REPORT

1st Central and Eastern European Proteomic Conference & 3rd Czech Proteomic Conference

29 – 31 October 2007, Prague, Czech Republic

Suresh Jivan Gadher[1], *Karel Bezouška*[2, 3] *and Hana Kovářová*[4, 5]

[1] Beckman Coulter International S.A., Nyon, Switzerland
[2] Department of Biochemistry, Faculty of Science, Charles University Prague, Prague, Czech Republic
[3] Institute of Microbiology v.v.i., Prague, Czech Republic
[4] Institute of Animal Physiology and Genetics AS CR v.v.i., Libechov, Czech Republic
[5] Joint Proteome Laboratory, Prague, Czech Republic

More than 130 delegates from all over the world attended the 1st Central and Eastern European Proteomic Conference organized together with the 3rd Czech Proteomic Conference in the TOP Hotel, Prague in the Czech Republic from 29th to 31st October, 2007. The autumn nostalgia of the historical city of Prague provided the stage for a fascinating meeting that reviewed rapidly emerging proteomic research in the countries of Central and Eastern Europe, and focused on proteomics driven discovery and applications.

Received: November 19, 2007
Accepted: November 21, 2007

Keywords:
Biomarkers / Mass spectrometry / Nanotechnology

This was the first time that such a comprehensive number of countries from Central and Eastern Europe including Russia participated jointly with the well established Czech Proteomic Society which was hosting their meeting for the third time. The aim was to strengthen links with these scientists, which until now had been weak or non existent. Additionally, the aim was to highlight the emergence of excellent proteomic studies from various countries which until now was not visible. Participating delegates from Central and Eastern Europe included researchers from Russia, Hungary, Poland, Slovakia, Slovenia, Greece, Bulgaria and Romania as well as International participation from far away places such as Canada, USA, India, Pakistan, Israel, Germany, France, Austria, U.K. and Italy (Fig. 1). A truly international panel of speakers was put together by Dr Hana Kovářová for presenting diverse proteomic sessions and cutting edge science. Beckman Coulter was the Platinum Sponsor of the meeting and together with various other exhibitors, displayed related technologies. The historical city of Prague was a befitting venue for such an important meeting in the scientific calendar.

On the first day, the *Clinical Proteomics* session started with Dr Michael Dunn (University College Dublin, Ireland) delivering the Opening Keynote Lecture on 'Proteomics of heart transplant rejection' and discussed proteomic approaches designed to look at protein expressions within the patient hearts that provide protection against the development of Tx-CAD. Their results demonstrated that vascular expression within the heart of a specific form of the small heat shock protein Hsp27, phosphorylated at serine residues 78 and 82, is associated with long-term freedom from chronic rejection. *In vitro* studies, using cells transfected with Hsp27 constructs, showed that phosphorylation of Hsp27 is involved in regulating endothelial and smooth muscle cell proliferation. An experimental animal model of heart transplantation using transgenic mice which constitutively over-express human Hsp27 is currently being used to investigate the ability of Hsp27 to protect against the development of graft vasculopathy *in vivo*.

Correspondence: Dr. Suresh Jivan Gadher, Beckman Coulter International S.A., CH-1260 Nyon, Switzerland
E-mail: SGADHER@BeckmanCoulter.com
Fax: +41-22-994-0703

Abbreviation: PF, protein fractionation

Figure 1. Participants during the opening session of the 1st Central and Eastern European Proteomic Conference in the TOP Hotel Prague, Czech Republic.

Dr David Graham (Johns Hopkins University School of Medicine, Baltimore, USA) discussed the goal of clinical proteomics and proteomics technologies to obtain viable biomarkers especially where cellular necrosis mechanisms are not the primary cause of disease. Dr Rudolf Oehler (Surgical Research Laboratories, University of Vienna, Austria) discussed human platelet proteomics in Ageing research. A central role might be played by platelets of the elderly which display increased generation of reactive oxygen species and an enhanced responsiveness to aggregating stimuli. The majority of significantly changed platelet proteins were found to have increased with age. This age-related platelet proteome may indicate increased oxidative stress in platelets which contributes to the prothrombotic state in the elderly population. Dr Tamas Janáky (University of Szeged, Hungary) discussed Psychiatric diseases including anxiety and depression, which represent a significant portion of neurological disorders. The altered "tuning" of the cells was put forward as one of the reasons for these diseases. Qualitative and quantitative differences in the protein composition in the brain of these anxious and control animals were analysed using specifically developed qualitative and quantitative systems.

Dr Bruno Domon (ETH Zurich, Switzerland) discussed the 'Paradigm shift in Proteomic Studies with reference to Biomarker Identification'. Two major challenges in biomarker discovery from plasma samples include the vast sample complexity due to a large number of proteins and their structural diversity, as well as the huge dynamic range of the protein concentrations spanning over several orders of magnitude (up to ten). Studies discussed included the discovery of proteomic markers based on the isolation of N-glycosites (N-glycosylated peptides) and extensive fractionation (at the protein and/or peptide levels) using LC-MS/MS. Dr Suresh Jivan Gadher (Beckman Coulter International, Nyon, Switzerland) discussed the immensity of the task of protein fractionation (PF) as well as quantitation. Despite the high-resolution power of PF in two dimensions (PF 2-D), UV-based quantitation could be compromised due to possible 'co-elution' of several proteins into one fraction. Hence, an optimised protocol for application of iTRAQ and MALDI-TOF/TOF-MS to obtain quantitative data from peptides derived by tryptic digestions of intact proteins fractionated by PF 2-D technique was discussed. Differentially expressed protein fractions selected by UV quantitation from the PF 2-D system were further subjected to iTRAQ coupled to tandem MS/MS. Based on the correlation between UV and iTRAQ quantitation, the potential biomarkers of anti-cancer activity of cyclin-dependent kinase inhibition and development of chemoresistance were selected.

Dr Hana Kovářová (Institute of Animal Physiology and Genetics AS CR v.v.i., Libechov, Czech Republic) discussed the 'Quest for protein biomarkers of neural stem cell differentiation'. Differentiation of neural cells was found to be accompanied by changes in the expression of proteins involved in DNA and RNA binding (paraspeckle protein –1, far upstream element-binding protein 1), mRNA processing and transport (hnRNPs A1 and A2/B1), stress responses (α-B crystallin), iron storage and redox regulation. High protein levels of signalling molecules such as Raf b, hemoxygenase-2, and low level of G-protein coupled receptor kinase 2 were typical for differentiated neural cells. Many of these proteins are closely related to various functions of nervous system.

The *Disease Proteomics* session covered several interesting topics including clinical proteomics for early cancer diagnostics by Dr Sergey Moshkovskiy (Institute of Biomedical Chemistry RAMS, Moscow, Russia). Ovarian cancer biomarker discovery using serum profiling by MALDI-TOF-MS was discussed as well as the limitations and perspectives of state-of-the-art proteome biomarker discovery. The *Proteomics Technologies and Bioinformatics* session included a fascinating insight into state-of-the-art quantitative proteomics by Dr Friedrich Lottspeich (Max-Planck-Institute, Martinsried, Germany). Many quantitative proteomics methods (*e.g.* iTRAQ, MudPit, GIST) require the digest of all proteins where information about the initial size and charge of different protein species is lost and the correct quantification of the proteins may become rather error prone. Additionally, in eukaryotes usually from a single gene many structurally closely related protein species are produced due to splicing and post-translational events. Enzymatic cleavage of such similar protein species produces many identical peptides which cannot be reliably used for quantification. Using a protein directed proteomics approach (*e.g.* ICPL) the stable isotopic label and quantification by MS was discussed for plasma of patients developing colon cancer.

The *Post-translational Modifications* session was opened by Dr Steven Pelech (Kinexus Bioinformatic Corporation and the University of British Columbia, Canada) who discussed phosphorylation and mapping cell signalling pathways in a cell and tissue-specific manner. Novel technologies for biomarker detection of expression and phosphorylation changes in cell signalling proteins in diverse experimental model

systems were presented. Dr Karel Bezouška (Department of Biochemistry, Faculty of Science, Charles University Prague, Czech Republic and Institute of Microbiology v.v.i., Praha, Czech Republic) provided a fascinating insight into 'Glycomic analysis of tumor cells and the molecular determinants critical for their sensitivity for natural killing and apoptosis'. Interesting studies used glycoproteomics, lectin and oligosaccharide arrays together with bioaffinity techniques to characterise the changes in cell surface glycosylation in tumor cells that are sensitive or resistant to natural killing and in tumor cells treated with carbohydrate dendrimers as compared to controls. Moreover, proteomic analyses of effector (killer) cells treated with mimetics of the tumor surface carbohydrates allowed identification of surface receptors driving the effector cells into apoptosis upon their contact with the tumor targets. Such findings have led to the development of efficient carbohydrate inhibitors that have proved effective in tumor immunotherapies *in vivo*.

Dr Martin Hubálek (Institute of Molecular Pathology, University of Defence, Hradec Kralove, Czech Republic) opened the *Bacterial Proteomes* session by presenting the 'Quantitative proteomic analysis of host-pathogen interaction by LC-MALDI-MS/MS iTRAQ analysis of *Francisella tularensis* murine macrophage infection'. The proteins were quantified by iTRAQ and analysed by LC-MALDI-MS/MS and the analysis yielded about 500 proteins identified from *Francisella* and about 400 from mouse. Additionally, Šedo *et al*. (Department of Functional Genomics and Proteomics, Masaryk University) discussed 'Bacteria profiling by MALDI-MS' and their study examined over a hundred of representatives of *Pseudomonas* and *Aeromonas* species, known for their complicated evolving taxonomy and problematically distinguishable species. They found that MALDI-MS bacteria profiling represents a highly promising tool for rapid and reliable classification of microorganisms. The *New Technologies* session ranged from plastic MALDI chips to protein microarrays and different ways of preparing samples. Dr Alexander Muck (Max Planck Institute for Chemical Ecology, Jena, Germany) presented the non-metallic disposable plastic MALDI sample platform for profiling different protein inhibitors and identifying higher selectivity surfaces for specific protein/peptides, such as phospho-proteins/peptides. An interesting presentation which also won the best oral presentation prize came from the *Beauty of Proteomics* session by Jiri Petrak (Institute of Hematology and Blood Transfusion, Prague) entitled 'Got Enolase? - A hit parade of notoriously identified differentially expressed proteins'. Study involved compilation of the identities of differentially expressed proteins identified in human, mouse and rat tissues by 2-DE-based experiments published in three recent volumes of Proteomics and calculated the appearance of the most predominant proteins in the dataset. The thinking behind this was that such a meta-analysis of proteomic data had the potential not only to dissect important biological pathways but also to discover eventual limitations of 2-D PAGE as a method and consequently help to improve and further develop a cornerstone technique for research. The *Proteomics of Cells, Tissues and Biological Fluids* session concluded the meeting with several interesting presentations – one of which was by Dr Martina Marchetti (Institute of Chemical Technology Vienna, Austria) on Snake venoms and showing these biological fluids to be complex mixtures of bioactive compounds below 2000 Da.

The conference was concluded by the *Closing Keynote Lecture* by Professor Alexander Archakov (Institute of Biomedical Chemistry RAMS, Russia). The lecture was on 'Analytical nanotechnologies in Proteomics'. It outlined sequencing of genes of biosystems and MS analysis which together, jointly converted protein biochemistry into the Proteomics of today. This combination made it possible to analyze hundreds and thousands of proteins at the same time by one scientist as opposed to the possibility of analyzing such amount of proteins by tens of hundreds of scientists. Additionally, the detection limit of existing high-throughput technologies does not exceed 10^{-12} M, while most part of protein molecules in tissue and biological fluid is lower than this limit. Concept of 'Nano-Detectors' that do not measure concentration of proteins but count single protein molecules and their complexes was introduced including atomic-force, electron scanning microscopes, nano-wire and nano-pore detectors. Molecular counting technology in combination with the technology that allows attachment and concentration of protein molecules on the surface consisting of high specific immobilized antibodies, aptamers and other ligates were discussed. Theoretical calculations showed that the combination of these two technologies allows the creation of new technologies having detection limit closer to reverse Avogadro number (10^{-20} – 10^{-21} M).

The closing keynote lecture concluded an exciting and informative Congress where the Czech Proteomic Society succeeded in hosting a joint 'Central and Eastern European Proteomic Conference' of an International Stature where numerous topics and technologies related to Science of Proteomics were discussed in great detail. For more details visit the conference website at: http://2007.czproteo.cz/. The next meeting will be hosted by Aleš Svatoš in Jena, Germany in 2008.

The authors have declared no conflict of interest.

