CECE 2015

12th International Interdisciplinary Meeting on Bioanalysis

"… bringing people and ideas together …"

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Find the meeting history and more at www.ce-ce.org

Foreword

Welcome to CECE 2015, the 12th CECE conference in a row. While we still firmly stay behind the goal of "bringing together scientists who may not meet at specialized meetings, promote informal communication of researchers from different disciplines and map the current status of the fields shaping the bioanalytical science" we have made few minor changes in the program. First, the conference date had to be shifted earlier to avoid collision with other events. In addition the invited speaker's lectures predate the CECE Junior. The poster sessions will be open during all three days. This year the meeting is not free of charge anymore; however, we hope that the small registration fee will be more than compensated by the conference program. The organizers want to thank all invited speakers, sponsors and participants for their continuing support.

Brno, August 18, 2015

The Medal of Jaroslav Janák

The Medal of Jaroslav Janák for contributions to the development of analytical sciences was established by the Institute of Analytical Chemistry. Named after the inventor of the gas chromatograph (patented in 1952), founder of the institute (1956) and its long term director, the medal is awarded to scientists who have significantly contributed to the development of separation sciences.

This year the medal goes to **Professor Pavel Jandera**, a recognized analytical chemist, working at the University of Pardubice. Prof. Jandera is recognized for his research work in modern separation sciences, especially high-performance liquid chromatography. He has focused on the study of principles of separation, retention mechanisms, development, optimization and application of methods for the analysis of various types of substances in the environment, food and industrial products. His research interests include comprehensive theory of chromatography with programmed elution, and development of new methods for evaluation and characterization of columns for liquid chromatography. He has been also engaged in micro column liquid chromatography techniques, particularly in development of efficient monolithic capillary columns and two-dimensional liquid chromatography. The results achieved in this area were published in over 250 papers in scientific journals with more than 6000 citations. He also presented his research in over 250 lectures at foreign universities and conferences and served as a member of scientific committees of a number of international symposia in the field of analytical separations. Previously he has received the Tswett Foundation Award, Hanuš Medal of the Czech Chemical Society, Commemorative Medal of Nicolaus Copernicus University in Torun, Commemorative Medal of the University of Messina, Waksmundzki Award for important achievements in the field of separation sciences from Polish Academy of Sciences, Commemorative Medal of University of Pardubice, and in 2015 the AJP Martin Medal awarded by the UK Chromatographic Society.

Program - CECE 2015

Monday, September 21

8:00 – 15:00	**Registration**
9:00 – 9:30	**CECE 2015 - Opening remarks** **Presentation of the Jaroslav Janak's Medal to Prof. Pavel Jandera**
9:30 – 10:00	**ON-LINE MULTIDIMENSIONAL LIQUID COLUMN CHROMATOGRAPHY** **Pavel Jandera** *University of Pardubice, Pardubice, Czech Republic*
10:00 - 10:30	**ENGINEERING TUNNELS AND GATES IN ENZYMES** **Jiri Damborsky** *Loschmidt Laboratories, Masaryk University, Brno, Czech Republic*
10:30 – 11:00	**Coffee break**
11:00 – 11:30	**ELECTROKINETICALLY DRIVEN BIOANALYSIS IN MICROFLUIDIC SYSTEMS** **Adam T. Woolley, Radim Knob, Suresh Kumar, Vishal Sahore, Anna V. Nielsen, Mukul Sonker** *Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah, USA*
11:30 – 12:00	**IONIZATION MICROCHIPS IN MASS SPECTROMETRY** **Risto Kostiainen** *Division of Pharmaceutical Chemistry, University of Helsinki, Finland*
12:00 - 12:30	**LIVING DROPLETS – BIOMEDICAL DISCOVERY AT HIGH THROUGHPUT** **Christoph Merten** *European Molecular Biology Laboratory, Heidelberg, Germany*
12:30 – 14:30	**Lunch break – poster session**

14:30 – 15:00 **DIGITAL HOLOGRAPHIC MICROSCOPY: A NOVEL APPROACH FOR ASSESSING CELLULAR DYNAMICS IN REAL TIME**
Vratislav Kostal, Jan Balvan, Aneta Krizova, Tomas Slaby, Drahomira Ctvrlikova-Knitlova
Tescan, Brno, Czech Republic

15:00 – 15:30 **INORGANIC BIOACTIVE MATERIALS, BIOACTIVITY THERMODYNAMICS AND ASSOCIATED DENTAL USE OF TITANIUM**
Jaroslav Šesták
New Technology - Research Center in the Westbohemian Region, West Bohemian University, Pilsen, Czech Republic, and Institute of Physics, Academy of Sciences of the Czech Republic, Prague, Czech Republic

16:00 **City walk with Franta** – meet in the hotel lobby

19:00 **CONFERENCE DINNER WITH THE TRADITIONAL MORAVIAN MUSIC**

Tuesday, September 22

9:00 – 15:00 **Registration**

09:30 – 10:00 **ALTERNATIVE APPROACHES FOR SAMPLE PREPARATION IN CAPILLARY ELECTROPHORESIS**
Rosanne Guijt
University of Tasmania, Hobart, Australia

10:00 – 10:30 **BIOANALYTICAL STUDY OF THE BACTERIAL TRANSGLYCOSYLATION REACTION**
Bart Blanchaert, Erwin Adams, Ann Van Schepdael
KU Leuven, Leuven, Belgium

10:30 – 11:00 **Coffee break**

11:00 – 11:30 **INDUSTRIAL PRODUCTION OF INORGANIC AND POLYMERIC NANOFIBERS MADE BY FORCESPINNING TECHNOLOGY**
Jan Buk, Miroslav Tejkl, Jana Růžičková, František Foret, Jana Křenková
PARDAM s.r.o., Roudnice nad Labem, Czech Republic

11:30 - 12:00 **A LOOK AT CANCER UP CLOSE AND PERSONAL: THE ART OF LIQUID BIOPSY**
Marek Minarik
Genomac Research Institute, Prague, Czech Republic

12:00 – 14:00 **Lunch break – poster session**

14:00 – 14:30 **MULTIPLE HEART-CUTTING 2D-LC FOR ENHANCED QUANTITATIVE ANALYSES USING UV AND MS DETECTION**
Tom van de Goor
Agilent Technologies, Waldbronn, Germany

14:30 – 15:00 **IMPACT OF CHROMATOGRAPHIC CHANNEL GEOMETRY ON PERFORMANCE OF MICROFLUIDIC LC DEVICES**
Martin Gilar, Thomas S. McDonald, Bernard Bunner, Fabrice Gritti
Waters Corporation, Milford, MA, USA

15:00 – 15:30 **NOVEL MICROEXTRACTION TECHNIQUES IN PRETREATMENT OF COMPLEX SAMPLES**
Pavel Kubáň, Pavla Pantůčková, Andrea Šlampová, Petr Boček
Institute of Analytical Chemistry of the CAS, v. v. i. Brno, Czech Republic

15:30 – 16:00 **SEARCHING FOR GLYCAN CANCER BIOMARKERS: A COMBINED USE OF MASS-SPECTROMETRIC AND MICROCHIP CZE DATA**
Milos V. Novotny[1,2], William R. Alley, Jr.[1], Christa M. Snyder[1], Stephen C. Jacobson[1], Margit I. Campos[1]
[1]Department of Chemistry, Indiana University, Bloomington, Indiana, USA
[2]RECAMO, Masaryk Memorial Oncological Institute, Brno, Czech Republic

Program - CECE Junior 2015

Wednesday, September 23

8:55 – 9:00 **CECE Junior 2015 – Opening remarks**

9:00 – 9:30 **ELECTROCHEMISTRY OF BIOMACROMOLECULES AND ITS USE IN BIOMEDICINE**

Emil Paleček, Veronika Ostatná, Hana Černocká, Mojmír Trefulka, Vlastimil Dorčák, Veronika Vargová

Institute of Biophysics of the CAS, v. v. i., Brno, Czech Republic

9:30 – 9:45 **TILTED MICROPILLARS: A NEW ALTERNATIVE TO INCREASE MICROFLUIDIC CELL CAPTURE EFFICIENCY**

G. Járvás[1], I. Rajta[2], R. Huszánk[2], A.T.T. Szabó[2], G.U.L. Nagy[2], S. Szilasi[2], P. Fürjes[3], E. Holczer[3], Z. Fekete[3], M. Szigeti[1, 4], L. Hajba[1], J. Bodnár[1], A. Guttman[1, 4]

[1]MTA-PE Translational Glycomics Group, MUKKI, University of Pannonia, Veszprem, Hungary

[2]MTA Atomki, Debrecen, Hungary

[3]Hungarian Academy of Sciences, Centre for Energy Research, Institute of Technical Physics and Materials Science, Budapest, Hungary

[4]Horvath Csaba Laboratory of Bioseparation Sciences, University of Debrecen, Hungary

9:45 – 10:00 **CAPILLARY ELECTROPHORESIS-MASS SPECTROMETRY: AN EFFICIENT TOOL FOR MIDDLE-UP CHARACTERIZATION OF MONOCLONAL ANTIBODIES AND ANTIBODY-DRUG CONJUGATES**

Rob Haselberg[1], Klara Petru[2], Elena Dominguez Vega[1], Govert W. Somsen[1]

[1]Division of Bioanalytical Chemistry, VU University Amsterdam, the Netherlands

[2]Department of Analytical Chemistry, Faculty of Pharmacy in Hradec Kralove, Charles University Prague, Czech Republic

10:00 – 10:15 **QUANTUM DOT-BASED IMMUNOPROBE FOR OPTICAL AND ELECTROCHEMICAL DETECTION**

Veronika Dvorakova[1,2], Michaela Cadkova[1,2], Vladimira Datinska[3], Andrzej Chałupniak[4], Lucie Korecka[2], Arben Merkoçi[4], Karel Kleparnik[3], Frantisek Foret[3], Zuzana Bilkova[2]

[1]Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic

[2]Department of Biological and Biochemical Sciences, Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic

[3]Institute of Analytical Chemistry of the CAS, v. v. i., Brno, Czech Republic

[4]Nanobioelectronics & Biosensors group, Institut Català de Nanociència i Nanotecnologia, Bellaterra, Spain

10:15 – 10:30 **NANOSTRUCTURED GOLD ELECTRODES FOR DETERMINATION OF GLUCOSE IN BLOOD**

Zdeněk Farka[1], Tomáš Juřík[1,2], David Kovář[1], Pavel Podešva[3], František Foret[1,3], Petr Skládal[1,2]

[1]CEITEC MU, Masaryk University, Brno, Czech Republic

[2]Department of Biochemistry, Faculty of Science, Masaryk University, Brno, Czech Republic

[3]Institute of Analytical Chemistry of the CAS, v. v. i., Brno, Czech Republic

10:30 – 11:00 **Coffee break**

11:00 – 11:15 **NOVEL PEMPDA β–CYCLODEXTRIN STATIONARY PHASE, STUDY OF ITS SEPARATION POTENTIAL**

Gabriela Kučerová[1], Květa Kalíková[1], Jindřich Jindřich[2], Eva Tesařová[1]

[1]Charles University in Prague, Faculty of Science, Department of Physical and Macromolecular Chemistry, Prague, Czech Republic

[2]Charles University in Prague, Faculty of Science, Department of Organic Chemistry, Prague, Czech Republic

11:15 – 11:30 **STRUCTURAL ANALYSIS AND RELATIVE QUANTIFICATION OF TIGHT JUNCTION PROTEINS: CLAUDIN-1 IN HUMAN SKIN BIOPSY USING CONFOCAL MICROSCOPE**

M. Svoboda[1,2], V.Pavlík[2], M.Hlobilová[2], T. Muthný[2]

[1]Department of Biological and Biochemical sciences, Faculty of Chemical-technology, University of Pardubice, Pardubice, Czech Republic

[2]Department of Research and Development, Contipro Biotech s.r.o., Dolní Dobrouč, Czech Republic

11:30 – 11:45 **COMPARISON OF MODELS USED FOR DESCRIPTION AND PREDICTION OF RETENTION BEHAVIOR OF OLIGOSACCHARIDES IN HYDROPHILIC INTERACTION LIQUID CHROMATOGRAPHY**

Nikola Vaňková, Petr Česla, Jan Fischer

Department of Analytical Chemistry, Faculty of Chemical Technology, University of Pardubice, Pardubice, Czech Republic

11:45 – 12:00 **INFORMATION ENTROPY CALCULATION TECHNIQUE FOR THE DETECTION OF THE PHASES IN THE SELF-ORGANIZING REACTION**

Anna Zhyrova[1,2], Dalibor Štys[1,2], Tomáš Náhlík[1,2], Petr Císař[1,2]

[1]University of South Bohemia in České Budějovice, Faculty of Fisheries and Protection of Waters, South Bohemian Research Centre of Aquaculture

[2]Biodiversity of Hydrocenoses, Institute of Complex Systems, Nové Hrady, Czech Republic

12:00 – 13:30 **Lunch break – poster session**

13:30 – 13:45 **PIECES OF KNOWLEDGE FROM THE STUDY ON THE ELECTROPHORETIC BEHAVIOUR OF SHORT OLIGODEOXYRIBONUCLEOTIDES IN FUSED SILICA CAPILLARIES**

Lada Vítová, Miroslav Fojta, Radim Vespalec

Institute of Biophysics, v. v. i., Academy of Sciences of the Czech Republic, Brno, Czech Republic

13:45 – 14:00 **LABEL FREE PROTEIN ANALYSIS AT CARBON ELECTRODES**

Veronika Vargová, Veronika Ostatná, Emil Paleček

Institute of Biophysics, Academy of Sciences of the Czech Republic, v.v.i., Brno, Czech Republic

14:00 – 14:15 **NOVEL POLYELCTROLYTES USED AS PHYSICALLY ADSORBED COATINGS – CAPILLARY ELECTROPHORESIS AND QUARTZ CRYSTAL MICROBALANCE STUDY**

Filip Duša[1], Joanna Witos[1], Erno Karjalainen[2], Tapani Viitala[3], Heikki Tenhu[2], Susanne K. Wiedmer[1]

[1]Department of Chemistry, University of Helsinki, Helsinki, Finland

[2]Laboratory of Polymer Chemistry, Department of Chemistry, University of Helsinki, Helsinki, Finland

[3]Centre for Drug Research, Division of Pharmaceutical Biosciences, Faculty of Pharmacy, University of Helsinki, Helsinki, Finland

14:15 – 14:30 **COMPARISON OF IONISATION PROPERTIES OF AETMA-LABELED SACCHARIDES WITH COMMON LABELS**

Jan Partyka[1,2], František Foret[1]

[1]Institute of Analytical Chemistry of the CAS, v. v. i., Brno, Czech Republic

[2]Department of Chemistry, Masaryk University, Brno, Czech Republic

14:30 – 14:45 **ONLINE CONNECTION OF FREE-FLOW ISOTACHOPHORESIS CHIP TO AN ELECTROSPRAY IONIZATION MASS-SPECTROMETER**

Jukyung Park[1], Rosanne Guijt[2], Andreas Manz[1]

[1]KIST Europe GmbH, Saarbrücken, Germany

[2]School of Medicine and Australian Centre for Research on Separation Science (ACROSS), University of Tasmania, Hobart, Australia

14:45 **Closing remarks**

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P2 AN AUTOMATED CAPILLARY ELECTROPHORESIS IN A SEPARATION SYSTEM WITH ENHANCED SAMPLE LOADABILITY
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P3 EFFECTS OF COMPLEXATION OF BUFFER CONSTITUENTS WITH CHARGED CYCLODEXTRINS
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P4 COMPARISON OF ANTIOXIDANT AND COLOUR CHARACTERISTICS OF DIFFERENT TYPES OF MEDICAL PLANTS ASSESSED BY MODERN SPECTROSCOPIC TECHNIQUES
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P5 SYSTEM FOR FAST ANALYSIS OF EXPLOSIVES IN THE ENVIRONMENT
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P9 STUDY OF THE CELL WALL OF STAPHYLOCOCCUS AUREUS AND ITS SENSITIVITY TO ENZYBIOTICS
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Jan Tříska, Naděžda Vrchotová, Olga Vilímková

About the invited speakers

Pavel Jandera, PhD, DSc. (Prof. Ing. DrSc.) is professor of Analytical Chemistry, University of Pardubice, Vice-chairman: Group for Chromatography and Electrophoresis of the Czech Chemical Society, Member of the International Committee of the Central European Group for Separation Sciences, International Board of the "Mediterranean Separation Science Foundation Research", Editorial Board of the "Journal of Chromatography A" „Journal of Separation Science"Analytical Letters". Research fields: High Performance Liquid Chromatography (HPLC). Theory, prediction and optimization of HPLC separations, separation mechanisms, gradient elution, multidimensional separations, development of monolithic columns. Book: "Gradient Elution in Column Liquid Chromatography", >20 chapters in monographs, >250 research papers, >6000 citations, h-index = 44. Awards: Hanus medal of the Czech Chemical Society, memorial medals: University of Turun, Poland, University of Messina, Italy. Waksmundzki medal of the Polish Academy of Sience, M. Sklodowska medal of the Polish Chemical Society, A. J. P. Martin gold medal of the Chromatographic Society (London).

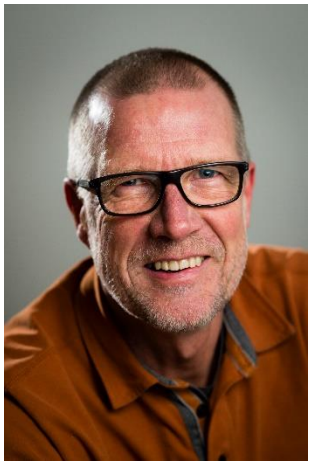
Jiri Damborsky is the Josef Loschmidt Chair Professor of Chemistry and Professor of Biochemistry at the Faculty of Science at Masaryk University in Brno, Czech Republic and a group leader at the International Centre for Clinical Research. Research of his group focuses on protein and metabolic engineering. His group develops new concepts and software tools for protein engineering (CAVER, HOTSPOT WIZARD, PREDICTSNP), and uses them for the rational design of enzymes and bacteria with improved properties for biocatalysis, biodegradation and biosensing. He has published >160 original articles, 12 book chapters and filed 6 international patents. He is a co-founder of the first biotechnology spin-off from Masaryk University Enantis Ltd. Among the awards and distinctions he has received is the award EMBO/HHMI Scientist of the European Molecular Biology Organisation and the Howard Hughes Medical Institute.

Adam T. Woolley graduated summa cum laude with a B.S. in Chemistry from Brigham Young University (BYU), Provo, Utah, USA in 1992. He received his Ph.D. in Chemistry in 1997 from the University of Californi - Berkeley under the direction of Professor Richard Mathies. His doctoral research involved the development of micromachined electrophoretic systems for rapid DNA analysis, and his work was recognized with the 1998 Fannie and John Hertz Foundation Thesis Prize. Woolley was a Cancer Research Fund Runyon-Winchell Foundation Postdoctoral Fellow in the group of Professor Charles Lieber at Harvard University from 1998-2000. His postdoctoral work focused on implementing carbon nanotube probes for high-resolution biological scanning probe microscopy. After postdoctoral studies, Woolley joined the Department of Chemistry and Biochemistry at BYU. He was promoted to Associate Professor in 2006 and to Professor in 2010. Prof. Woolley has also served as an Associate Department Chair since 2010.

Professor Woolley is author or co-author of more than 100 peer-reviewed papers, has given over 130 scientific presentations and has received 10 patents related to his work. He has received several recognitions, including the American Chemical Society Division of Analytical Chemistry Award for Young Investigators in Separation Science (2007), Presidential Early Career Award for Scientists and Engineers (2007), BYU Young Scholar Award (2008), BYU Reed M. Izatt and James J. Christensen Faculty Excellence in Research Award (2012), and BYU Karl G. Maeser Research and Creative Arts Award (2014).

The overarching theme of Professor Woolley's research is the interrelationship between biological molecules and miniaturization: he uses microfabrication techniques to create microfluidic systems to quantify clinically relevant biomolecules, and also utilizes biological molecules (in particular DNA) in designing and preparing nanoscale materials. He has trained over 30 undergraduate students, more than 20 graduate students, and 6 postdoctoral scholars in his group.

Woolley's current research is concentrated in three general areas: biotemplated nanofabrication, the creation of novel and sophisticated integrated microfluidic systems for enhanced biomarker quantitation, and the design of simple, miniaturized biomolecular assays. His group is developing ways to fold DNA into controlled nanoscale designs and convert these structures into functional nanomaterial systems through self-assembly and selective metallization. He is also combining affinity purification and solid-phase enrichment with electrophoretic separation in miniaturized devices to enable biomarker quantitation. Finally, his group is working to develop easy-to-use micro- and nano-fluidic chips for molecular analysis. These projects are pushing new frontiers in chemistry, medicine and engineering.



Risto Kostiainen
Professor, Vice Dean of Research Affairs
(risto.kostiainen@helsinki.fi)
Risto Kostiainen has acted as the professor of pharmaceutical chemistry at the University of Helsinki within the Faculty of Pharmacy since 1997. In addition he is acting as the Vice Dean of research affairs at the Faculty of Pharmacy. His research interest is focused to mass spectrometry, bioanalysis, microchip technology and separation sciences. The main application areas are metabolite analysis and metabolomics focused to brain research. Kostiainen has large national and international co-operation and he has headed and acted in several projects funded by the Academy of Finland, the Finnish Funding Agency for Technology and Innovation and European Union.

Christoph A. Merten (merten@embl.de) studied biochemistry at the University of Frankfurt and obtained his PhD on directed evolution of retroviruses at the Paul Ehrlich Institute in Langen, Germany. Subsequently he did a postdoc at the MRC Laboratory of Molecular Biology in Cambridge (UK), working on in vitro compartmentalization techniques. In 2005 he moved to the Institut de Science et d'Ingénierie Supramoléculaires (ISIS) in Strasbourg, France, where he became a junior group leader in 2007. Christoph joined the European Molecular Biology Laboratory in Heidelberg, Germany, as a Principal Investigator in 2010, focusing on microfluidic technology for HTS, diagnostics and genomics. His lab is particularly interested in the development of droplet-based microfluidics for cell-based screens. Christoph Merten is an inventor on a total of 13 patents (four of them with him as the sole inventor) and collaborates with many academic groups (e.g. The International AIDS Vaccine Initiative) and industrial companies (e.g. Diagenode, Fluidigm, Roche and GSK) in Europe, Asia and the US.

Vratislav Kostal is an applications specialist and a segment manager for Life Science at TESCAN, one of the leading manufacturers of scanning electron microscopes and focused ion beam systems.

Vratislav graduated with honors with M.S. in Environmental Chemistry from the Brno University of Technology, Czech Republic in 2003. In the same year, he joined the Institute of Analytical Chemistry, Brno, Czech Republic as a research assistant, where he was developing miniaturized fluorescence detectors for capillary separation methods. Vratislav received his Ph.D. in Analytical chemistry in 2007 from the Palacky University in Olomouc, Czech Republic. In 2007 he joined the laboratory of Prof. Edgar Arriaga at the University of Minnesota, Minneapolis, MN, USA. His postdoctoral work was focused on developing new technologies for the analyses of mitochondrial subpopulations using capillary electrophoresis and fluorescence microscopy. In 2012 Vratislav started his career at TESCAN, working as an Applications Specialist. Since 2015, he also works as a market segment manager for Life Science. In this new role, he oversees product support and marketing for TESCAN solutions dedicated to biotechnology and biomedicine, including correlative microscopy, cryotechniques and advanced FIB-SEM technology.

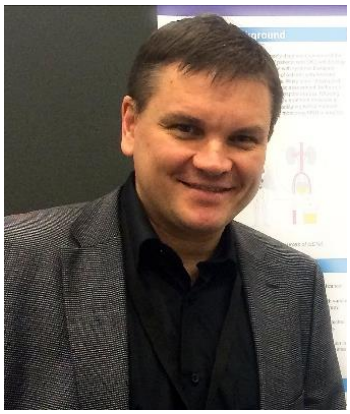
Jaroslav Šesták devoted his scientific proficiency in experimental and theoretical studies related to the fields of materials, applied thermodynamics and thermal analysis. As a full professor he has experience teaching not only in the field of material sciences and engineering but also in the interdisciplinary areas of philosophy and humanities. He edited and authored 14 books and monographs, published almost 300 papers (30 during the past five years) that have received about 2500 citations (Hirsh citation factor 24). Jaroslav gave over 150 invited key lectures and was presented with various scientific awards and assisted the underpinning of the School of Energy Sciences of Kyoto University (1996), Faculty of Humanities of Charles University in Prague (1999), Institute of Interdisciplinary Studies of West Bohemian University in Pilsen (2000) and Prague branch of the New York University (2000). Jaroslav was a co-founding member of both the ICTAC confederation (1965), Thermochimica Acta (1970), Journal of Mining and Metallurgy (1995) and recently the Global Journal of Analytical Chemistry (2010). Among important books belong his “Thermophysical properties of solids” (Elsevier, 1988); “Kinetic phase diagrams: nonequilibrium phase transitions” (Elsevier, 1991), “Special materials and their advanced technologies” (Academia, 1993), „Vitrification, transformation and crystallization of glasses“(Elsevier, 1996),

"Heat, thermal analysis and society" (Nucleus, 2004), "Science of heat and thermohysical study" (Elsevier, 2005), "Thermodynamics, structure and behavior of materials" (Pilsen 2009) and "Glassy, amorphous and nanocrystalline materials: Thermal physics, analysis, structure and properties" (Springer 2011 and "Thermal physics of micro-, nano- and non-crystalline materials (Springer 2013). Since 2010 is the doctor honoris causa of Pardubice University and a year later became the Emeritus Scientist of the Academy of Sciences. Beside his scientific career he was a league basketball player, mountaineer (Himalaya, Caucasus, Asian Pamir, South American Andes and the European Alps – earning needed funds as an occasional window-cleaner roping down tall buildings), ski instructor, politician (deputy and member of the Prague 5 government 1994-1998 and in 1996 a candidate for the seat in the Czech parliament) and enthusiastic globetrotter (notoriously carrying a sleeping sack in his backpack while participating at scientific conferences). Within this hobby he has also became a recognized photographer who held twenty three photo-exhibitions.

Rosanne Guijt completed her undergraduate degree in Biopharmaceutical Sciences at Leiden University, the Netherlands, and her PhD degree from Delft University of Technology where she worked between the Kluyver Institute for Biotechnology and the Institute de Microtechnique (IMT) at the Université de Neuchâtel in Switzerland. Her research involved the development of miniaturized total analytical systems (μTAS) for real-time monitoring of fermentations, and focused on the development of devices for capillary electrophoresis with integrated conductivity detection. She was awarded a fellowship from the Dutch Science and Technology Foundation STW to initiate Lab on a Chip research at the University of Tasmania (μTAS@UTAS). In Australia, she received a 4 year postdoctoral fellowship from the Australian Research Council to work in the Australian Centre for Research on Separation Science (ACROSS), and her research focus broadened to also include the development of flow-through microreactors. Her research strength is in the development of portable and field deployable analytical instrumentation, with applications in counterterrorism and environmental and bioprocess monitoring. She has also explored the use of 3D printing in microfluidics. Following her appointment in the School of Medicine focusing on the development of new technologies for point of care diagnostics and therapeutic drug monitoring. Currently, she resides at the Korean Institute for Science and Technology – Europe in Saarbrucken on an Alexander von Humboldt Fellowship for Experienced Researchers. She has published 67 articles in peer-reviewed journals with an average impact factor over 4.

Ann Van Schepdael (1964) obtained her diploma of Pharmacist in 1986, and PhD in 1990 (KU Leuven, pharmaceutical chemistry). Following a post-doc at KU Leuven and the Barnett Institute (Northeastern University, Boston, 1993) she was appointed as a lecturer in Leuven in 1997. Since 2007 she is a full professor at the Faculty of Pharmaceutical Sciences in the Pharmaceutical Analysis division, and heads the latter lab since 2010. Since 1990 her research focus is on analytical techniques, mainly capillary electrophoresis. One of her current interests lies in electrophoretically mediated microanalysis (EMMA) for enzyme studies and for chemical derivatization. She teaches courses on instrumental analysis and separation and absorption techniques, as well as practical pharmaceutical analytical chemistry exercises in the bachelor and master programs at the school of pharmacy KU Leuven. She has published over 200 publications in international peer-reviewed journals, has been the (co)-promoter of 25 PhD theses and is currently promoter of 4 PhD students and co-promoter of 4 PhD students.

Jan Buk – Chief Operating Officer, PARDAM s.r.o. at company focusing on Development and Production of Inorganic and Polymer nanofibers by Forcespinning technology. Member of the Executive Board of the Czech Association of Nanotechnology Industry. Member of the board of The Czech Society for Applied Photocatalysis. Experienced professional on the field of Development, Production and Application of nanofibrous materials with great experiences in technology Development, Commercialization and Project management at nanofiber industry.



Marek Minarik (1970) received his Ph.D. in bioanalytical chemistry from the Northeastern University in Boston in 2001 with Barry Karger at the Barnett Institute. The topic of his Ph.D. thesis was development of capillary-array electrophoresis instrumentation for micropreparative bioanalysis. Between 2000 and 2002 he worked in R&D at Molecular-Dynamics (later Amersham Biosciences) in Sunnyvale, CA developing applications for clinical and forensic DNA testing. He has authored over 40 scientific papers and 4 issued patents (3 US, 1 International).

His main area of research interest is in development and application of tools and technologies for DNA and RNA analysis with main emphasis on clinical cancer genomics.

Currently, he is a President and CEO of Genomac Research Institute in Prague, Czech Republic that he co-founded in 2001. Genomac is a private genomic research center funded partially from government research grants (domestic and EU). The center has developed own technology for screening and detection of molecular markers including liquid-biopsy technology for monitoring of cancer treatment and progression. Genomac is also an expert institute and a leading provider of DNA testing for medical and forensic genetics as well as direct to customer services such as genetic genealogy, traits and paternity testing.

Aside from commercial affiliation Marek currently holds an assistant professor position at the Department of Analytical Chemistry, Faculty of Sciences, Charles University in Prague lecturing on Genomic analysis in clinical practice.

Tom van de Goor studied Chemical Engineering at Eindhoven University of Technology in The Netherlands where after obtaining his engineering degree (1987), he also completed his Doctor degree in Analytical Chemistry with Prof. Frans Everaerts, Prof. Carel Cramers and Prof. Pat Sandra in the field of Capillary Electrophoresis in 1992.

He then joined Hewlett-Packard in their Central Research Laboratories in Palo Alto, California. During this time he worked on and lead research teams in several technology fields related to micro scale separation technologies, such as capillary and chip based electrophoresis, low flow chromatography systems, HPLC-chip-MS, Time of Flight Mass Spectrometry and electrospray interfacing. Many of these have found their way into HP and Agilent Technologies products.

In 2002 he joined the Mass Spectrometry division within Agilent in Santa Clara, California where he lead teams both in R&D and Marketing in product and application development leading towards the introduction of the new 6000 MS series instruments in 2006. His specific technology focus was on ionization techniques such as nanospray, multimode ESI/APCI and API MALDI and applications focus in the Omics fields (Metabolomics and Proteomics) as well as small molecule Pharmaceutical development.

Since 2007 he is R&D section Manager at the Liquid Phase Separations Business in Waldbronn Germany, responsible for System Validation, Application and Research Collaborations for Chromatography systems and Capillary Electrophoresis and more focused on Pharmaceutical and Biopharmaceutical Analysis.

He leads the Agilent Core Technology University Relations (ACT-UR) Program in Europe, a grant program to support top research in areas of interest to the company and leads the German University relations program focused on

collaboration, instrument donation for teaching in separation science and talent search for internships and employee hiring.

Since 3 years he is teaching a Master Curriculum Course at the University of Marburg entitled: Bioanalytical Separation & Detection on Microchip Platforms.

He is author of more than 40 peer reviewed publications, 3 book chapters, 10 patents and (invited) speaker and contributor to over 100 International conferences and has been reviewer for numerous journals as well as the NIH and other grant agencies.

Martin Gilar is a principal investigator in Core Research group at Waters Corporation. He has more than 20 years of experience in the separation sciences, including chromatography, electrophoresis, and mass spectrometry. His research interest is analysis of biopolymers, and 2D LC. He has published over 40 peer reviewed papers.

Dr. Gilar's received his Ph.D. in analytical chemistry from Institute of Chemical Technology in Prague (1996). He spent postdoc years in Hybridon Inc. (1996-1998) and Northeastern University in Boston (1998) developing separation methods for antisense oligonucleotides and fraction collector for DNA molecules. Since 1998 he works at Waters Corp. in Milford, Massachusetts.

Pavel Kubáň has graduated in Chemistry and Mathematics at Masaryk University, Brno, Czech Republic in 1998, obtained his Ph.D. degree at Mendel University, Brno, Czech Republic in 2001 and RNDr. degree at Palacký University, Olomouc, Czech Republic in 2010. In 2003-2006 he worked as a postdoctoral fellow at University of Basel, Switzerland and spent 2 months in Australian Centre for Research on Separation Science (ACROSS) at University of Tasmania, Australia as a visiting scientist. Since 2006 until now he has been working at the Department of Electromigration Methods, Institute of Analytical Chemistry, Czech Academy of Sciences, where he is currently the head of the Department. His work is mainly devoted to capillary electrophoretic analysis of low-molecular weight compounds, to fundamental research and applications of novel microextraction techniques for pretreatment of samples with complex matrices and to direct coupling of these techniques to capillary electrophoresis. He is author or co-author of more than 70 scientific papers, reviews and book chapters and of nearly 40 contributions on international scientific conferences. In 2007 he was awarded Otto Wichterle Award (Czech Academy of Sciences) for outstanding young researchers.

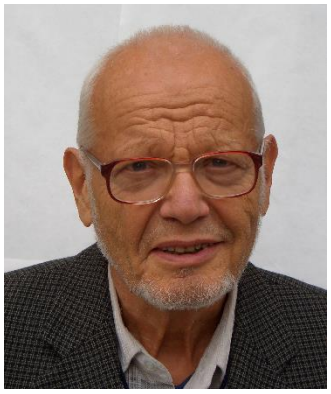
Milos Novotny has been a faculty member at Indiana University (Bloomington, Indiana, USA) for 43 years. He holds there the titles of Distinguished Professor and the Lilly Chemistry Alumni Chair. He is also an Adjunct Professor of Medicine and the Director of Institute for Pheromone Research. A native of Brno, Czech Republic, he received his undergraduate education and a doctoral degree in biochemistry at the University of Brno (now Masaryk University). Subsequently, Dr. Novotny held research appointments at the Czechoslovak Academy of Sciences in Brno (now the Institute of Analytical Chemistry of the Academy of Sciences) and the Royal Karolinska Institute (Sweden). He was a Robert A. Welch Postdoctoral Fellow at the University of Houston (under the direction of Albert Zlatkis) for two years.

He has been best known for his major role in developing modern chromatographic and electrophoretic methods of analysis. However, his general research interests are wide-ranging, including separation science and structural analysis of biological molecules, proteomics and glycoscience, and chemical communication in mammals. Dr. Novotny and his associates are known for structural identification of the first definitive mammalian pheromones. As a member of the Viking 1975 Science Team, Novotny designed the miniaturized GC column to search for organic molecules on the surface of Mars. He was a pioneer in the preparation of glass capillary columns for GC and coupling of capillary GC-MS during the late 1960s. A decade later, Novotny was responsible for the onset of the field of capillary LC, coming up with novel types of microcolumns, miniaturized detectors, and instrumentation. Capillary LC is now being routinely used under the names of "microflow LC" and "nanoflow LC" as an integral part of proteomics, lipidomics, glycomics, and metabolomics analytical platforms. Together with his former student, Milton Lee, Novotny was responsible for the renaissance of supercritical fluid chromatography during the 1980s. Milos Novotny made also major contributions to the development of capillary electrophoresis and capillary electrochromatography in the areas of protein, peptide and carbohydrate separations, including the design of unique fluorescent tags to assist these separations.

More recently, his group has been known for identification of disease biomarkers through glycomics and glycoproteomics. During his 43 years on the Indiana University faculty, Dr. Novotny has trained numerous students and visiting scientists who have become scientific leaders in separation science and bioanalytical chemistry, in both industry and academia.

Milos Novotny has authored over 500 journal articles, reviews, books and patents. He has received around 40 awards, medals and distinctions, including three honorary doctorates from European universities. His many awards include the American Chemical Society (ACS) Award in Chromatography (1986); the ACS Chemical Instrumentation Award (1988); the ACS Separation Science and Technology Award (1992); Eastern Analytical Symposium Awards in Separation

Science (1988) and Outstanding Achievements in the Field of Analytical Chemistry (2001), the Anachem Award (1992), the Dal Nogare Award (2004), the ACS Award in Analytical Chemistry (2005), and the Ralph N. Adams Award in Bioanalytical Chemistry (2008). Internationally, Dr. Novotny received the M. J. E. Golay Medal and was recognized by the Czech Academy (J. E. Purkynje Medal), the Russian Academy (M. S. Tswett Memorial Medal), the Royal Society of Chemistry of Great Britain (Theophilus Redwood Lectureship and the A. J. P. Martin Gold Medal) and Congreso Latinoamericano de Cromatografia Merit Medal (Argentina), and Giorgio Nota Award in Capillary Liquid Chromatography (2012) in Italy. He is a foreign member of two academies: The Royal Society for Sciences (Sweden) and the Learned Society of Czech Republic. Professor Novotny is a recipient of The J. Heyrovsky Honorary Medal for Chemical Sciences in 2015.



Emil Paleček received his PhD in biochemistry from Masaryk University in Brno, Czechoslovakia, in 1959. After working 5 years at the Institute of Biophysics of the Czechoslovak Academy of Sciences in Brno, he was a postdoctoral fellow with Professor Julius Marmur at the Graduate Department of Biochemistry, Brandeis University, Waltham, MA (1962-63). In 1967, he founded the Department of Biophysics of Macromolecules at the Institute of Biophysics in Brno and in 1969 he was promoted to Associate Professor. In 1989, he became a Corresponding Member of the Czechoslovak Academy of Sciences and in 1994 a Founding Member of the Learned Society of the Czech Republic. In 1993-1997, he was a Member of the Academy Council and in 2001-2005 and since 2013 a Member of the Scientific Board of the Academy of Sciences of the Czech Republic. He is Full Professor of Molecular Biology and Honorary Member of the Bioelectrochemical Society. In 2014 he was awarded a Medal of the Senate of the Parliament of the Czech Republic and the highest State award “The Czech Head” for his achievements in science. His research interests are in structure and chemical reactivity of nucleic acids and in electrochemistry of biomacromolecules and electrochemical biosensors.

Abstracts of oral presentations – Invited speakers

ON-LINE MULTIDIMENSIONAL LIQUID COLUMN CHROMATOGRAPHY

Pavel Jandera

Department of Analytical Chemistry, University of Pardubice, Pardubice, Czech Republic

Summary

The main objective of multidimensional (MD) chromatography is increasing the number of resolved compounds in a complex sample (peak capacity of the separation system). While off-line MD by TLC, GC and CLC techniques have been used for long time, on-line comprehensive technique where the whole sample is subject to separation in each dimension are relatively recent, especially as HPLC separations are concerned. The main issue in on-line two-dimensional (2D) separations is the necessity of accomplishing the second-dimension separation of the fraction collected from the first-dimension column in the short time (1-2 min or even less) available for the collection of the next fraction. For maximum peak capacity, different separation mechanisms should be used in each dimension of an MD system (RP, HILIC, Ion Exchange), however the mobile phases in each system should be compatible. For fast 2D separations, it is advantageous using gradient elution simultaneously in each dimension. The choice of optimum column combinations, dimensions and separation conditions (stationary and mobile phases, flow rate, fraction cycle time, etc.,) are discussed and examples of practical applications of on-line MD techniques are shown.

ENGINEERING TUNNELS AND GATES IN ENZYMES

Jiri Damborsky, Zbynek Prokop, Radka Chaloupkova, Jan Brezovsky

Loschmidt Laboratories, Department of Experimental Biology and the Research Centre for Toxic Compounds in the Environment, Masaryk University, Brno, Czech Republic
jiri@chemi.muni.cz

Summary

Protein structures contain a complex system of voids, making up specific features - clefts, pockets, cavities, channels and tunnels. These features are essential for the migration of solvents, ions and small molecules through the protein structure and

represent the natural hot spots for protein engineering. This migration is often controlled by highly dynamical structures called molecular gates. In this lecture, we will present: (i) examples of protein families possessing tunnels1 and gates2, (ii) software tools3 available for detection and analysis of tunnels and gates, (iii) success stories from engineering tunnels for catalytic activity4,5, enantioselectivity6 and stability7. We will demonstrate applicability of the software tools HOTSPOT WIZARD8 and CAVER9 for analysis and design of dynamical access pathways10 and will advocate the design of tunnels and gates as a powerful strategy for construction of novel biocatalysts. Moreover, we will also illustrate the importance of high-throughput bioanalytical techniques for directed evolution studies.

References

[1] Prokop, Z., et al., Engineering of Protein Tunnels. In. Lutz, S., Bornscheuer, U.T. (Eds.), Protein Engineering Handbook, Wiley-VCH, Weinheim, 421-464, 2012.

[2] Gora, A., et al., Gates of Enzymes, Chemical Reviews, 113, 5871-5923, 2013.

[3] Brezovsky, J., et al., Software Tools for Identification, Visualization and Analysis of Protein Tunnels and Channels, Biotechnology Advances, 31, 38-49, 2012.

[4] Pavlova, M., et al., Redesigning Dehalogenase Access Tunnels as a Strategy for Degrading an Anthropogenic Substrate, Nature Chemical Biology, 5, 727-733, 2009.

[5] Liskova, V., et al., Balancing the Stability-Activity Trade-off by Fine-Tuning Dehalogenase Access Tunnels. ChemCatChem, 7, 648-659, 2015.

[6] Prokop, Z., et al., Enantioselectivity of Haloalkane Dehalogenases and its Modulation by Surface Loop Engineering, Angewandte Chemie, 49, 6111-6115, 2010.

[7] Koudelakova, T., et al., Engineering Protein Resistance to Organic Co-solvent and Elevated Temperature by Access Tunnel Modification, Angewandte Chemie International Edition, 52, 1959-1963, 2013.

[8] Pavelka, A., et al., HotSpot Wizard: a Web Server for Identification of Hot Spots in Protein Engineering, Nucleic Acids Research, 37, W376-W383, 2009.

[9] Chovancova, E., et al., CAVER 3.0: A Tool for Analysis of Transport Pathways in Dynamic Protein Structures, PloS Computational Biology, 8, e1002708, 2012.

[10] Sykora, J., et al., Dynamics and Hydration Explain Failed Functional Transformation in Dehalogenase Design, Nature Chemical Biology, 10, 428, 2014.

ELECTROKINETICALLY DRIVEN BIOANALYSIS IN MICROFLUIDIC SYSTEMS

Adam T. Woolley, Radim Knob, Suresh Kumar, Vishal Sahore, Anna V. Nielsen, Mukul Sonker

Department of Chemistry and Biochemistry, Brigham Young University, Provo, Utah, USA

Summary

My research group is making micromachined devices that combine multiple analysis processes, which are used to quantify biomarkers linked to diseases. Our approach combines photopolymerized monolithic supports that carry out solid-phase affinity or reversed-phase extraction of target analytes, and microchip electrophoresis for separation and quantitation of selected components. In earlier work, we showed that electrically driven immunoaffinity extraction could be combined with microchip electrophoresis [1], and further, that solid-phase extraction and fluorescent labeling could be performed in monoliths in microfluidic devices [2]. We are utilizing both electrically driven and pressure-actuated methods for fluid manipulation within our microchips. We have recently developed microdevices with integrated pumps and valves for controlled injection of defined volumes of samples. These microchips also facilitate field-amplified stacking and injection of non-aqueous samples. We are now analyzing biomarkers relating to preterm birth in microfluidic systems that integrate immunoaffinity extraction, preconcentration, fluorescent labeling and electrophoretic separation. Our integrated microdevices have strong potential for broad application in studying biomarkers, especially where sample size is limited.

We are grateful to the United States National Institutes of Health (R01 EB006124) for partial support of this work.

References

[1] Yang, W.; Yu, M.; Sun, X.; Woolley, A.T. *Lab Chip* 10, 2527–2533 (2010).

[2] Yang, R.; Pagaduan, J.V.; Yu, M.; Woolley, A.T. *Anal. Bioanal. Chem.* 407, 737-747 (2015).

IONIZATION MICROCHIPS IN MASS SPECTROMETRY

Risto Kostiainen

Division of Pharmaceutical Chemistry, University of Helsinki, Finland

Summary

Miniaturization of analytical instruments utilizing micro-fabrication technology has been one of the hottest research topics in analytical chemistry over the past decade. Driving force to this is an increasing demand for low-cost instruments capable of rapidly analyzing very small amounts of samples with a high level of automation. A concept termed both “Miniaturized total analysis systems (μ-TAS)" and "Lab-on-a-chip" aims to develop integrated micro-analytical systems to perform complete analysis cycles (e.g. sample pre-treatment, chemical reactions, analytical separation, detection and data handling steps) on a single micro-device.

In the most micro-fluidic applications so far, on-chip detection has relied on optical detection, and for sensitivity reasons, fluorescence (FL) detection has been the most commonly utilized. Among the detection techniques alternative to optical detection, mass spectrometry (MS) has gained rapidly enhanced interest in chip-based analysis, and during the last few years great amount of reports have been published in the field. At present, the main focus is in integrating ionization methods to micro separation systems with MS.

Miniaturization of atmospheric pressure ionization techniques has gained rapidly enhanced interest in chip-based analysis. Electrospray ionization (ESI) is currently the method of choice to connect a microchip with mass spectrometry (MS). The flow rates used with microfluidic devices (nl-μl/min scale) are ideal for optimal sensitivity in ESI-MS. Different materials, such as silicon, glass, polymers have been used in fabrication of microchips. Recently SU-8 polymer has been shown to be highly suitable material for microfluidic separations and electrospray ionization.

Even though ESI is an excellent method for polar and ionic compounds, its sensitivity for neutral and non-polar compounds may be poor. Atmospheric pressure chemical ionization (APCI) and especially atmospheric pressure photoionization (APPI) offer alternative ionization techniques that is capable to ionize with high efficiency non-polar compounds. Recently we presented microchip APCI and APPI, which allow flow rates down to 50 nl/min making it directly compatible with microseparation systems. The chips provide excellent sensitivity, robust analysis, good reproducibility and cost efficient manufacturing. The feasibility of the APCI and APPI microchips in coupling of micro liquid chromatography (LC), gas chromatography and microchip LC to mass spectrometry is presented.

LIVING DROPLETS – BIOMEDICAL DISCOVERY AT HIGH THROUGHPUT

Christoph Merten

European Molecular Biology Laboratory, Heidelberg, Germany

Summary

We have developed fully integrated droplet-based microfluidic platforms for single-cell assays. In these systems tiny aqueous droplets (picoliter volumes) surrounded by oil serve as independent assay vessels. The technology allows the direct screening of >1 million primary, non-immortalized B-cells for the secretion of therapeutic antibodies. Furthermore, the technology can also be used for genomics applications and vaccine development, as we now show in collaboration with the International AIDS Vaccine Initiative.

DIGITAL HOLOGRAPHIC MICROSCOPY: A NOVEL APPROACH FOR ASSESSING CELLULAR DYNAMICS IN REAL TIME

Vratislav Kostal, Jan Balvan, Aneta Krizova, Tomas Slaby, Drahomira Ctvrlikova-Knitlova

TESCAN, Brno, Czech Republic

Summary

Cellular morphology and motility and their changes over time have been hallmarks of many cellular processes, so as a response to pathological processes, toxic stimuli and viral attacks. Invasive behavior of cancer cells can be also assessed by direct observations of cell proliferation.

Digital holographic microscopy (DHM) is an emerging microscopic technique for high resolution, label-free observations of living cells. Compared to standard microscopy, DHM has the unique ability to record complete information about the light waves passing through the sample. Intensity, DIC contrast and importantly phase shift can be easily computed from each hologram in real time. In particular, the phase shift images provide information of the mass distribution (i.e. thickness and shape) of the cells.

Here, we present the latest version of a multimodal holographic microscope (Tescan QPHASE) for long term studies of cellular dynamics and morphology. With the incoherent illumination setup, the system allows contrast imaging of cell boundaries with high clarity and excellent lateral resolution. Moreover axial sensitivity of phase shift detection enables observing very small changes in cell mass distribution. Due to the absence of halo-effect, images of cells can be easily

segmented, which is essential for reliable data analysis. In combination with the fluorescence module, the system becomes a comprehensive tool for investigating relationships between the morphological changes and involved biochemical processes.

The unique capabilities of the system are demonstrated by time lapse studies of cellular death, cancer cell behavior in 3D environments, and by observing dynamics of cancer development.

INORGANIC BIOACTIVE MATERIALS, BIOACTIVITY THERMODYNAMICS AND ASSOCIATED DENTAL USE OF TITANIUM

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Summary

Since 1970 two material groups have been identified capable to form a mechanically stable and functional interface with bone. One group consisted of certain soda-lime-silica glasses, which are exhibiting bone bonding ability (defined as "the bioactivity is the characteristics of an implant material which allows it to form a bond with living tissues"). Another material found to exhibit the bone–bonding ability was machined titanium. The phenomenon of attachment to bone was named osseointegration (defined as "osseointegration represents the formation of a direct contact of a material with bone without intermediate fibrous tissue layer, when observed using light microscope"). Apparently, surface quality determines tissue reactions to an oral implant and its assets can be classified regarding (i) mechanical (j) topographic (roughness, porosity, fractality) and (v) chemical properties. Bio-chemical bonding is related to bioactivity, which existence, however, has often been questioned because there is not a clear evidence of separated effects of surface roughness and interfacial chemical reactions. In modern dental and spinal implantology, advanced treatment protocols (e.g. early or immediate loading) are frequently used to enable reduction of the treatment time. A shorter healing period and shorter time of unloading, entails new demands on both the primary and secondary stability of the implant. The bioactivated surface, which is rich in hydroxyl groups, in contrast to machined surface, rapidly induces adsorption of calcium and phosphate ions on contact with the ions of the blood plasma. The calcium phosphate-rich layer promotes adsorption and concentration of proteins and constitutes a suitable substrate for the first apatite structures of the bone matrix, which are synthesized by the osteogenic cells at the beginning of the formation of the new bone tissue. The clinical study on dental implants was designed as a comparative study of two commonly used surfaces:

classical machined titanium surface and bioactivated titanium surface (LASAK©). The bio-surface is created by sand-blasting, acid etching and a final treatment in an alkaline solution andexhibits more favorable values of the major surface characteristics compared to the machined surface and other commercially available implant surfaces studied so far studied, such as the resonance frequency analysis method used to measure the implant stability quotient. A more easily wettable hydrophilic bio-surface allowed the contact formation between the body environment (blood) and the complicated rough and porous structure of the implant surface, and thus contributes to cell and bio-molecule migration and adhesion.

References

N. Koga, Z. Strnad, J. Šesták, J. Strnad "Thermodynamics of non-bridging oxygen in silica bio-compatible glass-ceramics" J. Thermal Anal.Calor.71 (2003) 927-937.

J. Strnad, Z. Strnad, J. Šesták „Physico-chemical properties and healing capacity of potentially bioactive surfaces" J. Thermal Anal. Calor. 88 (2007) 775

J. Strnad, Z. Strnad, J. Šesták, K. Urban "Bio-activated Ti-surface utilizable for mimetic implantation in dentistry: implant contact formation" J. Phys. Chem. Solids 68 (2007) 841

J. Šesták J. Strnad, Z. Strnad, N. Koga, M. Holeček „Biomedical thermodynamics of bio-compatible glass-ceramics and otherwise modified inorganic material surfaces" Advanced Materials Research 39/40 (2008) 329

J. Šesták, N.Koga, P. Šimon, B. Foller, P. Roubıcek, N-L.Wu. Amorphous Inorganic Polysialates: Geopolymeric Composites and the Bioactivity of Hydroxyl Groups. Chapter 22 in Book: Thermal Analysis of Micro, Nano- and Non-Crystalline Materials (J. Šesták, P.Šimon, editors), Springer (Berlin 2013), ISBN 978-90-481-3149-5

ALTERNATIVE APPROACHES FOR SAMPLE PREPARATION IN CAPILLARY ELECTROPHORESIS

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Summary

In separation science, especially in capillary electrophoresis (CE), the process of sample preparation is often lengthier than the actual separation time and requires specialised equipment and trained personnel in a laboratory environment. Aiming to reduce analytical turn around times by bringing the analytical instrumentation to the sample, we developed a number of alternatives to commonly used sample preparation techniques. Because CE is instrumentally most compatible with the development of

portable and field deployable instrumentation, the focus was on the use of CE as separation technique.

First, the direct sample injection from fruits and vegetables will be presented [1]. Hydrodynamic effects caused by the separation capillary piercing the fruit were effectively excluded by increasing the hydrodynamic resistance of the separation capillary, enabling the electrokinetic injection from various fruits including zucchini, apple and mushroom. The analytical results correlated well with analysis by ICP-MS. Second, we developed a system for on-line monitoring of suspension cultures by sequential injection capillary electrophoresis [2]. Using only 8.1 mL of media (41 µL per run), the metabolic status and cell density were recorded every 30 minutes over 4 days. Thirdly, an electrokinetic size mobility trap was developed to selectively extract, concentrate and purify pharmaceuticals from whole blood [3]. Using nanojunctions of decreasing poresize, ampicillin, an antibiotic used for the treatment of sepsis, could be analysed from whole blood in less than 5 minutes.

With significant differences in their application, these examples demonstrate the feasibility of simplifying sample processing to enable fast and on-site analysis by CE with minimal human intervention.

References

[1] U. Kalsoom, R.M. Guijt, M.C. Boyce, A. Townsend, R. Haselberg, M.C. Breadmore, Electrokinetic injection of minerals from whole fruits and vegetables for capillary electrophoresis analysis, Journal of Chromatography A, under review (2015).

[2] A.A. Alhusban, M.C. Breadmore, N. Gueven, R.M. Guijt, Automated on-line monitoring of suspension cultures: correlating cell density, nutrients and metabolites in near real-time, Scientific Reports, under review (2015).

[3] A.I. Shallan, R.M. Guijt, M.C. Breadmore, Electrokinetic size and mobility traps for on-site therapeutic drug monitoring, Angew. Chem. Int. Ed., (2015).

BIOANALYTICAL STUDY OF THE BACTERIAL TRANSGLYCOSYLATION REACTION

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Summary

Since the discovery of penicillin in the first half of the 20th century, antibiotics have contributed immensely to the improvement of general human health. However, soon after their mass introduction in medicine, the first signs of bacterial resistance towards these drugs have been noticed. Nowadays, the situation is alarming as more and more resistant strains are reported. One of the best known cases is the dangerous and

clinically important pathogen *S. aureus* of which 50 % of infections are caused by the MRSA strain which is resistant to all types of the β-lactam antibiotics. Only a few types of antibacterial therapeutics are left to treat patients infected with this strain. Therefore, research towards new antibacterial therapeutics is of the utmost importance. The bacterial cell wall and more specifically peptidoglycan, which already was the target of the first antibiotic, is still an interesting structure as not all processes involved in its production have been targeted.

The monomeric building block for the peptidoglycan is Lipid II. This is in essence a disaccharide unit to which a pentapeptide is attached, docked on an undecaprenyl pyrophosphate which serves as an anchor in the cell membrane. Lipid II is incorporated into peptidoglycan in two extracellular reactions. First the disaccharide unit is connected to a growing strand in a reaction called 'transglycosylation'. The undecaprenyl pyrophosphate anchor is disconnected in this step. In a second phase, transpeptidation, the cross-linking of saccharide strands by their pentapeptides, occurs after the linear glycan strands are formed. Both reactions are catalyzed by a group of membrane bound proteins called 'penicillin binding proteins' (PBPs). In contrast to the transpeptidation function of the PBPs, which is very intensively studied and is a well-known therapeutic target, the transglycosylation function has not led to any human therapeutic agents so far.

The study towards transglycosylation has been hampered by the unavailability of the substrate, Lipid II. Since Lipid II became available due to efforts in chemical and enzymatic synthesis about a decade ago, a wide variety of assays and high-throughput screens have been developed. The difficulty in the development of such an assay lies in the absence of a UV-chromophore in Lipid II causing the need for other alternatives, mostly radioactive or fluorescent labeling. Since work with radioactive labels is tedious and time-consuming and the attachment of fluorescent groups might alter affinity of inhibitors towards Lipid II, a label-free assay would be most interesting. Therefore, the goal of this project was to develop a label-free LC/MS assay for the bacterial transglycosylation reaction which could be used in a later stage to test inhibitors. In this study, PBP2 from *S. aureus* is used because it is the most important transglycosylase in this species. PBP2 had to be recombinantly produced and Lipid II was produced enzymatically. The development and validation of an LC/MS method for Lipid II will be described. In addition, the influences of the composition of the incubation mixture and incubation conditions on enzymatic activity have been evaluated and optimal conditions were selected. Under these circumstances, repeatable enzyme activity was obtained.

INDUSTRIAL PRODUCTION OF INORGANIC AND POLYMERIC NANOFIBERS MADE BY FORCESPINNING TECHNOLOGY

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Summary
Pardam a company located in the Czech Republic is focused on development and production of nanofibrous materials and products based on nanofibers. Pardam has great experiences with two different production technologies Electrospinning and Forcespinning. Both technologies are used for development of new nanofibrous materials as well as for mass production. There are two different types of nanofibrous materials in the product portfolio of the company. NnF CERAM – inorganic nanofibrous materials in powder like or cotton like structures and NnF MBRANE – polymeric nanofibrous membranes. Pardam has filed several patents and developed several commercial products based on nanofibers made by its technology. Presentation will be focused on introduction of Forcespinning technology, comparisons with Electrospinning, explaining challenges met during the technology development and optimization, on introduction of the final products based on nanofiber materials made by Pardam as well as sharing some experiences on post-treatment of nanofibrous materials in order to get them into the final product. Special attention will be dedicated to the phosphopeptide enrichment product based on inorganic nanofibers.

A LOOK AT CANCER UP CLOSE AND PERSONAL: THE ART OF LIQUID BIOPSY

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Summary
According to WHO in 2012 a total of 32.6 million people lived with cancer within 5 years from diagnosis (1). From the survival standpoint the most efficient therapy is an early surgical intervention in combination with efficient selection of other therapy modalities such as chemo-or radio- therapy. During the past decade the survival of some patients has significantly been prolonged with the arrival of targeted biological therapies. The effect of these new generation drugs is often directed by molecular signatures such as presence of specific somatic mutations within the tumor (2). The knowledge of tumors molecular makeup from tissue biopsy is therefore essential prior

to administering biological therapies. Histopathology evaluation of tissue biopsy has long been the primary tool in cancer diagnosis. More recently a testing for presence of molecular predictors (gene mutations or amplifications) from tissue biopsy samples has also become standard (3). The main problem for subsequent therapy decision comes when the tumor is either undetectable, inaccessible or the patient is incapable of undergoing an invasive procedure.

Short fragmented DNA has historically been observed in blood circulation of patients suffering from metastatic stages of cancers (4). Circulating DNA is often referred to as cell-free DNA to emphasize its exogenous nature in comparison to DNA originating from nuclei of the blood cells. Due to its exclusive origin in the cancerous cells, ctDNA retains the fundamental imprint of its cancer genome including cancer-specific aberrations such as somatic mutations. The most important uses include tumor diagnosis, early detection of tumor relapse or progression and, more recently, therapy prediction and survival prognosis (11). This new promising alternative, generally termed a "liquid biopsy", has immediately become one of the hot topics in cancer research (5). With liquid-biopsy the sampling can be done repeatedly with minimum invasivity. In addition to plasma, a former exploration of the ctDNA phenomena lead to its discovery inurine of patients with solid cancers (12). Subsequently, a transrenal passage of short DNA fragments has been verified offering an invaluable potential for further shift towards truly molecular cancer diagnosis involving repeated non-invasive sample acquisitions (6).

References

[1] http://www.who.int/mediacentre/factsheets/fs297/en

[2] Vogelstein B, Papadopoulos N, Velculescu VE, Zhou S, Diaz LA Jr, Kinzler KW. Cancer genome landscapes. Science. 2013, 339, 1546-1558.

[3] The NCCN Clinical Practice Guidelines in Oncology (NCCN Guidelines®) v 2.2015 National Comprehensive Cancer Network, Inc 2015. Accessed June 18, 2015.

[4] Bendlich A. et al. Circulating DNA as a possible factor in oncogenesis. Science, 1965, 148, 374-6.

[5] Diaz LA Jr, Bardelli A. Liquid biopsies: genotyping circulating tumor DNA J Clin Oncol., 2014, 32, 579-586.

[6] Vibat CR et al. Monitoring Minimal Residual Disease (MRD) by KRAS Mutation Burden in Urinary or Plasma Circulating Tumor (ct) DNA in Colorectal Cancer (CRC) Patients withResectable Liver Metastases, ASCO 2015 meeting, poster no. 3594.

MULTIPLE HEART-CUTTING 2D-LC FOR ENHANCED QUANTITATIVE ANALYSES USING UV AND MS DETECTION

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Summary

Two-dimensional liquid chromatography (2D-LC) has been demonstrated to greatly enhance separation performance compared to conventional 1D-LC. Using orthogonal separation mechanisms delivers much higher peak capacity than optimizing a one-dimensional method.

In contrast to full comprehensive 2D-LC, multiple heart-cutting (MHC) focusses on interesting regions of the 1D chromatogram and is predominantly used for targeted, quantitative analysis.

Factors influencing quantitation in 2D-LC using MHC approaches coupled to UV as well as mass spectrometry (MS) detection are discussed and examples of low level quantitation in complex matrices shown. The advantage of a multidimensional approach to approve LOQ in MS-quantitation obtained with strongly, electrospray-ionization suppressing matrix is also demonstrated.

The peak (heart-cut) parking functionality of MHC 2D-LC breaks the link between 1D and 2D time scales, which allows for addition of a second dimension to existing 1D-methods, the use of longer 2D-cycle times and columns with higher separation efficiency, and the use of 2D-flow rates that are acceptable to mass spectrometry detection.

IMPACT OF CHROMATOGRAPHIC CHANNEL GEOMETRY ON PERFORMANCE OF MICROFLUIDIC LC DEVICES

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Summary

Microfluidic LC is promising alternative to conventional scale chromatography especially for high sensitivity applications. However, chromatographic efficiency of microfluidic LC devices (tiles) packed with sub two micron sorbents is often lower compared to equivalent UHPLC columns. This is in part due to extra column band dispersion and in part due to the channel geometry of microfluidic tile columns adding to column dispersion. We evaluated the impact of turns in the chromatographic bed (including right angle turns at the entry and exit points of planar tile) on chromatographic performance of 0.3 and 0.5 mm ID's microfluidic tiles. The

measurement was performed using optimized LC system with minimal pre-column (sample injector) and post-column (detector cell) band dispersion. Direct efficiency measurement for 0.5 and 0.3 mm ID straight tiles showed that their efficiency is comparable to 2.1-mm ID UPLC columns. However, a distinct loss of efficiency was observed for tiles that included turns in the chromatographic bed. We optimized the tile geometry (turn radius, turn tapering) to improve the tile performance. Computational fluidic dynamic modeling using COMSOL software was used to predict the impact of tile geometry on efficiency and to improve the tile design. Predicted losses in efficiency were in a good agreement with experimentally measured data.

NOVEL MICROEXTRACTION TECHNIQUES IN PRETREATMENT OF COMPLEX SAMPLES

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Summary
Fundamental considerations and practical applications of various microextraction techniques for pretreatment of samples with complex matrices are summarized in this contribution. Special emphasis is devoted to electrically driven microextractions, such as electromembrane extraction (EME) across supported liquid membranes (SLMs), their down-scaling to micro- and nano- format and their applications in analyses of biological samples. New phase interfaces, such as free liquid membranes (FLMs) and polymer inclusion membranes (PIMs) are described and their potential for microextractions of undiluted biological samples is demonstrated. Various approaches for in-line coupling of the presented microextraction techniques to home-made and commercial capillary electrophoresis (CE) instrumentation are also presented. Full automation of the entire process, low instrumental requirements, low costs of the developed microextraction devices and membranes, their disposability and other aspects make the application of such hyphenated microextraction/CE techniques very attractive for routine clinical analyses.

1 Introduction

Analysis of complex, particularly biological, samples is burdened by their inherent properties. Matrix of biological samples, such as human blood, is highly complex and contains large quantities of salts, proteins, lipids and fatty acids. These matrix components usually adhere onto the inner surface of analytical instrumentation and result into deteriorated analytical performance and/or analytical system poisoning.

Standard extraction techniques, such as liquid-liquid extraction (LLE) and solid-phase extraction (SPE), are usually employed to eliminate the detrimental effects of matrix components. In academia, LLE and SPE have been recently replaced with micro-scaled sample pretreatment techniques [1,2]. Liquid-phase microextraction (LPME) [1] and solid phase microextraction technique [2] are most frequently used these days. In this contribution, novel LPME approaches will be presented, which were developed in our laboratory with special emphasis on simplification of sample pretreatment, reduction of sample volume and extraction time and on full automation of the entire microextraction/analytical procedure.

2 Experimental

Instrumental equipment and basic operational principles for EMEs, micro-electromembrane extractions (μ-EMEs) and extractions across SLMs, PIMs and FLMs were described previously [3-7].

3 Results and Discussion

Standard EMEs are performed with mL volumes of body fluids. Extractions of mL volumes of body fluids may, however, be complicated in certain cases due to their limited availability. Concept of a down-scaled μ-EME was proposed recently, where nL to μL volumes of body fluids can be extracted across FLMs in transparent perfluoroalkoxy (PFA) tubing [4]. Figure 1A depicts the basic instrumental arrangement of μ-EME across FLM and Figure 1B demonstrates applicability of such arrangement in μ-EMEs of basic drugs from 1.5 μL of undiluted human body fluids.

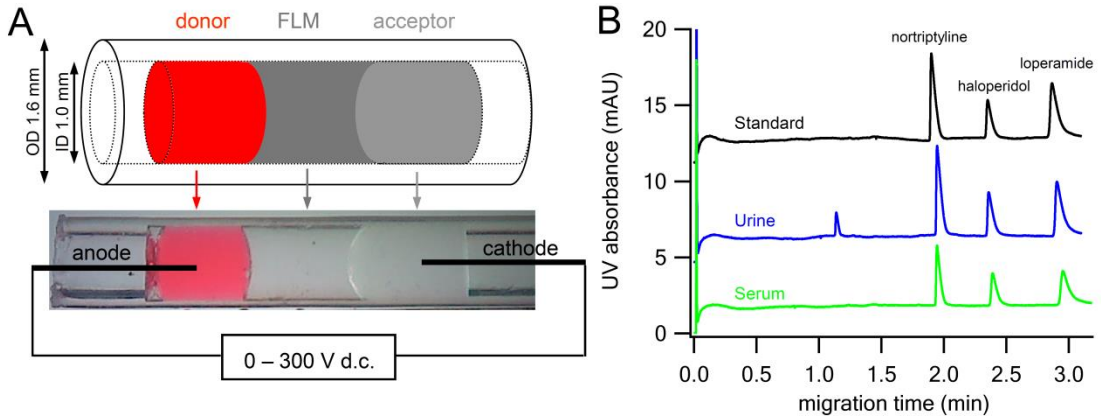

Fig. 1. A. Instrumental arrangement of μ-EME across FLM in PFA tubing (volume of each phase is 1.5 μL). B. μ-EMEs of three basic drugs from undiluted human body fluids followed by CE-UV of the acceptor solutions.

Off-line handling of acceptor solutions and their manual transfer to analytical instrument belong among the most frequently reported drawbacks of newly developed microextraction procedures. Alternative ways for direct combination of microextractions with analytical techniques are thus desirable and various approaches

for in-line coupling of EME and SLM/PIM extractions to commercial CE have been presented recently in our laboratory [6-8]. A simple, disposable microextraction device based on planar membranes (dialysis, SLM, PIM), which is compatible with injection systems of commercial CE instruments, is shown in Figure 2A. A fully automated extraction, injection, separation and quantification of target analytes in µL volumes of undiluted body fluids can be performed in a few minutes. Corresponding electropherograms for PIM extractions coupled in-line to CE analyses for determination of formate (the major metabolite in methanol poisoning) using this device are depicted in Figure 2B.

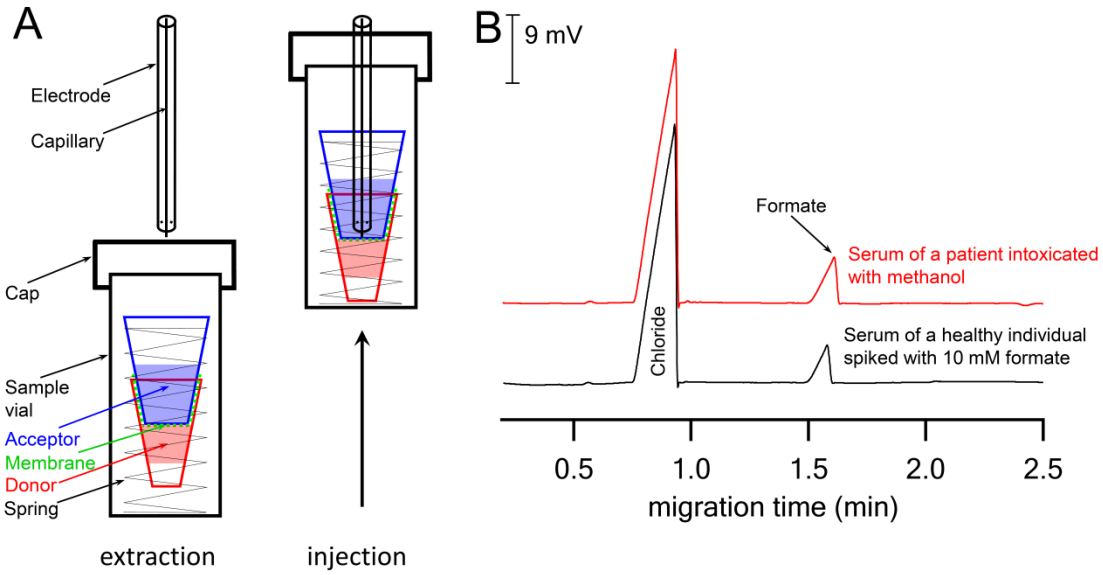

Fig. 2. A. Microextraction device with planar membrane in-line coupled to injection system of Agilent 7100 CE instrument. B. Direct analysis of formate in undiluted serum using PIM microextraction device in-line coupled to CE.

4 Conclusions

Novel LPME approaches for direct analyses of untreated biological samples were presented. They involved development and applications of new phase interfaces, such as FLMs and PIMs, down-scaling of EMEs to micro- and nano-format and development of disposable microextraction devices with planar membranes compatible with injection systems of home-made and commercial CE instruments. Particularly, in-line coupling of microextractions to commercial CE, which ensures full automation of the extraction, injection, separation and quantitation of target analytes, renders the developed techniques very attractive for routine clinical analyses.

Acknowledgement

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References

[1] Pedersen-Bjergaard, S., Rasmussen, K.E., *Anal. Chem.* 1999, *71*, 2650-2656.

[2] Arthur, C.L., Pawliszyn, J., *Anal. Chem.* 1990, 62, 2145-2148.

[3] Kubáň, P., Strieglerová, L., Gebauer, P., Boček, P., *Electrophoresis* 2011, *32*, 1025-1032.

[4] Kubáň, P., Boček, P., *J. Chromatogr. A* 2014, *1346*, 25-33.

[5] Kubáň, P., Boček, P., *J. Chromatogr. A* 2012, *1234*, 2-8.

[6] Pantůčková, P., Kubáň, P., Boček, P., *Electrophoresis* 2013, *34*, 289-296.

[7] Pantůčková, P., Kubáň, P., Boček, P., *Anal. Chim. Acta* 2015, http://dx.doi.org/10.1016/j.aca.2015.07.004.

[8] Kubáň, P., Kiplagat, I.K., Boček, P., *Electrophoresis* 2012, 33, 2695–2702.

SEARCHING FOR GLYCAN CANCER BIOMARKERS: A COMBINED USE OF MASS-SPECTROMETRIC AND MICROCHIP CZE DATA

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Summary

Aberrant glycosylation for a number of proteins in physiological fluids has been observed in different human cancers. During most of the recent discoveries in cancer glycobiology, the moderm mass- spectrometric (MS) measurement technologies have played a decisive role in structural identification of the aberrant glycans and glycopeptides. Our recent studies using MALDI/MS-based glycomic profiling implicate various tri- and tetra-antennary N-glycans, in different quantitative ratios, to be indicative of several types of malignancy. Since certain fucosylated and multiply sialylated structures may occur as different isomeric glycan forms, MS alone is insufficient to distinguish isomerism. Capillary zone electrophoresis (CZE) with laser-induced fluorescence (LIF) of fluorescently labeled glycans can complement MS-based profiling with its capability to resolve isomeric oligosaccharides. The combined uses of MALDI/MS and microchip-based CZE on fairly extensive datasets from the ovarian cancer and colorectal cancer patients' samples could be statistically compared and quantified in our laboratories.

ELECTROCHEMISTRY OF BIOMACROMOLECULES AND ITS USE IN BIOMEDICINE

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Summary
In this review progress in electrochemistry of nucleic acids will be briefly summarized and new trends in electrochemical analysis of non-conjugated proteins and glycoproteins will be discussed particularly in relation to their application in biomedicine.

1 Introduction

First paper on electrochemistry of proteins was published in 1930 showing the ability of proteins to catalyze hydrogen evolution at Hg electrodes [1]. About 30 years later it was shown that DNA produced reduction signal reflecting DNA structure [2,3]. At present electrochemistry of nucleic acids (NAs) and proteins are booming fields (reviewed in [3,4]) NAs sensing deals predominantly with DNA hybridization and damage [3] with application in various areas in practical life, including biomedicine.

2 Experimental

Details of the experimental arrangements are given in the quoted literature [5-7].

3 Results and Discussion

In the recent decades electrochemistry of proteins was oriented mainly on a small group of conjugated proteins yielding reversible reactions of their non-protein components (e.g. metals in metalloproteins) while thousands of proteins important in proteomics, biomedicine, etc. were neglected. Recently we have shown that using constant current chronopotentiometric stripping (CPS) practically any protein produces electrocatalytic peak H at Hg and solid amalgam electrodes (SAEs). Using peak H at low current densities, proteins can be determined down to nM and subnanomolar concentrations. We have shown that proteins do not denature when adsorbed to Hg electrodes or SAE close to the potential of zero charge but can be denatured at negative potentials [4]. Enzymatic activity of urease attached to Hg electrodes was retained while prolonged exposure to negative potentials resulted in the enzyme denaturation. At higher current densities (where the rate of potential changes is extremely fast) CPS protein structure-sensitive analysis was developed [5,6]. At thiol-modified electrodes, changes in properties of mutant proteins could be detected [6]. Using CPS, detection of sequence-specific DNA-protein binding was possible [8].

In humans about 70% of proteins are glycosylated. Differences in protein glycosylation can be involved in pathological processes including cancer [9]. Combination of mass spectrometry with separation techniques has been successfully applied in studies of glycans and their glycosylation sites in glycoproteins [9,10]. For better understanding of progression of various forms of different diseases and for identification of new glycan-containing biomarkers for early diagnostics, simpler and less expensive methods are sought. For decades polysaccharides (PSs) were considered as electroinactive biopolymers. Recently it was shown that some PSs produce peak H_{PS} [7], similar to peak H of proteins. Very recently we have shown glycans containing N-acetylated glucosamine residues (which are electroinactive) can be easily deacetylated and transformed into electroactive species. Moreover, facile modification of PSs and oligosaccharides with osmium(VI) complexes transformed the electroinactive carbohydrates in electroactive Os(VI) adducts, detectable down to pM concentrations. Glycan detection in glycoproteins without deglycosylation was shown [11]. Our results show new possibilities in glycoprotein analysis [4].

4 Conclusions

Methods of electrochemical analysis are relatively simple and inexpensive. They can be easily miniaturized and adapted for parallel analysis. These methods can be applied not only for biomacromolecule determination but also for studies of their mutual interactions and of their properties at electrically charged surfaces.

Acknowledgement

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References

[1] J. Heyrovsky, J. Babicka, Coll. Czech. Chem. Commun. 1930, 2, 370.

[2] E. Palecek, Nature 1960, 188, 656.

[3] E. Palecek, M. Bartosik, Chem. Rev. 2012, 112, 3427.

[4] E. Palecek, J. Tkac, M. Bartosik, T. Bertok, V. Ostatna, J. Palecek, Chem. Rev. 2015, 115, 2045.

[5] V. Ostatna, H. Cernocka, E. Palecek, J. Am. Chem. Soc. 2010, 132, 9408.

[6] E. Palecek, V. Ostatna, H. Cernocka, A. C. Joerger, A. R. Fersht, J. Am. Chem. Soc. 2011, 133, 7190.

[7] E. Palecek, L. Rimankova, Electrochem. Commun. 2014, 44, 59.

[8] E. Palecek, H. Cernocka, V. Ostatna, L. Navratilova, M. Brazdova, Anal. Chim. Acta 2014, 828, 1.

[9] I. Mitra, W. R. Alley, Jr., J. A. Goetz, J. A. Vasseur, M. V. Novotny, S. C. Jacobson, J. Proteome Res. 2013, 12, 4490.

[10] W. R. Alley, B. F. Mann, M. V. Novotny, Chem. Rev. 2013, 113, 2668.

[11] M. Trefulka, E. Palecek, Electrochem. Commun. 2014, 48, 52.

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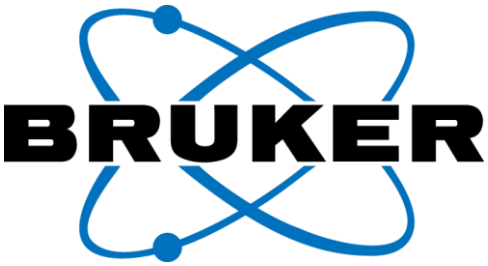

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