revealed a bipolar resistive switching event in the film, when the film behavior changes abruptly and reversely from a high resistance state (4 GΩ cm^2) to a low resistance state (78 kΩ cm^2) in a low voltage range. Electrochemical impedance spectroscopy measurements of the MIM-configured films are in progress now, the results to be reported in due course.

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[1] N. Borisenko et al., Electrochimica Acta 54 (2009) 1519-1528

145. Mesoporous Metallosilicates Prepared by Nonhydrolytic Acetamide Elimination

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Non-hydrolytic sol-gel reactions are efficient alternatives to classical aqueous techniques for synthesis of multimetallic oxides and inorganic-organic hybrid materials. These processes provides better reaction control and higher homogeneity of prepared materials. We developed non-hydrolytic sol-gel routes to several groups of metallosilicate materials based on acetamide elimination reactions.

This approach is based on the condensation reaction of silicon tetraacetate Si(OAc)4 and metal amides (Ti(NEt2)4, Zr(NEt2)4). Nonhydrolytic condensation leads to formation the Si–O–Ti and Si–O–Zr bonds in the xerogel framework. Diethylacetamide and acetanhydride were confirmed (GC-MS, 1H NMR) as the reaction byproducts. To achieve the mesoporous nature of the material, the reaction was successfully modified by the addition of the Pluronic P123. This copolymer has structure-directing and protecting feature and allows to prepare tough gels. After heat treatment the Pluronic P123 is burned out and xerogels are mesoporous with high surface areas. The resulting xerogels and volatile byproducts were characterized by liquid and solid-state NMR, IR, GC-MS, surface area analysis, TGA, XRD and DRUV-Vis.

The catalytic activity of titanosilicate xerogels for epoxidation of cyclohexene was studied. It was observed, that samples with 10 mol % of Ti contain high amount of four-coordinated Ti atoms. These species are the active sites of catalyst. In the case of the xerogels synthesized with Pluronic P123, the best results are achieved for calcined xerogels (500 °C). In the model epoxidation reaction the yields of cyclohexene oxide were 96 %.

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146. Silicon nanowires grown by thermal annealing of Si with gold catalytic layer

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Silicon nanowires (Si NWs) have many possible applications in electronics, such as solar cells [1] or lithium battery anodes [2]. Several methods of Si NWs growth have been reported since the first publication on their growth was published [3]. Si NWs can be fabricated through both 'top-down' or 'bottom-up' approaches. In our contribution, we focus on Si NWs growth from Si wafer upon thermal annealing in the presence of catalytic gold layer.

The Si substrate coated with 100 nm thick Au sputtered layer was thermally annealed at 1000 °C for 60 min in various atmospheres at 8 kPa. The catalytic layer morphology and composition were examined using atomic force microscopy and laser desorption-ionization time of flight mass spectrometry, while Si NWs structure was observed through scanning electron microscope and energy dispersive X-ray spectroscopy.

During thermal annealing, the gold layer reconstructs and forms together with silicon droplets with various diameters. It has been found, that both size of the droplets and growth of Si NWs strongly depends on gas atmosphere.

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References
[1] L. Tsakalakos, J. Balch, J. Fronheiser, B. Korevaar, O. Sulima, J. Rand, Silicon nanowire solar cells, Applied Physics Letters 91 (2007) 233117.
[2] C. K. Chan, H. Peng, G. Liu, K. McIlwrath, X. F. Zhang, R. A. Huggins, Y. Cui, High-performance lithium battery anodes using silicon nanowires, Nature nanotechnology 3 (2007) 31–35.
[3] R. Treuting, S. Arnold, Orientation habits of metal whiskers, Acta Metallurgica 5 (1957) 598.

147. BsoBI endonuclease conformatonal behavior modelled by molecular dynamics

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BsoBI is a type II restriction endonuclease belonging to the EcoRI family. The enzyme was isolated from the bacteria Geobacillus stearothermophilus and crystallized in complex with short DNA duplex. The dimer structure contains a tunnel suitable for DNA binding and recognition. The DNA molecule is completely encircled inside the tunnel. This suggests that a conformational change is needed to enable the DNA binding. The most probable scenario is the separation of two catalytic domains, which would cause a formation of a gap large enough to allow entering the DNA into the active site.

In this project, the investigation was focused on the process of opening of the BsoBI dimer enzyme studied by molecular dynamics simulations. Our goal was also to find structure or set of structures representing apo BsoBI, which has not been determined experimentally yet. Sampling of opening process was improved by Metadynamics approach. Moreover the influence of cofactor ions in the active site and in bulk on the process of enzyme opening was studied too.

148. Gelatin Nanofibers Modified with Oxidized Cellulose with Significant Bactericidal Effects

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This work is focused on the preparation of gelatin nanofibers modified with salts of oxidized cellulose. Gelatin is a biocompatible, biodegradable protein-based material with high absorption capacity, which is obtained by partial hydrolysis of natural protein - collagen. Oxidized cellulose and its salts are biocompatible as well, biodegradable and they have antibacterial and hemostatic effects. Electrospun novel nanofibers, combining properties of both gelatin and oxycellulose exhibited unique bactericide efficiency. Chemical composition of the fibers was studied by ATR-FTIR spectroscopy and EDX elemental analysis. Obtained results confirmed the presence of oxidized cellulose salt in prepared nanofibers. Diameter and morphology of nanofibers were determined by scanning electron microscopy (SEM). Bactericidal effect was examined by the method of chemical bioluminescence on modified bacterial strain of *E. coli*. This method proved complete mortality of *E. coli* in most of cases. Newly prepared material may have a potential application in surgery, regenerative medicine of soft tissue (burns) and in tissue engineering.

149. Early neonatal modification accompanies the late behavioral and morphological changes in a neurodevelopmental model of schizophrenia

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In schizophrenic patients cerebral morphological changes such as ventricular enlargement, volume reduction in medial temporal lobe and white-matter deficits have been demonstrated by structural and functional magnetic resonance imaging (MRI) studies [1]. Evidence for a neurodevelopmental disruption is based largely on follow-back, cohort and population studies in which the pre-morbid history of schizophrenia patients indicates a prevalence of subtle prenatal perturbations such as maternal infection, hypoxia, malnutrition that may interact with genetic predisposition to result in a schizophrenic phenotype [2]. Even though the symptoms and brain morphological changes, resulting from this detrimental neuronal development remain relatively dormant until the psychosis in adulthood is manifested, a possible identification of certain premorbid neurodevelopmental signs has been suggested.In the present study, we aimed to investigate the potential effects of prenatal administration of the mitotoxin methylazoxymethanol acetate (MAM) on early neurophenotypic presentations and morphological changes using a set of behavioral test battery and MRI, respectively. Timed-pregnant Sprague Dawley rats were treated with MAM (22 mg/kg) or vehicle intraperitoneally on gestational day 17 [3]. To assess the development of neonatal behavior, starting on postnatal day 1, newborn pups were observed for neonatal reflexes (i.e.: righting, cliff aversion, forelimb placing, bar holding, forelimb grasping, negative geotaxis) as index of brain maturation, until maximum appearance was scored (i.e. 100% of the brood was found to exhibit the full repertoire of reflexes). During adolescence they were subjected to different behavioral tests such as the social interaction test or the novel object recognition and the Y-maze tests to assess the negative-like symptoms or the various cognitive aspects affected, respectively. At birth, neonatal reflexes had a delayed onset (i.e. percent of appearance) in prenatally MAM exposed rats, as compared to the control group ($P<0.05$; $P<0.01$; $P<0.001$). At adolescent age prenatally MAM exposed rats engaged in less social behavior as suggested by the reduced time of interaction ($P<0.05$). No difference in the number of interactions as index of locomotor activity was found. In the novel object recognition test prenatally MAM exposed rats showed an impaired cognitive performance, as described by the decreased discrimination index ($P<0.001$). By contrast, spatial recognition memory was not affected by prenatally MAM exposure since no difference between the two groups of rats (MAM vs. VHC) was found in the Y-Maze test. Interestingly, the behavioral alterations correlated with morphological changes (i.e. enlargement of ventricles), as revealed by MRI. These results suggest that behavioral abnormalities at neonatal age as index of impaired brain maturation resulting from a MAM environmental challenge, could represent a predictive factor for behavioral and structural abnormalities which resemble to a schizophrenia-like phenotype.

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References

[1] Shenton, M.E., Dickey, C.C., Frumin, M., McCarley, R.W., 2001. A review of MRI findings in schizophrenia. Schizophr Res 49, 1-52.
[2] Jones, C.A., Watson, D.J.G., Fone, K.C.F., 2011. Animal model of schizophrenia. Br. J. Phramacol. 164, 1162-1194.
[3] Lodge, D.J., Grace, A.A., 2009. Gestational methylazoxymethanol acetate administration: A developmental disruption model of schizophrenia. Behav. Brain Res. 204, 306-312.

150. To decipher the possible presence of auxin transport machinery in chloroplast

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Environmental stresses adversely affect plant growth and development leading to worldwide yield losses. The plant hormone and signaling molecule auxin is a key player in plant development and plays an important role in plant stress responses. Lesser photosynthesis is partially responsible for some of the observed symptoms in stress adapted plants. This symptoms such as growth retardation, reduced metabolism and increased antioxidant activities helps to maximize plant survival. Auxin modifies chloroplast structure, chlorophyll synthesis, nuclear photosynthetic genes expression, and chloroplast transcription in response to environmental changes[1]. Thus auxin could hypothetically affect the remodeling of the photosynthetic apparatus to minimize photo oxidative damage induced upon stress[1]. Reciprocally, chloroplasts synthesize tryptophan-derived precursors for IAA biosynthesis and in some plants can actively biosynthesize auxin[1]. Moreover, recently chloroplast redox state was shown to modulate auxin homeostasis[2]. These data show that there is a strong connection between chloroplast processes and auxin homeostasis. However, the connection between auxin and photosynthesis is rarely described.

To investigate whether the Arabidopsis chloroplast can transport auxin, chloroplast localized putative auxin transporters were selected. *In silico* promoter gene analysis shows that our target genes share some similarities with the promoters of ATP Binding Cassette auxin transporters. Auxin transport machinery in chloroplasts will be studied using a novel ratiometric auxin biosensor[3]. Owing to the vital importance of photosynthesis for plant growth and survival, understanding how chloroplast and auxin metabolism are interconnected will bring novel insights into plant stress biology applicable to improve the performance of crops under field conditions.

Tognetti et al. (2011). Plant Cell & Environment. 35: 321-333.
Ferrández et al. (2012). Plant Signaling and Behavior. 7: 1177-1179.
Wend et al. (2013) Scientific Reports 3: 2052.

151. The study of encapsulation magnetic nano- and microparticles using real time polymerase chain reaction

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Nano- and microparticles for biotechnology applications usually consist of a number of components: superparamagnetic core, protective coating and the surface functionality. Magnetic cores are covered with a variety of functionalized low-or high-molecular compounds (polymer layer). Iron oxides and some substances used to stabilize magnetic cores can reduce biocompatibility of the proposed particles; therefore perfectly encapsulated cores are very important for their application. The aim of work was the development of simple method for the study of magnetic cores encapsulation of nano- and microparticles suitable for applications in molecular diagnostics.

The degree of magnetic particle's coverage with a variety of low-or high molecular weight compounds (polymer layer) was verified by real time polymerase chain reaction (qPCR). The proposed method is based on estimation of DNA amplification effectiveness in qPCR. Serial dilutions of different types of magnetic particles, such as (hydrophilic non-porous poly (2-hydroxyethyl methacrylate-*co*-glycidyl methacrylate) P(HEMA-*co*-GMA) and poly(glycidy lmethacrylate) P(GMA), poly[(glycidyl methacrylate)-co-[2-(methacryloyloxy)ethoxy]acetic-*co*-ethylene dimethacrylate]P(GMA-MOEAA)-PEG, poly[(2-hydroxyethyl methacrylate)-*co*-[2-(methacryloyloxy)ethoxy]acetic-*co*-ethylene dimethacrylate] P(HEMA-MOEAA)-PEG and some individual components used for their preparation were tested. The results of study have shown that the real-time PCR can be used for the study of characteristics of magnetic particles according to their compatibility with DNA amplification.

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152. Catalytic Mechanism of the ppGalNAcT2 Retaining Glycosyltransferase Inferred from QM/MM Calculations

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The glycosylation of cell surface proteins plays a crucial role in a multitude of biological processes, such as cell adhesion and recognition. To understand the process of protein glycosylation, the reaction mechanisms of the participating enzymes need to be known. However, the reaction mechanism of retaining glycosyltransferases has not yet been sufficiently explained.

Here we investigated the catalytic mechanism of human isoform 2 of the retaining glycosyltransferase polypeptide UDP-GalNAc transferase by coupling two different QM/MM-based approaches, namely a potential energy surface scan in two distance difference dimensions and a minimum energy reaction path optimisation using the Nudged Elastic Band method.

Potential energy scan studies often suffer from inadequate sampling of reactive processes due to a predefined scan coordinate system. At the same time, path optimisation methods enable the sampling of a virtually unlimited number of dimensions, but their results cannot be unambiguously interpreted without knowledge of the potential energy surface. By combining these methods, we have been able to eliminate the most significant sources of potential errors inherent to each of these approaches.

We found that ppGalNAcT2 catalyzes a same-face nucleophilic substitution with internal return (S_Ni). The optimized transition state for the reaction is 13.8 kcal mol^{-1} higher in energy than the reactant while the energy of the product complex is 6.7 kcal mol^{-1} lower. During the process of nucleophilic attack, a proton is synchronously transferred to the leaving phosphate.

The presence of a short-lived metastable oxocarbenium intermediate is likely, as indicated by the reaction energy profiles obtained using high-level density functionals.

153. Investigating the effects of different amino acid residues at the key position (Trp373) on maize ?-glucosidase Zm-p60.1 enzyme activity

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Cytokinins are hormones directing plant growth and development. In these processes enzymes are included in control of biosynthesis, storage and activation of plant hormones. A maize β-glucosidase Zm-p60.1 is an enzyme able to convert glucosylated (non-active) cytokinins into their active forms.

Saturation mutagenesis is a cornerstone technique in protein engineering because of its utility (in conjunction with appropriate analytical techniques) for assessing effects of varying residues at selected positions on proteins' structures and functions. Site-directed mutagenesis with degenerate primers is the simplest and most rapid saturation mutagenesis technique. Thus, it is highly appropriate for assessing whether or not variation at certain sites is permissible, but not necessarily the most time- and cost-effective technique for detailed assessment of variations' effects. Thus, in the presented study we applied the technique to randomize position Trp373 in β-glucosidase Zm-p60.1, which is highly conserved among β-glucosidases. Unexpectedly, β-glucosidase activity screening of the generated variants showed that most variants were active, although they generally had significantly lower activity than the wild type enzyme.

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154. Initial Structural Analysis of Frog Virus 3 by Electron Microscopy and Tomography Shows Composition and Morphology of its Large Virion with Inner Membrane

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Nucleocytoplasmic large dsDNA viruses (NCLDV) are among the largest and most complex viruses known – their capsids are 200–800 nm large and the dsDNA genome is replicated in the cell nucleus and cytoplasm with only little help of the host organism [1]. Here, we focused on ranaviruses (~200 nm in diameter) that have caused significant economic losses to fish industry and threaten biodiversity of amphibian species worldwide [2]. Despite increasing importance of ranaviruses as global pathogens relatively little is known about the structural and mechanistic features of their replication cycle. The ranaviruses exist in two infectious forms (naked capsids and enveloped virions), each with a different way of cell entry and cell egress [3]. In order to determine the structural basis for cell entry by the two respective forms of virions, we purified and imaged virions of the type ranavirus, frog virus 3 (FV3), by cryo-electron microscopy and tomography. Electron micrographs showed large capsids with three distinguishable layers enclosing the electron-dense core of packaged dsDNA. The inner shell presumably corresponds to an internal membrane, the intermediate layer to a proteinous capsid shell and the outer layer to an external lipid envelope. Initial 3D reconstruction of FV3 revealed an icosahedral shell with large flat triangular facets between the 5-fold vertices and small turrets at the 5-fold vertices of the shell. The capsid diameter varies from 157 nm at the 3-fold axis and 159 nm at the 2-fold axis to 175 nm at the 5-fold icosahedral axis. Overall, the FV3 capsid exhibits similar morphology and structural features as related viruses from the family *Iridoviridae*, PBCV-1 and CIV [4]. Electron tomograms of FV3 virions additionally revealed small spikes at the outer surface of enveloped virions that likely correspond to viral glycoproteins. Subtomogram averaging of individual extracted virions was done in *Bsoft* [5] using icosahedral symmetry. The average is consistent with the single particle reconstruction and further image analysis will be undertaken to identify the putative special vertex for genome ejection.

[1] La Scola, B. et al. (2003) *Science*, 299, 2033.
[2] Gray, M.J. et al. (2009) *Dis. Aquat. Organ.*, 87, 243–266.
[3] Chinchar, V.G. et al. (2009) *Curr. Top. Microbiol. Immunol.*, 328, 123–170.
[4] Xiao, C. and Rossmann, M.G. (2011) *Curr. Opin. Virol.*, 1, 101–109.
[5] Heymann, J.B. et al. (2008) *J. Struct. Biol.*, 161, 232–242

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155. Double-belt a Novel Structure of Membrane Pore

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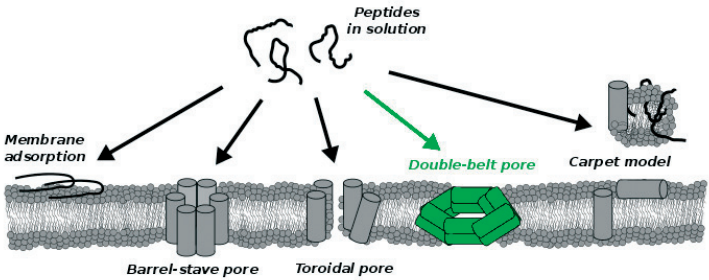
Amphiphilic proteins and peptides can induce formation of stable and metastable pores in phospholipid membranes, which has been associated with toxicity or antimicrobial activity. Using coarse-grained simulations we have studied peptide orientation within the pores and have found that peptides can be oriented perpendicular, parallel, or tilted with respect to the membrane plane. The orientation depends on the length of the peptide and its hydrophobicity distribution, which we rationalized in terms of the hydrophobic mismatch. Apart from well-known barrel-stave or toroidal pores our simulations suggest a novel 'double-belt' pore structure, where peptides within the membrane pore are oriented parallel to the membrane plane. This result was verified using more detailed simulations with the MARTINI force field, where the double-belt structure was stable in micro-second time scale of our simulation.



156. Charge transport in DNA

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In this contribution we compare the charge transfer mechanism of the standard DNA/DNA and hybrid DNA/RNA duplexes and investigate the impact of mercury bonded on DNA mismatched bases on the charge transfer process. We studied the temperature dependence of steady-state and time-resolved fluorescence and compared the experimental results with a theoretical approaches within Density Functional Theory (TD-DFT). The studied systems included DNA/DNA and DNA/RNA duplexes with various separations of Ap (donor) and G (acceptor) by adenosine units and DNA/DNA duplexes with a mismatched (T-T) or T-Hg-T base pair. The results show that the RNA/DNA system flexibility improves the conditions for polaron creation and facilitates rapid (coherent) charge transport. DFT modelling revealed that in the case of hybrid duplex HOMO orbitals were more delocalized along the strand and that spatial overlap between adjacent bases was better, which all resulted in large values of electronic coupling. Calculations indicated that the changes in charge transfer due to the presence of Hg were likely owing to the differences in local geometry (overlap of bases) rather than due to the electronic structure. Models showed that Hg orbitals did not contribute to HOMO and HOMO-1, and therefore Hg orbitals could not directly influence the charge transport properties.

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157. Dynamic Collection of Electrochemical Impedance Data During a Voltammetric Sweep

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Data obtained from impedance measurements enjoy lot of attention in electrochemical studies, because they provide wealth of information relevant to static, as well as dynamic, parameters of the system. The nature of proper impedance data collection (and successful interpretation) requires that the data be collected while the system under observation is stable, at equilibrium, although the equilibrium can be static, as well as dynamic. With this requirement in mind, and also due to some complexity, should the impedance analyzer be required to perform other tasks during impedance data collection, most instruments are designed not to perform other operations while impedance is taken.

We present here data taken with Solartron ModuLab setup, which we programmed to perform extremely slow potential scans, and to collect simultaneously impedance data. The electrochemical experiment performed was cyclic voltammetry on a rotated disk electrode and voltammetry of vanadium(IV) in sulfuric acid. This is a system in which we are interested further for the use in redox flow cells. The very slow voltammetric scan (1 mV/s), coupled with rapid data collection using the multi-sine impedance function, was successfully used to collect impedance data without distortion caused by the changing voltage bias during the voltammetric scan.

While the instrument facilitates collection of the data series as described, it does not have a built in data processing, that would allow sequential evaluation of all the impedance data series collected. We have developed such an evaluation routine and we are presenting, for the first time, detailed impedance data results running in parallel with the data for voltammetric measurements.

158. Electrochemical analysis of Anthraquinone-Labeled nucleotides and oligonucleotides

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Electroanalytical methods are used for analysis of natural as well as chemically modified nucleic acids more than 50 years. Nucleic acids are electroactive species that can be reduced and/or oxidized at various electrodes. For application of electroanalytical techniques in analysis of DNA sequences or DNA interactions it is convenient to use DNA probes containing chemically modified nucleosides [1] Attachment of a new electroactive group can change electrochemical behaviour of DNA or/and can give rise to a new signal. Electrochemically labelled DNA can be prepared using primer extension with DNA polymerase and dNTPs bearing different electroactive groups. Very useful redox label for DNA detection is antraquinone. In cyclic and square wave voltammetry at mercury based, carbon and gold electrodes antraquinone give a well developed characteristic signal suitable for electrochemical analysis of labelled DNA [2].

References

[1] M. Fojta, L. Havran, H. Pivonkova, P. Horakova, M. Hocek: Current Organic Chemistry 15 (2011), 2936-2949
[2] J. Balintova, R. Pohl, P. Horakova, P. Vidlakova, L. Havran, M. Hocek, M. Fojta: Chem-Eur. J. 17 (2011), 14063-14073

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159. Examples of Contributions of Capillary Electrophoresis for Analyses of Short Oligodeoxynucleotides and of their Osmate Derivatives

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Electrochemically active probes and labels of DNA, e.g. stabilized osmate adducts, are useful tools for studies of structure structural changes and interactions of DNA. Short synthetic oligodeoxynucleotides (ODNs) serve as model molecules for studies of properties of such adducts and reactions leading to their formation. Preparation of osmate adducts is achieved by derivatization of a chosen reactive nucleic base in a short synthetic ODN with osmium tetroxide (OsO_4) in the presence of a nitrogen organic base that stabilizes the given adduct.

ODNs and their osmate adducts are charged. Capillary electrophoresis (CE) is therefore a proper technique for analyses of their reaction mixtures. Due to length of model molecules used in this work, sieving media are not suitable for determination of short ODNs and of their derivatives. Applicability of CE in free solution has been therefore investigated for both tasks.

Separation system was designed for separation of a pentanucleotide AAAAT from its osmate derivative stabilized with 2, 2´-bipyridine. The separation system is highly selective and is suitable for analogous separations: osmates stabilized with different ligands (e. g. bathophenanthrolinedi sulfonic acid or neocuproine) can be separated due to ligand-characteristic difference in mobility of the mother ODN and of its stabilized osmate. Using CE, it is also possible to predict the number of derivatization products in ODNs containing more than one reactive base.

The separation system is also suitable for mutual separations of very short ODNs. CE allows for a fast, simple and indicative check on quality and on chemical identity of synthetic ODNs before their further use.

160. Numerical modeling of electromagnetic field in the biological cell

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The electric and magnetic field interactions with biological cells are of significant interest in various biophysical and biomedical applications. One reason why it is extremely important to deeply understand the true mode of action of electric or magnetic fields on living organisms, is the need to protect human health in consideration of the probably future introduction of new technologies such the therapeutic use of magnetic and electric field. The second reason is also important. It is important to know how cells will responds to external stimullation without disrupting the living cell structure (especially cell membrane). In order to study such important aspect, it is necessary to evaluate the time constant in order to estimate the response time of living cells in the electric or magnetic field. In the present study, the time constant is evaluated by considering the hypothesis of electrical analog of spherical shaped cells and assuming realistic values for capacitance and resistivity properties of cell – nuclear membrane, cytoplasm, and nucleus. This work demonstrates how a stochastic model can be implemented to obtain a realistic description of the interaction of a biological cell with an external magnetic or electrical fields. In our model formulation, the stochasticity is adopted by introducing various levels of forcing intensities in model parameters. The increasing amount of data reporting on the biological effects of magnetic and electric fields is leading researchers to understanding of how important it is to fully understand the mode of action of magnetic or electric fields to living organisms. Indeed, even if the perturbations of biological systems by magnetic or electric fields are sublethal at shorter times of exposure, these perturbations could, especially at longer times of exposure, evolve into a progressive accumulation of modifications, whose ultimate effects still need to be clarified.

161. 3D Imaging of Biopolymeric Scaffolds Using X-Ray Computed Micro-Tomography and Stereoscopy

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In this work we have used X-ray computed micro-tomography (μCT) as suitable method for observing the morphology of the polymer scaffold for bone tissue engineering in the entire volume of the sample without its destruction, and also for the determination of porosity, pore size and their interconnectivity. Obtained 3D image of the collagen/hydroxyapatite (HA) scaffold was binarized. Subsequently, the porosity (88%) and total pore interconnection with each other (48%, the remaining 52% of the pores do not affect the second pore walls but collagen) was calculated and the pore size was determined from both the printed circuit sections (148 ± 89 μm) and from the volume by two methods: semi-automated method (138 ± 38 μm) and fully automated method (216 ± 86 μm). The fully automated method was used to determine as well the average pore volume (8.106±1.107 μm^3). Moreover, scaffold images were stereospectroscopicaly visualized in order to better see the 3D polymer composite scaffold morphology.

This new technology will help us to better understand the methods of seeding the scaffold by cells, the behavior of cells in the scaffold (proliferation and differentiation in different time intervals), or changes in the morphology of the scaffold after some time exposed to the cells, which is essential in the modern tissue engineering for tissue regeneration.

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162. Ab initio study of impurity-decorated grain boundaries in ferromagnetic metals

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Macroscopic mechanical behavior of polycrystalline metallic materials is largely determined by cohesion of the grain boundaries (GB) which is strongly affected by segregated impurities. We present a systematic ab initio study of segregation of 12 non-magnetic sp-impurities (Al, Si, P, S, Ga, Ge, As, Se, In, Sn, Sb and Te) at Σ5(210) grain boundary (GB) and (210) free surface (FS) in fcc ferromagnetic cobalt and nickel and analyze their effect on structure, magnetic and mechanical properties.

We investigate the changes in geometric configuration as well as in the distribution of magnetic moments. We find magnetically dead layers at many sp-impurity-decorated GB and FS in nickel. Futher we analyze charge density and density of states and changes in the charges in Bader volumes induced by segregated impurities at the GB and FS. We also determine the preferred segregation sites of the impurity atoms at the Σ5(2 1 0) GB and their segregation enthalpies. On the basis of the RiceWang model, we calculate a change of the ideal work of interfacial separation called here the strengthening/embrittling energy and evaluate both chemical and mechanical contributions to this energy. We find interstitially segregated Si as a GB cohesion enhancer, and interstitially segregated S, Ge, As, Se and substitutionally segregated Ga, In, Sn, Sb and Te as GB embrittlers in nickel and cobalt. For substitutionally segregated Al and interstitially segregated P at grain boundary in nickel we find none or minimum strengthening effect. However, they are embrittlers when they segregate at grain boundary in cobalt. As there is very little experimental information on GB segregation in nickel and cobalt most of the present results are theoretical predictions which may motivate future experimental work.

163. How does human staufen1 recognize dsRNA targets?

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It is known that double-stranded RNA (dsRNA) binding proteins are involved in many biological processes such as gene regulation, transport of mRNA and translation. These biological functions have also been described for the human dsRNA binding protein staufen homologues Stau1 and Stau2. The human Stau1 protein consist of multiple dsRNA binding domains (dsRBD) but mainly dsRBD3 and dsRBD4 are known as true RBDs involved in RNA binding. Binding of Stau1 with a 19 basepair stem-loop within ADP-ribosylation factor1 (ARF1) mRNA regulates its cytoplasmic levels by a process called staufen-mediated mRNA decay (SMD), but how this target RNA as well as others are recognized by Stau1 remained elusive. To reveal the RNA target specificity of Stau1, we are determining the solution structure of the ARF1 dsRNA – stau1 RDB3+4 complex by NMR spectroscopy using a 'divide and conquer' approach. We construct stau1 RBD3+4 and individual RBDs for expression in E. Coli, and purify the proteins using biochemical techniques. ARF1 dsRNA and subdomains thereof are prepared by in vitro transcription and purified using chromatographic techniques. Fluorescent anisotropy (FA) measurements show binding affinity of stau1 RBD3 and RBD4 for ARF1 dsRNA in the nano-molar range. Preliminary NMR measurements indicate that purified stau1 RBD3 and RBD4 as well as RDB3+4 are well folded and bind ARF1 dsRNA in the intermediate to slow exchange regime forming stable protein-RNA complexes suitable for further structural studies.

164. Preliminary Research on Change on Rat Nervous System in the ECoG BMI Environment

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Recently Brain-Machine Interface (BMI) is expected as one of the treatment methods for patients with paralysis or limb amputation. However, the changes in the nervous system that occurs in the course of the adaptation to a BMI system areis not known in detail. It is very important to investigate the details towards the clinical application. We constructed an experimental system to study plastic changes in the nervous system in a BMIBrain Machine Interface environment based on electrocorticogram (ECoG) using rats. Then, we have confirmed the utility of this experimental system in preliminary experiments. In this system we used signal recorded by one channel of electrodes (from this electrode has 32 channels on the probe) as a trigger to provide the reward of water. Rats didon't know howwhy to get the reward comes out. However, the rats adapted to this system and they couldan gain the reward. Moreover, the change showed athe manner tendency to in which converges onto the channel that we have attentionused as the trigger.

165. Chromosomal association of the SMC5-6 complex is dependent on interaction of its Nse1-Nse3-Nse4 subcomplex with DNA

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SMC5-6 is a highly conserved protein complex related to cohesin and condensin complexes which are the key components of higher-order chromatin structures. The SMC5-6 complex is essential for proliferation in yeast and is involved in the homologous recombination-based DNA repair processes, including repair of DNA double strand breaks, restart of stalled replication forks etc. However, the precise mechanism of SMC5-6 function is not known. We will present the evidence for direct physical interaction of its part, Nse1-Nse3-Nse4 sub-complex, to DNA and its essential role for the function of the whole SMC5-6 complex.

The Nse1-Nse3-Nse4 sub-complex is rich in winged-helix domain motifs and at least one of them form a putative DNA-binding cleft. The purified Nse1-Nse3-Nse4 sub-complexes shift different DNA substrates in electrophoretic mobility shift assays (EMSA) proving their ability to bind DNA in vitro. Mutations of the key basic residues within the putative DNA-binding cleft reduce *in vitro* binding to DNA. Introduction of these mutations into Schizosaccharomyces pombe genome results in cell death or hypersensitivity to hydroxyurea. The chromatin immunepreciptation (ChIP) analysis of the DNA-binding mutant shows reduced association of SMC5-6 with chromatin. The above data and our genetic analysis indicate the essential role of the interaction between Nse1-Nse3-Nse4 and DNA for the loading and/or maintenance of the SMC5-6 complex on chromatin.

References

1. Palecek, J., S. Vidot, M. Feng, A. J. Doherty, and A. R. Lehmann. 2006. The SMC5-6 DNA repair complex: Bridging of the SMC5-6 heads by the Kleisin, NSE4, and non-Kleisin subunits. *J Biol Chem* 281:36952-36959.
2. Hudson, J. J. R., K. Bednarova, L. Kozakova, C. Liao, M. Guerineau, R. Colnaghi, S. Vidot, J. Marek, S. R. Bathula, A. R. Lehmann, and J. Palecek. 2011. Interactions between the Nse3 and Nse4 components of the SMC5-6 complex identify evolutionarily conserved interactions between MAGE and EID families. PLOS One **6**: e17270.

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166. Characterisation of Internal Motions of 2 Isoforms of Intrinsically Disordered Malaria Surface Protein MSP2 and Comparison for the 2 Isoforms

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Plasmodium falciparum causes one of the most important infectious disease, malaria, resulting in two million deaths worldwide. For this reason, antigens on the surface of the merozoite stage of *Plasmodium falciparum* represent attractive candidates for components of malaria vaccine. Merozoite surface protein 2 (MSP2), one of the most abundant proteins on the merozoite surface, is a 23.5 kDa protein anchored to the merozoite membrane by a C-terminal glycosylphospha tidylinositol (GPI) moiety consisting of conserved N- and C-terminal domains and a variable central region. The central region consists of repetitive sequences surrounded by non-repetitive sequences. Given the diversity of the variable region, two major allelic forms are formed by MSP2, FC27 and 3D7. MSP2 is intrinsically disordered protein with a high propensity for amyloid-like fibril formation, in which the conserved N-terminal region (MSP21-25) plays a key role. Agregation and fibril formation properties of 3D7 are weaker than those of the FC27, thus suggesting that the downstream central region may have some influence on the N-terminus and thus determining the fibril formation propensities.

5D experiments, HN(CA)CONH and HabCabCONH, were employed to assign the resonance frequencies of the 3D7 isoform due to the low dispersion of amide proton chemical shifts. All the non-proline residues were successfully assigned. Assignment of the FC27 isoform was achieved using a set of 3D experiments, HNCACB, CBCA(CO)NH, HNCA, HN(CO)CA, and HNCO. Resonance frequencies of 204 out of 208 non-proline residues were assigned.

In order to characterize the backbone dynamics, we analyzed relaxation of the amide 15N-1H spin system. Auto-relaxation rates R1 and R2, longitudinal and transverse cross-correlated relaxation rates, and steady-state Nuclear Overhauser enhancement were evaluated. Spectral density mapping was performed for different combinations of measured relaxation rates. As expected, the major difference was found in the region of highly repetitive sequences, but some additional differences were observed in other regions. Although it was assumed that relaxation rates would differ in the conserved N-terminal region, the results suggest that the repetitive sequences do not have significant influence on the motional behavior of the N-terminal part of the MSP2.

167. Hybrid Composites of Electrospun Polymer Nanofibers Coated with Organosilicon Plasma Polymers

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Production of polymer nanofibers by electrospinning has high application potential but it is often necessary to perform their additional surface treatment. Plasma enhanced chemical vapor deposition can be used with advantages for modification of nanofibrous polymer foils because it offers wide range of plasma sources at low or atmospheric pressure allowing to set-up the processes according to technological needs. In this work, organosilicon plasma polymers were deposited on PVA and PA6 electrospun nanofibers from hexamethyldisiloxane/Ar mixtures in low pressure RF discharges and by cold RF plasma multi-jet at atmospheric pressure. The experiments were designed also to investigate the penetration depth of plasma modifications into the polymer substrate. The influence of plasma parameters on chemical composition of the resulting layers was investigated by IR spectroscopy and XPS. The chemical composition was strongly influenced by the plasma chemistry, i.e. the energy input into the plasma. Incorporation of C=O and COOR groups into films due to oxidation of CH groups occurred. Significant variations in microstructure of resulting composites were obtained when comparing two different plasma sources. The wettability, i.e. the values of water contact angles, were strongly influenced by both the chemical composition of deposited layers and the overall surface structure.

168. Characterization of new mutants affected in auxin-dependent protein degradation identified by a forward genetic screen in *Arabidopsis thaliana*

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The plant hormone auxin is a major player for the regulation of plant growth development including embryo and root patterning, lateral organ formation and growth responses to environmental stimuli. Auxin is polarly cell-to-cell transported by the action of specific auxin influx (AUXIN-RESISTANT1 (AUX1) proteins) and efflux (PIN-FORMED (PIN) proteins) carriers, whose subcellular localizations indicate the direction of the auxin flow. Auxin itself regulates its own transport by modulation of the expression and subcellular localization of the auxin transporters. Short auxin treatment activates the transcription of *PIN* and *AUX1* genes and stabilizes PIN proteins at the plasma membrane, whereas prolonged auxin application promotes the turnover of PIN proteins and their vacuolar degradation. In this study we took advantage of forward genetics, which opens up the possibility of identifying molecular components playing a role in these processes. In order to identify new mutants with impaired auxin transport or showing disorders with routing of auxin carriers, we used EMS mutagenized *Arabidopsis* transgenic line *PIN2::PIN2-GFP AUX1::AUX1-YFP eir1 aux1* and we looked for mutants with stronger fluorescent signals after prolonged treatment with the synthetic auxin 2, 4-dichlorophenoxyacetic acid (2, 4-D). The detailed analysis of these mutant lines will be presented.

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169. High-contrast 3D imaging biological tissues: embryos

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Understanding developmental processes requires an accurate visualisation and parameterization of three dimensional embryos. There exist a few methods for non-destructive whole-volume imaging of animal tissues. X-ray microtomography (microCT) has the potential to produce quantitative 3D images of small biological samples. The microCT imaging has been limited by the low inherent contrast of non-mineralized tissues. Although X-ray contrast enhancement agents are used routinely in clinical radiography, only a few technique have appeared for imaging soft tissues in preserved animal specimens. We present high-contrast imaging of embryonic tissues using a commercial high-resolution lab-based microCT system. The overall contrast and differential tissue contrast is demonstrated on 14th day mouse embryo stained by phosphotungstic acid. Using the staining method, the microCT imaging is established as a useful tool for comparative developmental studies, embryo phenotyping, and quantitative studies of morphology.

170. Synthesis, characterisation and effect on cell viability of AgCu nanoparticles

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AgCu aqueous colloids were synthesized by reduction of Ag and Cu nitrates by sodium borohydride under action of different stabilization substances (e.g. 1, 10-phenanthroline, polyvinyl alcohol (PVA) or triethylentetraamine). Chemical composition of synthesized AgCu NPs was done using inductively-coupled plasma-optical emission spectrometry (ICP-OES). Optical properties of nanoparticles were monitored using UV-VIS spectrophotometer. Size of colloid nanoparticles was measured by dynamic light scattering method (DLS). Zeta potential of AgCu colloids was measured by electrophoretic method. Size and shape of metal core of nanoparticles and morphology of aggregates were investigated by electron microscopy (SEM, TEM and HRTEM). Thermal properties of AgCu nanoparticles were evaluated by differential scanning calorimetry (DSC). Hydrodynamic size of AgCu NPs measured by DLS was compared with the metallic core size obtained by electron microscopy. Surface effects on AgCu nanoparticles synthesized in aqueous solution of 1, 10-phenathroline were investigated by Knudsen effusion mass spectrometry (KEMS) during slow heating in order to understand their thermal stability and behaviour of organic compounds present on the surface. Direct inlet probe mass spectrometry (DIP/MS) applied prior the KEMS method yielded necessary information for mass spectra monitoring. Toxicity of the synthesized nanoparticles was consequently tested because metallic nanoparticles are widely known as cytotoxic or antibacterial reagent. The effect of application of therapeutical ultrasonic field in the presence of metallic nanoparticles AgCu < 100 nm was examined on human ovarian carcinoma cells A2780. The cell viability was tested by MTT test. Significant decrease of cell viability was observed. The maximum decrease of cells viability was observed for nanoparticles modified by 1, 10-phenanthroline.

171. Magnetism and elasticity of selected ordered Fe-Pd and Fe-Pt systems using ab initio methods

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The aim of this theoretical density-functional theory (DFT) study is to deposit a magnetic element, iron, into a non-magnetic matrix of either Pd or Pt and examine the effect of such additions on mechanical and magnetic properties of the resulting composite. A similar study has already been performed in case of Mn-Pt system [1]. The inserted Fe atoms form nanowires (NWs) in the matrix, hence the structures studied represent examples of low-dimensional systems and relate to an actively pursued research field that is motivated by recent discoveries of unique properties of carbon-based low-dimensional materials [2].

We consider selected ordered Fe-X systems (X is either Pd or Pt) that are based on face-centred cubic (FCC) lattice and contain Fe NWs in the matrix of the element X. They can be characterised using an at most 3x3x1 FCC supercell and differ e.g. in Fe concentration, distance of the NWs etc. We study stoichiometries FeX_{15}, FeX_8, FeX_7, FeX_3 (L12 structure), FeX ($L1_0$ structure) and include also elemental Fe, Pd and Pt in the FCC configurations.

To determine structural parameters, we employ the Vienna Ab initio Simulation Package (VASP) code in non-relativistic approximation and generalised gradient approximation (GGA-PBE). In order to better understand the effect of magnetism, we analyse ferromagnetic (FM), non-magnetic (NM) and several antiferromagnetic (AFM) configurations.

We find that the FM configurations are the lowest energy states in most of the cases studied and that the structural parameters of all FM, NM and AFM are close to each other for given stoichiometry and matrix element. The tetragonality ratio differs from 1 only in case of the $L1_0$ structures and the results for FePt system are in fair agreement with an experimental study [3]. With increasing iron content, both atomic volume and magnetic moment on individual iron atoms decrease but the trend of magnetic moment induced on the matrix atoms is more complicated due to different symmetry of various Fe-X systems.

[1] T. Kana and M. Sob, Phys. Rev. B **85**, 214438 (2012)
[2] A. K. Geim, K. S. Novoselov, Nature Materials **6**, 189-191 (2007)
[3] G. Brown et al., Phys. Rev. B **68**, 052405 (2003)

172. Are the T-cell repertoires of genetically identical twins similar?

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The adaptive immune system protects our organisms from the invasion of other organisms (infection) and suppresses the multiplication of own transformed cells. Specific hypervariable molecules possessed by key cells of adaptive immunity - T and B lymphocytes, potentially are able to recognize any alien or abnormal structures. It's provided at most by huge diversity of hypervariable CDR3 regions of the receptors forming by somatic recombination of genome encoded V, D and J gene segments with template-independent incorporation of several nucleotides at the genes junctions during T-cells and B-cells differentiation. Thus each T-cell clone has a TCR with unique structure, and the overall potential diversity of alpha/beta TCRs is estimated as 10^{16}-10^{18}. But only about 10^{7} - 10^{8} variants of TCR beta chains exist in a healthy individual in contrast to 10^{11} predicted theoretically. The main factors which influence the actual T-cell repertoire formation is the negative selection of newly formed T-cells in thymus and the environment in particular pathogens presented to the immune system during ontogenesis. In both of the processes products of many genes act in concert and individual variability or similarity in their structure might be reflected in actual repertoire. Indeed several studies provide evidence for the generation of identical or very similar T-cell receptors recognizing the same antigen at least in the same MHC context. On the other hand, it is known that individuals with different genotypes can vary in the ability to suppress particular pathogens. Thus the individual T-cell repertoire is the result of a complex interaction between genetic context and environmental factors. It remained unknown to what extent these factors influences on actual T-repertoire diversity realizing in each of us and what is the extent of similarity between genetically identical individuals comparing with non-identical.

In this study we analyzed alpha-beta TCR repertoires in several pairs of monozygotic twins. We clearly demonstrate that the number of CDR3 clonotypes shared between any pair of individuals is strictly dependent on the product of the intersected cloneset sizes for both α and β TCR chains. In general, deep TCR repertoires (mainly composed by low abundant T cells) of MZ twins have the same amount of shared CDR3 clonotypes compared with unrelated individuals. However, the most abundant clonotypes (supposed to mainly reflect the antigen experienced T cells) shared by identical twins show some characteristics of β chain that clearly distinguish them from the ones shared by random individuals. The proportion of shared CDR3 sequences among abundant β clonotypes is significantly higher in twin pairs. Also abundant clonotypes shared by twins have a higher number of added nucleotides compared with unrelated humans. Finally, the most abundant clonotypes with identical CDR3s share the same Vβ segments in twin pairs for β TCR chains more often.

We have also found that V gene segment selection for TCR β and α chains is very similar in twin pairs. This result could be expected because twins have identical sets of MHC molecules and V segments coding for MHC recognizing motifs. In contrast, the selection of J gene segments for both α and β TCR chains at the initial recombination step is not different in twin pairs and random pairs of individuals. This finding indicates that the se- lection of J gene segments for recombination is not strictly determined by genetic factors.

173. Damage and recovery in a model of hybrid physical and covalent hydrogels

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Double-network hydrogels composed of different types of crosslinks became attractive recently because of their mechanical performance. The primary network provides the functional properties of the hydrogel such as pH-, or thermo-responsivity. The secondary network usually prevents material failure. Using Molecular Dynamics simulation of a model hybrid cross-link hydrogel, the network damage evolution and the related structure transformations was investigated. We model the hydrogel structure as a self-assembly of crosslinked clusters whereby deformation-induced damage is considered along with network recovery. The two principal mechanisms involved in hydrogel recovery from deformation include segment hops of the building structure units (segments) between clusters and cluster reconstruction. These mechanisms act either instantaneously, or with a certain time delay after the onset of deformation. By elucidating the conditions under which one of the mechanisms prevails, one may design hydrogel materials with a desired response to deformation.

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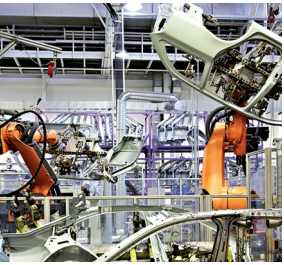

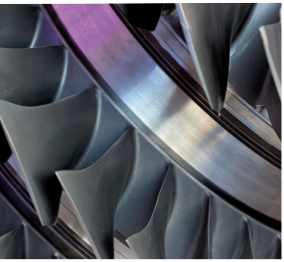

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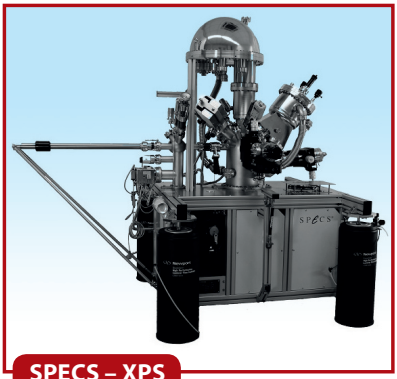
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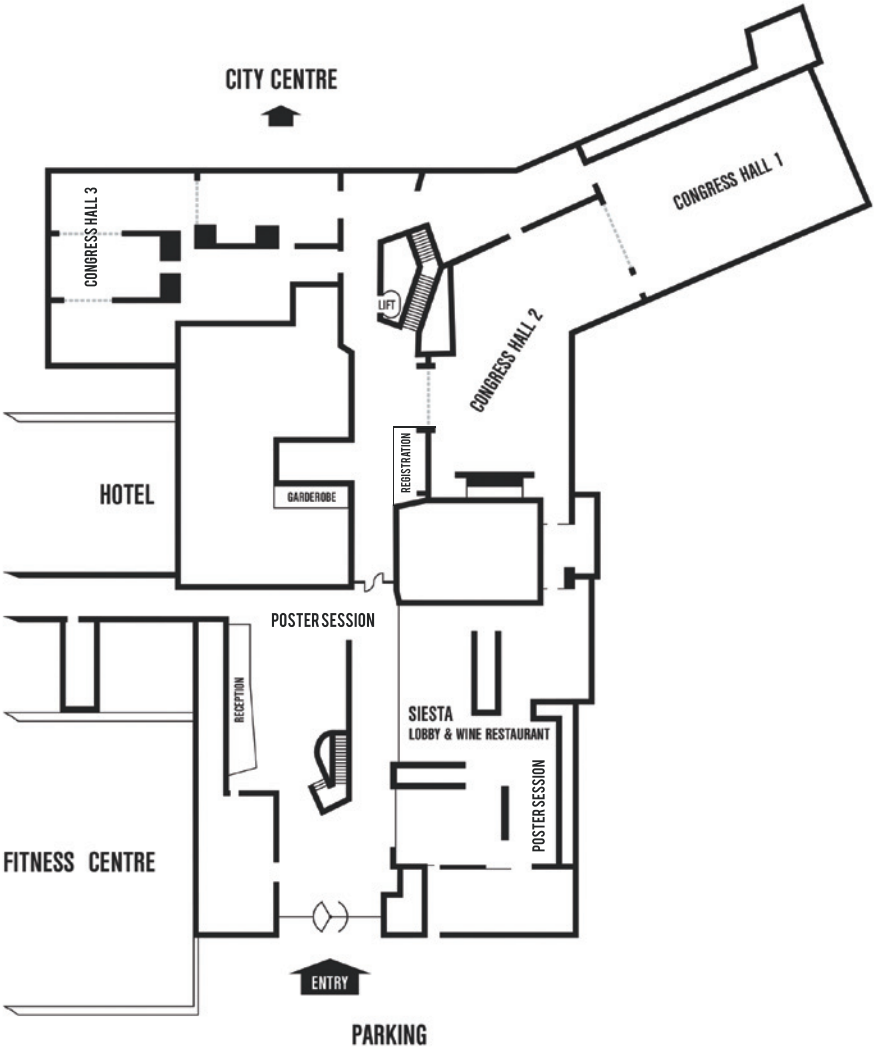

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