CEITEC news

EXTERNAL NEWSLETTER No. 1/2013

Dear CEITEC friends,

This last quarter has been one of the most productive times in CEITEC's short history. We are starting to see many positive outcomes from the groundwork that has been laid by all of the members of the CEITEC community. Many of the most advanced pieces of equipment that exist in Europe are now filling our laboratories. This is evident by the opening of the Josef Dadok National NMR Centre, with many more Core Facilities to be opened. New group leaders from different parts of the world are signing on with CEITEC to bring their expertise and build on the collaborate spirit that will shape our organization's culture. Our future success will depend on scientists and staff members working toward the common goals of the project, in order to realize the great potential that exists throughout our consortium. As we are still in the beginning of building the foundations for our Institute, there is still much logistical work to be done. Through all of this, we will not forget our mission which is dedicated to the pursuit of scientific excellence at the fundamental level and in the application sphere. I am grateful to all of those that have put in the hard-work and long-hours to make CEITEC a choice place for scientific discovery.

Markus Dettenhofer
Executive Director

Best wishes,

Markus Dettenhofer

CEITEC news

No. 1/2013

Content

In Memoriam - Assoc. Prof. RNDr. Petr Lukáš, CSc. dr. h. c.

Petr Lukáš

In mid-January, Assoc. Prof. RNDr. Petr Lukáš, CSc. dr. h. c. passed away unexpectedly. He was a member of the CEITEC Coordination Board and a Statutory Representative of one of the six CEITEC partners from December 2009 to June 2012. He made substantial contributions to the successful preparation and start-up period for the European centre of scientific excellence in Brno, and for the integration of the Institute of Physics of Materials Academy of Sciences into this project.

Dr. Lukáš's main areas of research were physical aspects of the mechanical behaviour of metallic materials with an emphasis on metal fatigue. He markedly contributed to current knowledge of basic mechanisms in the fatigue damage of metals and alloys. He was author of approximately 120 publications in scientific journals indexed in ISI Web of Knowledge, several monographs, and more than 160 contributions in the proceedings of prestigious international scientific conferences. Moreover, he published numerous papers in domestic scientific journals. The most important of his monographs is the book "Fatigue of Metallic Materials", which has been published in Czech, Japanese and twice in English. He was the acting Subject Editor of the ENCYCLOPEDIA of Materials, published by Elsevier in 2001. According to SCI, his papers have been quoted in excess of 2000 times and his Hirsch index is 24. •●

L. Kunz and L. Náhlík

CEITEC acquires the most powerful NMR spectrometer in Central Europe

As of January 2013 CEITEC has had the use of the most powerful nuclear magnetic resonance (NMR) spectrometer in Central and Eastern Europe. The NMR spectrometer is a part of the Josef Dadok National NMR Centre's equipment. The Centre was officially opened on 23 January 2013. Currently, this state-of-the-art workplace has the use of six NMR spectrometers in total, with the most powerful NMR spectrometer working at a frequency of 950 MHz. There are only eight of these devices operating at this frequency in use worldwide.

950 MHz NMR spectrometer for high-resolution spectroscopy in liquids

Prof. Josef Dadok

The Josef Dadok National NMR Centre is one of the Core Facilities of the Structural Biology Research Programme with its focus on the key technology of NMR spectroscopy. NMR spectroscopy deals with detailed studies of biomolecule structure and how it changes over time on an atomic level. The use of this method contributes to detailed descriptions and explanations of a wide range of biologically interesting processes – from the regulation of the transcription of genetic information up to processes at a cellular level. The new workplace has been named after the Czech professor, Josef Dadok, whose scientific research has put him in a prominent position in the history of NMR spectroscopy.

You can watch video from the ceremonial inauguration of the Josef Dadok National NMR Centre **HERE**.

More information and photos can be found **HERE**.

CEITEC scientists discover a new method for the rapid diagnosis of methanol poisoning

More information about the method and Petr Kubáň´s research can be found in the interview on page 10 of this Newsletter.

The press release can be found **HERE.**

Scientists from CEITEC MU led by Assoc. Prof. RNDr. Petr Kubáň, PhD, in cooperation with doctors from Havířov Hospital, have developed a unique method for the rapid diagnosis of methanol poisoning by determining levels of formic acid in blood serum. Formic acid is the final product when methanol is broken down. This new method can determine whether there are higher than normal levels of formic acid in the blood within 1-2 minutes, and thus whether treatment is needed. Rapidly determining levels of formic acid in serum is, therefore, more important than determining methanol levels when it comes to deciding to initiate treatment. The advantages of the new capillary electrophoresis method are simplicity, small sample size (a few microlitres of diluted blood serum is sufficient) and quickness. Compared to current methods, such as gas chromatography (only available in selected toxicological laboratories) this eliminates the necessity of transport, derivatization and pre-treatment of blood samples, which inevitably lead to significant delays. The research results were published in the prestigious Journal of Chromatography A at the beginning of 2013.

Petr Kubáň graduated from Masaryk University in Brno in analytical chemistry. As a scientist, he worked, among other places, at Stockholm University, Sweden, where he obtained his PhD, at Texas Tech University, Lubbock, TX, USA, and at Tallinn University of Technology, Estonia. In his research, he focuses on separation techniques – capillary electrophoresis, ion chromatography, development of analytical instrumentation and its uses in environmental and clinical practice. He is the author of more than 50 publications in reputable scientific magazines (Analytical Chemistry, Journal of Chromatography A, Electrophoresis), several chapters of international books and a co-author of one US patent.

Petr Kubáň

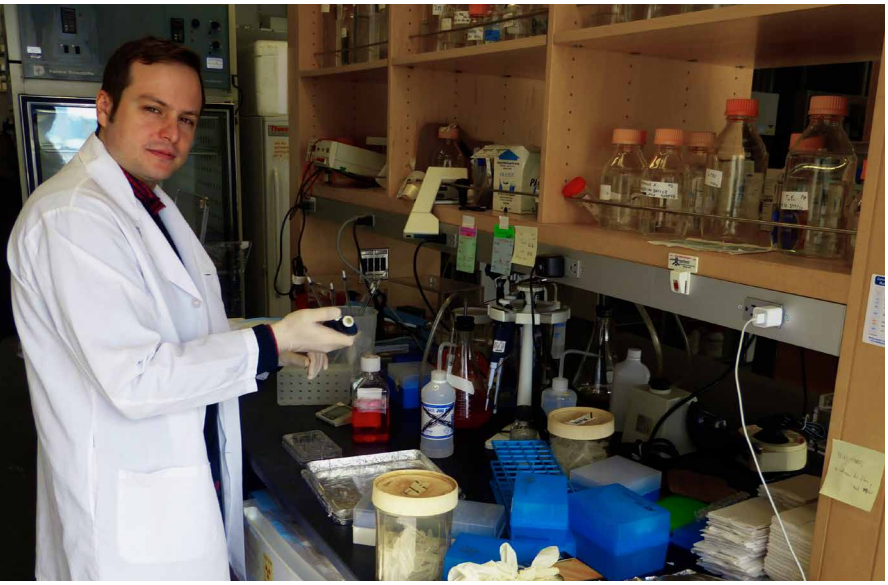
Michal Zimmermann from CEITEC MU discovers the characteristics of the Rif1 gene

Michal Zimmermann, a young scientist from CEITEC MU working in the laboratories of the prestigious American Rockefeller University, has discovered a new genetic factor that is pivotal to the repair mechanism for damaged DNA. The research follows the behaviour of tumour cells in hereditary cancers of the breast and ovaries during the course of chemotherapy, which purposefully causes damage to DNA in these cells – so-called double-strand breaks. During treatment both strands of the DNA helix are interrupted and these breaks need to be repaired. Hereditary breast cancer cells often carry a genetic mutation which prevents the correct repair of breaks so that, after chemotherapy, these cells die and the tumours recede. Obviously, if the tumour cells find a way to repair breaks without errors, the tumour becomes resistant to treatment. It is precisely the Rif1 gene, or rather its mutation, that decides how the DNA is repaired, and thus the success of the treatment.

The research findings were published in January 2013 in the prestigious journal Science.

More information can be found HERE.

Michal Zimmermann is a postgraduate student in the field of Genomics and Proteomics at the Faculty of Science of Masaryk University, while at the same time being a research scientist at CEITEC MU in the laboratory of Ctirad Hofr, PhD. As part of his postgraduate studies Michal Zimmermann has also been part of an international team led by Prof. Titia de Lange at Rockefeller University in New York since 2010. He was engaged at this prestigious international workplace thanks to long-term cooperation between the laboratories of Dr. Hofr and Prof. Fajkus of CEITEC MU with the Laboratory for Cell Biology and Genetics at Rockefeller University in New York.



Michal Zimmermann

CEITEC is kicking off its first PhD programme

Starting in the new academic year, CEITEC BUT is opening a new inter-disciplinary doctoral study programme "Advanced Materials and Nanosciences", which is simultaneously accredited at the Brno University of Technology, Masaryk University, and the Institute of Physics of Materials of the ASCR. Thus, the students will have the opportunity to draw on the expertise of professionals from all three institutions at the same time. They will also have a unique opportunity to use the state-of-the-art devices and technological equipment that already is available or that will be acquired by CEITEC in the course of the next two years.

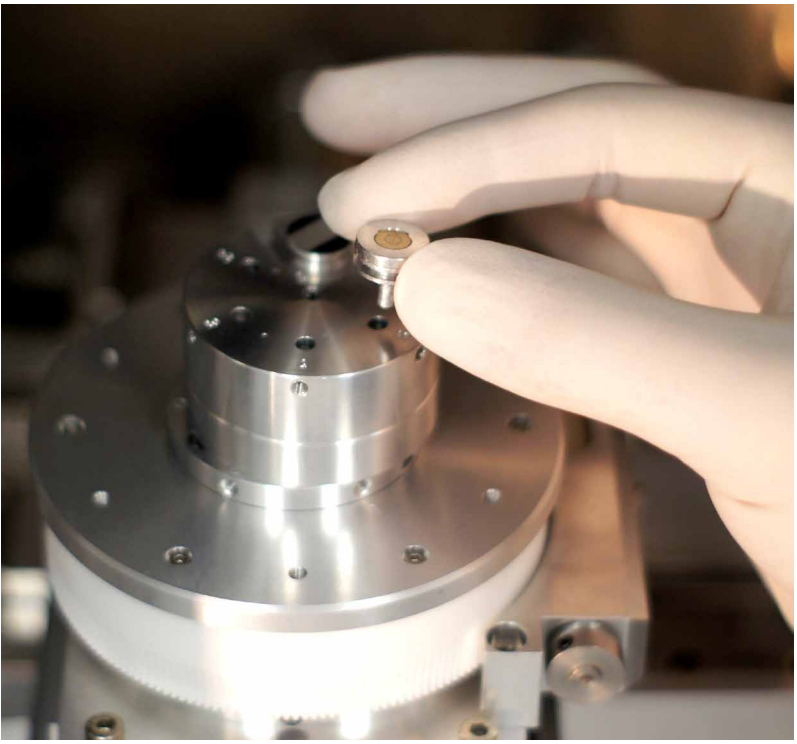
In the future, there will be two more inter-disciplinary doctoral study programmes opened at CEITEC. The first one will focus solely on living nature sciences. The second one will be designed as a multi-disciplinary programme interconnecting living and non-living nature sciences, and utilising the natural cooperation between all seven Research Programmes within CEITEC.

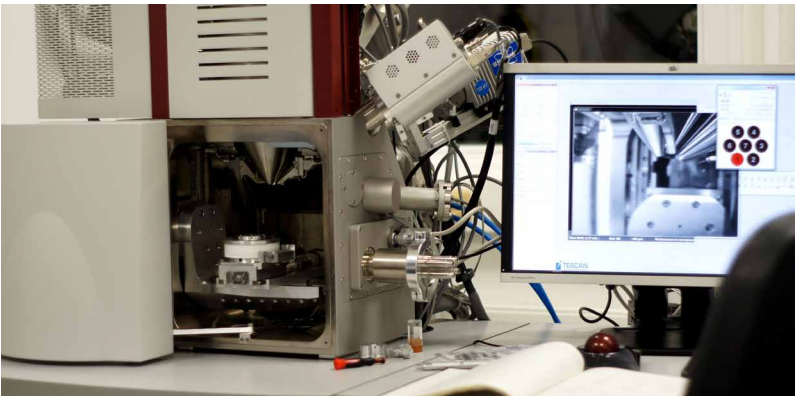
More information can be found **HERE**.

A new CEITEC device enables a deeper insight into the nanoworld

At the turn of the year, the equipment of the Core Facility Nanolithography and Nanofabrication at CEITEC BUT was complemented with the addition of a new device, a two-beam combined LYRA3 XMH microscope. It combines the technology of Scanning Electron Microscopy (SEM) with the Focused Ion Beam method (FIB). In the microscope, beams of ions and electrons are crossed at the desired angle at the researched impact point. Thus, scientists can analyse, modify, prototype and even micro-process samples having the dimensions of 1000 times less than a human hair. LYRA3 XMH was custom made by the Tescan company for scientists working in the Reserach Programme Advanced Nanotechnologies and Microtechnologies.

The combination of the FIB method and SEM technology has been intensively developed and used as an aspect of cooperation between the Research Group Fabrication and Characterisation of Nanostructures led by Prof. Tomáš Šikola and Tescan. The combination of the FIB and SEM methods also enables visual display during the course of ion cauterisation in real time with the use of electron microscopy. Thanks to its high-definition capability resulting from the diameter of the ion beam trace, the device can be widely utilised in the field of materials research.





Interested in using CEITEC Core Facilities? Make use of CEITEC – open access project

MINISTRY OF EDUCATION, YOUTH AND SPORTS

While the new CEITEC buildings, which will house most of the Core Facilities, are still under construction, some of the Core Facilities have already acquired new equipment and have started providing services. The CEITEC Core Facilities are to be open not just to CEITEC researchers but also to external users including Czech and international researchers or companies. The dedicated funding from the Czech Ministry of Education, Youth and Sports for the CEITEC – open access project makes it possible to offer Core Facilities free of charge to the wider scientific community based on a feasibility assessment and peer review of research projects received. Calls for proposals are published every year to invite projects from interested scientists.

In this year's call, 7 of 10 CEITEC Core Facilities will participate and offer their services: Nanolithography and Nanofabrication, Nanocharacterisation, Biomolecular Interaction, Single Crystal X-ray Diffraction, Josef Dadok National NMR Centre, Proteomics and Genomics. The call for proposals for research projects will be published in the coming weeks. Please check the project website for up-to-date information.

Awards

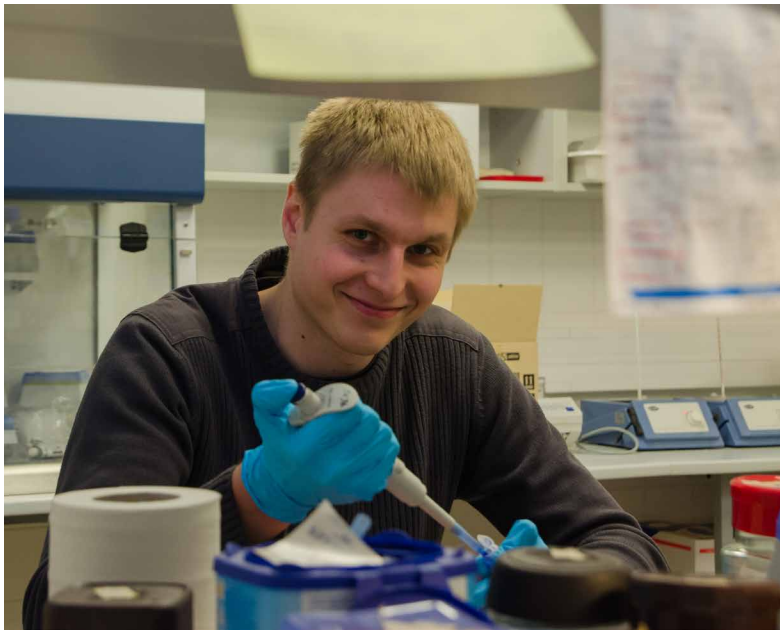
Prof. Radimír Vrba, the CEITEC Scientific Director for Materal Sciences, was honoured by the City of Brno for his activities in the field of technological development. He received the award from the Mayor of Brno on 22 January 2013 at a special ceremony in the council chambers of the Brno City Hall.

"*I was surprised by the award, I take it as a reward for my whole career. At the same time, I perceive it as recognition for the entire team of people around me because cooperation is a key aspect in science*," remarked Professor Vrba, who was, among others, one of the founders of the Department of Microelectronics and the Faculty of Information Technologies at the Brno University of Technology.

As an academic, Professor Vrba works in the field of applied electronics and sensors and, as a principal investigator, has received dozens of domestic and international research grants, the results of which have found real world industrial applications. •●

Peter Holub, PhD, was awarded *The Minister of Education, Youth and Sports Prize for Excellent Students and Graduates 2012* in Prague. He received the award for the exceptional results in RNA metabolism and RNA quality control research, publications in world renowned journals and presentation of results on international conferences in Europe, USA and Japan.

Peter Holub conducted the research in the CEITEC's RNA Quality Control Research Group of Assoc. Prof. Štěpánka Vaňáčová, as a PhD student. He focused on the biochemical mechanisms by which the cell recognizes aberrant RNA transcripts in the nucleus and targets them for degradation in a process conserved from yeast to humans. Recently he successfully defended the thesis on this topic and received his PhD degree. •●



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World Cancer Day

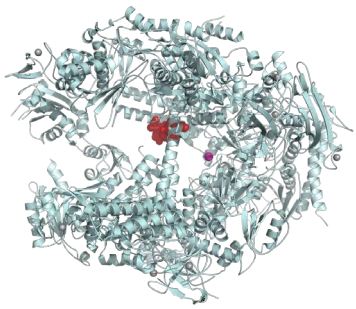
CEITEC Research Group Smart Nanodevices, the Laboratory of Metallomics and Nanotechnologies of MENDELU and the Work Group Anti-Cancer League held on 8 February 2013 a working seminar and an Open House Day at the research laboratories to mark the occasion of the World Cancer Day.

More information, presentations and photos can be found **HERE**.

The agenda for the day was divided into two parts. In the morning, the participants could attend nine topical lectures and then, at noon, the laboratories were opened allowing visitors not only to take a look at the premises and the equipment, but also to be informed of the main experiments carried out in these laboratories. The lectures and the subsequent laboratory openings for the general public focused mainly on issues related to tumour diseases, potential markers for their detection, the impacts of cytostatic drugs on the change of DNA structures and their interactions with amino-acids.

Structural Biology Seminar Series

You are cordially invited to the CEITEC Structural Biology Seminar Series organized by the Structural Biology Research Programme Coordinator, Prof. Vladimír Sklenář.



WHEN: the event takes place regularly on the third Thursday of each month, at 2 p.m.
WHERE: in lecture room 132 of pavilion A11 on the University Campus Bohunice, Brno

The speakers have been carefully selected based on the quality and diversity of proposed lectures in order to keep the Series as interesting and beneficial as possible for all CEITEC members.

The next lecture in the Series will be delivered by Prof. Dr. Patrick Cramer, Director of Gene Center Munich, Ludwig-Maximilians-Universität München, who is a leading figure in the fields of structural and molecular biology of transcription and gene regulation. In his talk, he will demonstrate the assembly and function of a large megadalton multisubunit complex of RNA polymerase II.

More information and the programme for year 2013 can be found **HERE**.

Workshop: Advances in Confocal Microscopy & High Content Screening

We would like to inform you of new trends in Confocal Microscopy and invite you to the international workshop:

WHEN: Thursday, April 4, 2013

WHERE: Veterinary Research Institute, Hudcova 70, Brno

More information and programme of the event can be found **HERE**.

Interview with CEITEC scientist

Assoc. Prof. RNDr. Petr Kubáň, PhD

Scientist, Research Group – Bioanalytical Instrumentation
Research Programme – Genomics and Proteomics of Plant Systems

Petr Kubáň graduated from Masaryk University in Brno in analytical chemistry. As a scientist, he worked, among other places, at Stockholm University, Sweden, where he obtained his PhD, at Texas Tech University, Lubbock, TX, USA, and at Tallinn University of Technology, Estonia. In his research, he focuses on separation techniques – capillary electrophoresis, ion chromatography, development of analytical instrumentation and its uses in environmental and clinical practice. He is the author of more than 50 publications in reputable scientific magazines (Analytical Chemistry, Journal of Chromatography A, Electrophoresis), several chapters of international books and a co-author of one US patent.

You have discovered a new method for the quick detection of methanol intoxication. Where did the idea of analysing formic acid in blood come from? Had you worked with this method in the past?

The idea of analysing formic acid came as a response to the methanol poisoning incidents in September 2012. We commonly use capillary electrophoresis in our laboratory, so we did not need to develop the device itself, as we had already been using it for several years. Coincidentally, we were just using formic acid for another method as an internal standard. And then it occurred to us, in connection with the methanol intoxication news in the media, that it should not be a problem to use our device for determining the presence of formic acid in blood.

What is the point of this method, what is its underlying principle? And how did you develop this method?

Capillary electrophoresis is a technique during which various substances of a given sample are separated in the electrical field. The separation takes place in a fused silica capillary with a very small internal diameter (approx. 50 µm). Blood serum contains a number of substances (ions, organic acids, proteins). Our technique allows selective separation of formic acid in the serum from the above substances and the determination of its concentration. It took us approximately a week from the initial idea to get the first successful determination of formic acid concentration in a model sample of lyophilized serum. Then, naturally, it was necessary to optimise the separation and to verify the method in practice, which we managed to do in cooperation with physicians from the Havířov hospital.

Note on methanol intoxication:

In the initial stages of methanol intoxication, it is not easy to determine methanol consumption, because there are almost no warning symptoms, except for slight inebriation, similar to what we would expect from ethanol consumption. A dramatic change occurs in the body during the course of methanol metabolism giving rise to formic acid, which typically occurs within 6 – 24 hours after the methanol consumption. Formic acid is responsible for some serious symptoms of methanol intoxication, such as sight disorders, CNS damage, etc.

Why did you choose Havířov hospital in particular as your partner? Do you still have continuing cooperation?

This was also a coincidence. I read an interview on the Internet with Dr. Bocek, the Head Physician at Havířov hospital, where they had, at that time, admitted an increased number of patients with methanol poisoning. At that time, our method had already been tested using the model sample of the serum and we knew it was working.

However, it was essential to verify in the period between the initial idea and its implementation in practice if it would be possible to determine the presence of formic acid in patients who were currently suffering from methanol intoxication. And for this purpose, we needed to cooperate with a hospital. I am very much thankful to Dr. Bocek of the Havířov hospital, who I called and who was very willing to help – together, we verified that our method does work in practice. And our cooperation continues very well to this day.

Your new method can detect methanol poisoning within two minutes based on an increased level of formic acid in blood. What is the normal level and what figure already indicates that a person has had methanol in their body?

In healthy individuals, formic acid is only present in the blood serum in small concentrations – the literature quotes figures in the range of 0.07 – 1.2 mM, but the usual concentrations tend to be lower than 0.2 mM and in some samples, it is not possible to detect formic acid at all. If the formic acid level exceeds approximately 4 - 5 mM, the intoxication is deemed severe. The actual concentrations of formic acid in the serum in very severe cases of methanol intoxication were even higher – between 10 - 15 mM, which is approximately 100 times higher than in the serum of a healthy individual.

How long does a common diagnosis of methanol intoxication take? What methods are used in hospitals today for detecting the presence of methanol?

I cannot answer this question precisely, because I only have second-hand information. For example, at the time of the methanol crisis, it took up to several hours to detect formic acid.

The common method for detecting methanol is gas chromatography. This method does indicate intoxication, but for clinical purposes, it is equally important to detect the presence of formic acid. It originates as a product of methanol metabolism and causes serious problems. Gas chromatography is also used in determining the presence of formic acid. For this purpose, it is necessary to derivatize the sample, which is the reason the entire analysis process takes approximately an hour. Besides, gas chromatography is not a standard laboratory technique available in all smaller hospitals, so it is necessary to add the time necessary for the blood sample to be transported to a suitably equipped laboratory.

Is your method / device used in practice? What will be the future development of the device? Can we expect "mass" production?

The method itself will definitely be usable in clinical practice, after the necessary validation, of course. In simple terms, the validation consists of comparing the results of the new method with the known values in certified reference materials (usually for example a blood serum with a certified level of formic acid). If no such reference material is available, validation can be performed by means of comparing results of several analytical methods in independent laboratories.

As for the device itself – capillary electrophoresis is commonly used in clinical laboratories for determining the presence of some proteins (e.g. albumin, γ-globulin), DNA profiling, etc. The device is commercially available and some clinical laboratories are definitely equipped with it. We have been currently working on the development of a small portable device that should be more affordable and easier to use than the existing devices that are commercially available and that could also be used for example in the ambulances.

What is the main contribution of this device in practice? How can it help the doctors?

Its contribution is two-fold. The first aspect is quick diagnostics. Timely commencement of treatment is one of the key factors for the patient's prognosis. However, it doesn't mean that if patients are treated faster, they won't go blind or experience other serious complications. This depends rather on the patient's stage of intoxication at the moment when the patient gets to the doctor. The second aspect is the possibility of monitoring the formic acid levels in blood during the treatment. With the use of capillary electrophoresis, it is possible to optimise the duration of the haemodialysis treatment necessary to eliminate formic acid from the blood, which is important both in terms of potential complications (bleeding, circulation) and, of course, in terms of economic costs.

But your research activities cover a much wider range. What else have you been working on?

The main topic of our research is the use of separation methods in the analysis of biological samples. In addition, we focus on the development of analytic instrumentation (portable devices, detectors, dispensing apparatuses, micro-fluidic chips, etc.), mainly for micro-column separation techniques, such as capillary electrophoresis, micro-column ion and liquid chromatography. Emphasis is also placed on an interdisciplinary approach to our research – we cooperate with physicians, engineers and biologists.

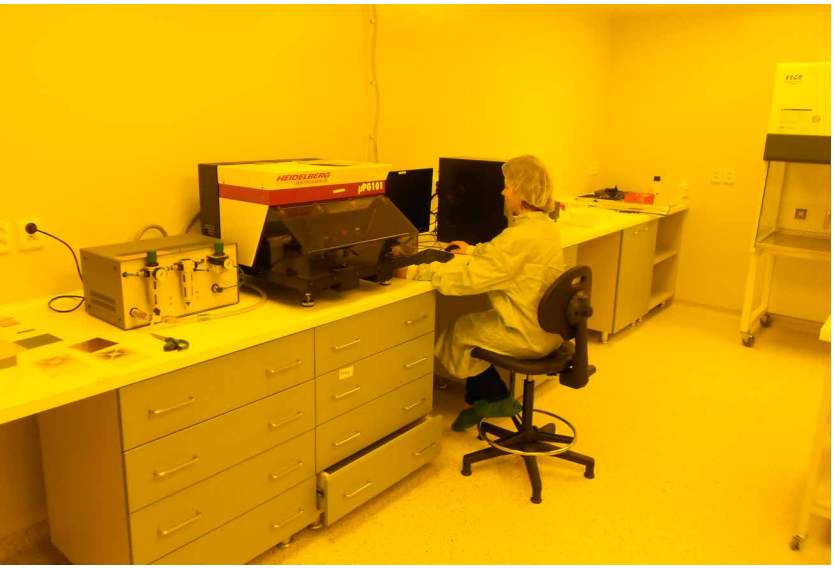
What has been the greatest accomplishment of your work – what are you most proud of?

It is difficult to name one example of a great accomplishment because there are several that are notable. In addition to the most recent research that helps us determine formic acid levels in the blood after methanol poisoning, which is significant especially for the general public, we have also been successful, for example, in the development of an on-line coupling between flow analysis and capillary electrophoresis, micro-fluidic ion suppressor based on immiscible liquids, and the development of new micro-columns for ion chromatography. But what currently brings me the most joy are my three children...

I have read you have gone through internships in Sweden, USA and Estonia. How did you perceive the different scientific environments? Which of the internships influenced you the most?

I think that different environments in general – I wouldn't call them different scientific environments because scientific environments themselves are not that much different – do have motivational impacts on a person. You try to prove your worth and you don't take the internship as a vacation. So in my opinion, such multi-year internships definitely have a great influence, mainly in terms of one's professional career – a person learns new things and gains new insights. This could involve solutions to certain problems, in one's field of specialisation that can subsequently be used in the future. In addition to scientific growth, a person also gets to know the way of life in other countries. I think that each scientist should undergo at least one internship abroad because it would definitely contribute to improving the scientific force in this country. The most influential of these stays abroad was definitely the stay in the laboratory of Prof. Sandy Dasgupta in the USA.

How did you get into chemistry and subsequently into your field of specialisation?



How I got into chemistry... well, chemistry has been a part of our family in a way, so that was probably the main reason. From early childhood, I had been in a process of decision-making trying to decide between becoming a physician or a chemist until finally, for certain personal reasons, I chose chemistry. However, I have always been and still am attracted to medicine – it's one of my little professional "hobbies". And that's why I am happy I can contribute with my expertise in analytical chemistry.

And how I got into my field of specialisation – analytical chemistry... that's simple as well. When I studied chemistry at Masaryk University from 1991-1996, I had the choice of several specialisations after I completed of my third year and, since I had always been keen on discovering and analysing new things (not only substances, but also things like life situations or financial markets...), I chose analytical chemistry as my area of specialisation and I haven't regretted that choice at all.

Within CEITEC, you work in the Research Group Bioanalytical Instrumentation – what is the focus of this Research Group and who will you be cooperating with the most?

The Research Group Bioanalytical Instrumentation, as implied by the name itself, focuses mainly on the development of analytical instrumentation for the analysis of small bio-active molecules, metabolites, nucleic acids, and proteins. The techniques we work with the most are in areas such as capillary electrophoresis, chromatography, and micro-fluidics in conjunction with new detection methods. We also work with nanotechnologies, study fluorescent quantum dots and structured magnetic nanoparticles, immobilisation of enzymes on monolithic materials and the connection of separation techniques with mass spectrometry (CE-MS, LC-MS) in the analysis of complex samples. We will be cooperating mostly with physicians, engineers and biologists but are open to any other collaboration.

Thank you for the interview!

Selected CEITEC publications

ACS Nano

Guided assembly of gold colloidal nanoparticles on silicon substrates prepatterned by charged particle beams

Kolíbal M.; Konečný M.; Ligmajer F.; Škoda D.; Vystavěl T.; Zlámal J.; Varga P.; Šikola T.

CEITEC Research Group: Fabrication and Characterisation of Nanostructures
Research Programme 1: Advanced Nanotechnologies and Microtechnologies

Summary

Colloidal gold nanoparticles represent technological building blocks which are easy to fabricate while keeping full control of their shape and dimensions. We report on a simple two-step maskless process to assemble gold nanoparticles from a water colloidal solution at specific sites of a silicon surface. First, the silicon substrate covered by native oxide is exposed to a charged particle beam (ions or electrons) and then immersed in a HF-modified solution of colloidal nanoparticles. The irradiation of the native oxide layer by a low-fluence charged particle beam causes changes in the type of surface-terminating groups, while the large fluences induce even more profound modification of surface composition. Hence, by a proper selection of the initial substrate termination, solution pH, and beam fluence, either positive or negative deposition of the colloidal nanoparticles can be achieved.

Journal of American Society for Mass Spectrometry

Analysis of the Formation Process of Gold Nanoparticles by Surface-Assisted Laser Desorption/Ionization Mass Spectrometry

Tomalová, I.; Lee, C.-H.; Chen ,W.-T.; Chiang, C.-K.; Chang, H.-T.; Preisler, J.

CEITEC Research Group: Synthesis and Analysis of Nanostructures
Research Programme 1: Advanced Nanotechnologies and Microtechnologies

Summary

Chemical reactions of reducing agents in the gold nanoparticle (AuNP) formation process were characterized using surface-assisted laser desorption/ionization mass spectrometry (SALDI-MS). As the reaction of the AuNPs progresses, the produced AuNPs can serve as an efficient SALDI substrate. SALDI-MS revealed that the reducing agents and their oxidation products can be determined in the mass spectra. With respect to the transmission electron microscopic and UV-Vis spectroscopic examination of AuNPs, SALDI-MS results confirm not only the tendency toward AuNPs formation, but also reflect the information of the redox reaction process. Our results provide useful information for developing SALDI-MS methods to explore the chemical information regarding the surface behavior between adsorbates and nanomaterials.

Physical Review B

Antiferrodistortive phase transition in $EuTiO_3$

Goian, V.; Kamba, S.; Pacherová, O.; Drahokoupil, J.; Palainus, L.; Dušek, M.; Rohlíček, J.; Savinov, M.; Laufek, F.; Schranz, W.; Fuith, A.; Kachlík, M.; Maca, K.; Shkabko, A.; Sagarna, L.; Weidenkaff, A.; Belik, A.A.

CEITEC Research Group: Advanced Ceramics Materials
Research Programme 2: Advanced Materials

Summary

X-ray diffraction, dynamical mechanical analysis, and infrared reflectivity studies revealed an antiferrodistortive phase transition in $EuTiO_3$ ceramics. Near 300 K, the perovskite structure changes from cubic *Pm3m* to tetragonal *I4/mcm* due to antiphase tilting of oxygen octahedra along the **c** axis ($a^0a^0c^-$ in Glazer notation). The phase transition is analogous to $SrTiO_3$. However, some ceramics as well as single crystals of $EuTiO_3$ show different infrared reflectivity spectra bringing evidence of a different crystal structure. In such samples, electron diffraction revealed an incommensurate tetragonal structure with modulation wave vector $\mathbf{q} \approx 0.38\,\mathbf{a}^*$. Extra phonons in samples with modulated structure are activated in the IR spectra due to folding of the Brillouin zone. We propose that defects such as Eu^{3+} and oxygen vacancies strongly influence the temperature of the phase transition to antiferrodistortive phase as well as the tendency to incommensurate modulation in $EuTiO_3$.

The EMBO Journal

In vivo SELEX reveals novel sequence and structural determinants of Nrd1-Nab3-Sen1-dependent transcription termination

Porrua, O.; Hobor, F.; Boulay, J.; Kubicek, K.; D'Aubenton-Carafa, Y.; Kanth Gudipati, R.; Stefl, R.; Libri, D.

CEITEC Research Group: Spectroscopy of Nucleic Acids and Proteins
Research Programme 3: Structural Biology

Summary

The Nrd1-Nab3-Sen1 (NNS) complex pathway is responsible for transcription termination of cryptic unstable transcripts and sn/snoRNAs. The NNS complex recognizes short motifs on the nascent RNA, but the presence of these sequences alone is not sufficient to define a functional terminator. We generated a homogeneous set of several hundreds of artificial, NNS-dependent terminators with an *in vivo* selection approach. Analysis of these terminators revealed novel and extended sequence determinants for transcription termination and NNS complex binding as well as supermotifs that are critical for termination. Biochemical and structural data revealed that affinity and specificity of RNA recognition by Nab3p relies on induced fit recognition implicating an α-helical extension of the RNA recognition motif. Interestingly, the same motifs can be recognized by the NNS or the mRNA termination complex depending on their position relative to the start of transcription, suggesting that they function as general transcriptional insulators to prevent interference between the non-coding and the coding yeast transcriptomes.

Nucleic Acids Research

Polymorphism of human telomeric quadruplex structure controlled by DNA concentration: a Raman study

Palacky, J.; Vorlickova, M.; Kejnovska, I.; Mojzes, P.

CEITEC Research Group: CD Spectroscopy of Nucleic Acids and Proteins
Research Programme 3: Structural Biology

Summary

DNA concentration has been recently suggested to be the reason why different arrangements are revealed for K^+-stabilized human telomere quadruplexes by experimental methods requiring DNA concentrations differing by orders of magnitude. As Raman spectroscopy can be applied to DNA samples ranging from those accessible by absorption and CD spectroscopies up to extremely concentrated solutions, gels and even crystals; it has been used here to clarify polymorphism of a core human telomeric sequence $G_3(TTAG_3)_3$ in the presence of K^+ and Na^+ ions throughout wide range of DNA concentrations. We demonstrate that the K^+-structure of $G_3(TTAG_3)_3$ at low DNA concentration is close to the antiparallel fold of Na^+-stabilized quadruplex. On the increase of $G_3(TTAG_3)_3$ concentration, a gradual transition from antiparallel to intramolecular parallel arrangement was observed, but only for thermodynamically equilibrated K^+-stabilized samples. The transition is synergically supported by increased K^+ concentration. However, even for extremely high $G_3(TTAG_3)_3$ and K^+concentrations, an intramolecular antiparallel quadruplex is spontaneously formed from desalted non-quadruplex single-strand after addition of K^+ ions. Thermal destabilization or long dwell time are necessary to induce interquadruplex transition. On the contrary, Na^+-stabilized $G_3(TTAG_3)_3$ retains its antiparallel folding regardless of the extremely high DNA and/or Na^+ concentrations, thermal destabilization or annealing.

Science

53BP1 regulates DSB repair using Rif1 to control 5´ end resection

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CEITEC Research Group: Chromatin Molecular Complexes
Research Programme 3: Genomics and Proteomics of Plant Systems

Summary

The choice between double-strand break (DSB) repair by either homology-directed repair (HDR) or nonhomologous end joining (NHEJ) is tightly regulated. Defects in this regulation can induce genome instability and cancer. 53BP1 is critical for the control of DSB repair, promoting NHEJ, and inhibiting the 5´ end resection needed for HDR. Using dysfunctional telomeres and genome-wide DSBs, we identify Rif1 as the main factor used by 53BP1 to impair 5´ end resection. Rif1 inhibits resection involving CtIP, BLM, and Exo1; limits accumulation of BRCA1/BARD1 complexes at sites of DNA damage; and defines one of the mechanisms by which 53BP1 causes chromosomal abnormalities in Brca1-deficient cells. These data establish Rif1 as an important contributor to the control of DSB repair by 53BP1.

Plant Physiology

Proteome analysis in arabidopsis reveals shoot- and root-specific rargets of cytokinin action and differential regulation of hormonal homeostasis

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CEITEC Research Group: **Functional Genomics and Proteomics of Plants**
Research Programme 4: **Genomics and Proteomics of Plant systems**

Summary

The plant hormones cytokinins (CKs) regulate multiple developmental and physiological processes in Arabidopsis (*Arabidopsis thaliana*). Responses to CKs vary in different organs and tissues (e.g. the response to CKs has been shown to be opposite in shoot and root samples). However, the tissue-specific targets of CKs and the mechanisms underlying such specificity remain largely unclear. Here, we show that the Arabidopsis proteome responds with strong tissue and time specificity to the aromatic CK 6-benzylaminopurine (BAP) and that fast posttranscriptional and/or posttranslational regulation of protein abundance is involved in the contrasting shoot and root proteome responses to BAP. We demonstrate that BAP predominantly regulates proteins involved in carbohydrate and energy metabolism in the shoot as well as protein synthesis and destination in the root. Furthermore, we found that BAP treatment affects endogenous hormonal homeostasis, again with strong tissue specificity. In the shoot, BAP up-regulates the abundance of proteins involved in abscisic acid (ABA) biosynthesis and the ABA response, whereas in the root, BAP rapidly and strongly up-regulates the majority of proteins in the ethylene biosynthetic pathway. This was further corroborated by direct measurements of hormone metabolites, showing that BAP increases ABA levels in the shoot and 1-aminocyclopropane-1-carboxylic acid, the rate-limiting precursor of ethylene biosynthesis, in the root. In support of the physiological importance of these findings, we identified the role of proteins mediating BAP-induced ethylene production, METHIONINE SYNTHASE1 and ACC OXIDASE2, in the early root growth response to BAP.

Clinica Chimica Acta

Hyperphenylalaninemia in the Czech Republic: Genotype–phenotype correlations and *in silico* analysis of novel missense mutations

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CEITEC Research Group: **Inherited Diseases I - Genetic Research**
Research Programme 5: **Molecular Medicine**

Summary

Hyperphenylalaninemia (HPA) is one of the most common inherited metabolic disorders caused by deficiency of the enzyme phenylalanine hydroxylase (PAH). HPA is associated with mutations in the PAH gene, which leads to reduced protein stability and/or impaired catalytic function. Currently, almost 700 different disease-causing mutations have been described. The impact of mutations on enzyme activity varies ranging from classical PKU, mild PKU, to non-PKU HPA phenotype.

Pharmacology & Therapeutics

Endocannabinoid system and mood disorders: Priming a target for new therapies

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CEITEC Research Group: Experimental and Applied Neuropsychopharmacology
Research Programme 6: Brain and Mind Research

Summary

The endocannabinoid system (ECS), comprising two G protein-coupled receptors (the cannabinoid receptors 1 and 2 [CB1 and CB2] for marijuana's psychoactive principle Δ^9-tetrahydrocannabinol [Δ^9-THC]), their endogenous small lipid ligands (namely anandamide [AEA] and 2-arachidonoylglycerol [2-AG], also known as endocannabinoids), and the proteins for endocannabinoid biosynthesis and degradation, has been suggested as a pro-homeostatic and pleiotropic signaling system activated in a time- and tissue-specific way during physiopathological conditions. In the brain activation of this system modulates the release of excitatory and inhibitory neurotransmitters and of cytokines from glial cells. As such, the ECS is strongly involved in neuropsychiatric disorders, particularly in affective disturbances such as anxiety and depression. It has been proposed that synthetic molecules that inhibit endocannabinoid degradation can exploit the selectivity of endocannabinoid action, thus activating cannabinoid receptors only in those tissues where there is perturbed endocannabinoid turnover due to the disorder, and avoiding the potential side effects of direct CB1 and CB2 activation. However, the realization that endocannabinoids, and AEA in particular, also act at other molecular targets, and that these mediators can be deactivated by redundant pathways, has recently led to question the efficacy of such approach, thus opening the way to new multi-target therapeutic strategies, and to the use of non-psychotropic cannabinoids, such as cannabidiol (CBD), which act via several parallel mechanisms, including indirect interactions with the ECS. The state of the art of the possible therapeutic use of endocannabinoid deactivation inhibitors and phytocannabinoids in mood disorders is discussed in this review article.

Human Brain Mapping

Brain functional connectivity of male patients in remission after the first episode of schizophrenia

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CEITEC Research Group: Behavioural and Social Neuroscience
Research Programme 6: Brain and Mind Research

Summary

Objectives: Abnormal task-related activation and connectivity is present in schizophrenia. The aim of this study was the analysis of functional networks in schizophrenia patients in remission after the first episode. *Experimental design*: Twenty-nine male patients in remission after the first episode of schizophrenia and 22 healthy controls underwent examination by functional magnetic resonance during verbal fluency tasks (VFT). The functional connectivity of brain networks was analysed using independent component analysis. *Results*: The patients showed lower activation of the salience network during VFT. They also showed lower deactivation of the default mode network (DMN) during VFT processing. Spectral analysis of the component time courses showed decreased power in slow frequencies of signal fluctuations in the salience and DMNs and increased power in higher frequencies in the left frontoparietal cortex reflecting higher fluctuations of the network activity. Moreover, there was decreased similarity of component time courses in schizophrenia—the patients had smaller negative correlation between VFT activated and deactivated networks, and smaller positive correlations between DMN subcomponents. *Conclusions*: There is still an abnormal functional connectivity of several brain networks in remission after the first episode of schizophrenia. The effect of different treatment modalities on brain connectivity, together with temporal dynamics of this functional abnormality should be the objective of further studies to assess its potential as a marker of disease stabilization.

Environmental Microbiology

Vancomycin-resistant enterococci in rooks (*Corvus frugilegus*) wintering throughout Europe

Oravcova, V.; Ghosh, A.; Zurek, L.; Bardon, J.; Guenther, S.; Cizek, A.; Literak, I.
CEITEC Research Group: **Molecular Bacteriology**
Research Programme 7: **Molecular Veterinary Medicine**

Summary

This study's aims were to assess the prevalence of, and to characterize, vancomycin-resistant enterococci (VRE) from rooks (*Corvus frugilegus*) wintering in Europe during 2010/2011. Faeces samples were cultivated selectively for VRE and characterized. Pulsed-field gel electrophoresis and multilocus sequence typing (MLST) were used to examine epidemiologic relationships of *vanA*-containing VRE. The *vanA*-carrying VRE were tested in vitro for mobility of vancomycin resistance traits. Eight VRE harboured the *vanA* and *ermB* genes. Seven *vanA*-carrying VRE originated from the Czech Republic and one from Germany. All *vanA*-carrying VRE were identified as *E. faecium*. Based on MLST analysis, six *vanA*-positive isolates were grouped as ST92 type, one isolate belonged to ST121, and the remaining one was described as a novel type ST671. Seven out of eight isolates were able to transfer the vancomycin resistance trait via filter mating with a transfer rate of $8.95 \pm 3.25 \times 10^{-7}$ transconjugants per donor. In conclusion, wintering rooks in some European countries may disseminate clinically important enterococci and pose a risk for environmental contamination.

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Mechanistic basis of infertility of mouse intersubspecific hybrids

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CEITEC Research Group: **Mammalian Reproduction**
Research Programme 7: **Molecular Veterinary Medicine**

Summary

According to the Dobzhansky–Muller model, hybrid sterility is a consequence of the independent evolution of related taxa resulting in incompatible genomic interactions of their hybrids. The model implies that the incompatibilities evolve randomly, unless a particular gene or nongenic sequence diverges much faster than the rest of the genome. Here we propose that asynapsis of heterospecific chromosomes in meiotic prophase provides a recurrently evolving trigger for the meiotic arrest of interspecific F1 hybrids. We observed extensive asynapsis of chromosomes and disturbance of the sex body in >95% of pachynemas of *Mus m. musculus* × *Mus m. domesticus* sterile F1 males. Asynapsis was not preceded by a failure of double-strand break induction, and the rate of meiotic crossing over was not affected in synapsed chromosomes. DNA double-strand break repair was delayed or failed in unsynapsed autosomes, and misexpression of chromosome X and chromosome Y genes was detected in single pachynemas and by genome-wide expression profiling. Oocytes of F1 hybrid females showed the same kind of synaptic problems but with the incidence reduced to half. Most of the oocytes with pachytene asynapsis were eliminated before birth. We propose the heterospecific pairing of homologous chromosomes as a pre-existing condition of asynapsis in interspecific hybrids. The asynapsis may represent a universal mechanistic basis of F1 hybrid sterility manifested by pachytene arrest. It is tempting to speculate that a fast-evolving subset of the noncoding genomic sequence important for chromosome pairing and synapsis may be the culprit.

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