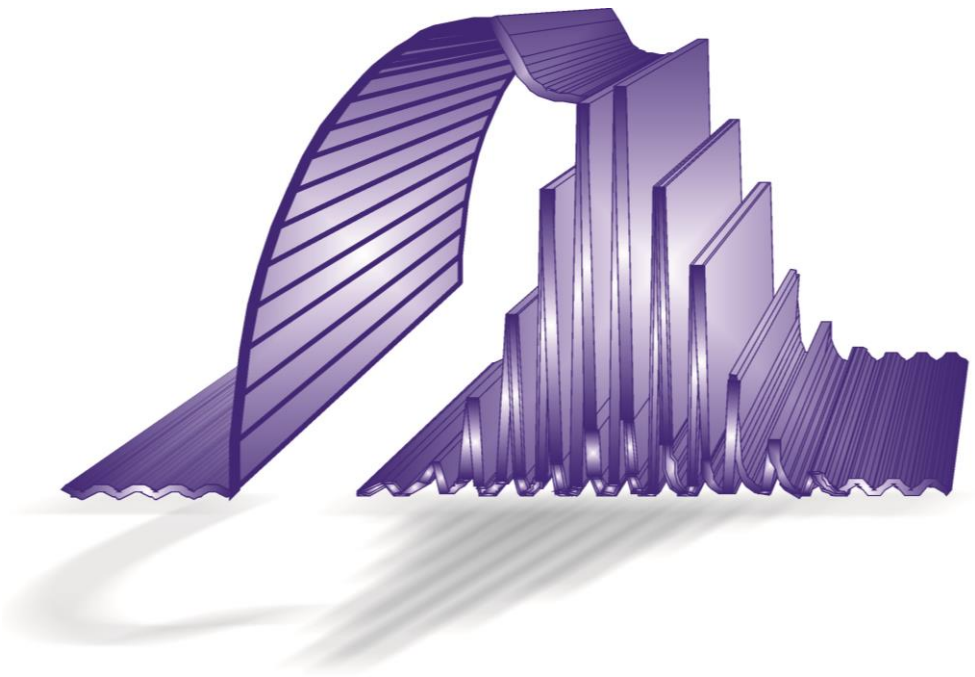
"Affinity - Mass Spectrometry" workshop at HUPO 2015



(http://www.affinityms.de)

Organizers

Christoph Borchers, Michael O. Glocker, and Michael Przybylski

"Affinity - Mass Spectrometry" workshop at HUPO 2015

Scope

This workshop focuses on research activities and methodology development of affinity isolation and determination strategies combined with mass spectrometry. Pre-fractionation methods are the key issue when it comes to addressing the complexity of biological samples and when analyzing low abundant analytes from biological/clinical material. Many applications of affinity-based separation methods have been used and are continuously developed to increase the selectivity and sensitivity in mass spectrometry-based proteomics; now spreading out into many scientific and medical application areas.

Lately, immuno-affinity approaches which make use of defined sets of specific capture molecules such as antibodies have enabled the rapid and unequivocal identification of binding structures and surfaces (e.g. epitopes) by mass spectrometry, particularly by new developments of online MS approaches. With novel state-of-the-art "wet-lab" as well as "dry-lab" developments quantitative and functional analyses in model studies and even in patient samples can be tackled using affinity-MS procedures, thereby opening the field of structure- /function correlations with broad application potentials in cutting-edge research areas.

Speakers

Christoph **Borchers**, Victoria, Canada (christoph@proteincentre.com)

Hans Christian **Eberl**, Heidelberg, Germany (hans-christian.h.eberl@gsk.com)

Anne-Claude **Gingras**, Toronto, Canada (gingras@lunenfeld.ca)

Michael O. **Glocker**, Rostock, Germany (michael.glocker@med.uni-rostock.de)

Petr **Novak**, Prague, Czech Republic (pnovak@biomed.cas.cz)

Oliver **Poetz**, Reutlingen, Germany (oliver.poetz@nmi.de)

Michael **Przybylski**, Rüsselsheim, Germany (michael.przybylski@stw.de)

Venue

Date: Tuesday, September 29, 2015

Time: 7:30 a.m. – 9:00 a.m.

Location: Vancouver Convention Centre, East Building, Room 18

Agenda of the "Affinity - Mass Spectrometry" workshop at HUPO 2015

7:30 – 7:35	**M.O. Glocker**, Proteome Center Rostock, Rostock Welcome and introduction to the workshop as well as a brief overview about Affinity-MS developments
7:35 – 7:50	**M.O. Glocker**, Proteome Center Rostock, Rostock Risk Assessment of Preterm Newborns by Affinity Mass Spectrometry
7:50 – 8:05	**P. Novak**, Laboratory of Structural Biology and Cell Signaling, Prague A Novel Mass Spectrometry-based Approach in Immuno-Diagnostics
8:05 – 8:20	**A.-L. Gingras**, Lunenfeld-Tanenbaum Research Institute, Toronto Computational Tools for Storing, Scoring and Showing Affinity Proteomics Data
8:20 – 8:25	**C. Borchers**, University of Victoria - Genome BC Proteomics Centre, Victoria Discussion
8:25 – 8:40	**C. Borchers**, University of Victoria - Genome BC Proteomics Centre, Victoria iMALDI for quantifying Akt1 and Akt2 expression and phosphorylation in colorectal cancer
8:40 – 8:55	**H.C. Eberl**, Cellzome – a GSK company, Heidelberg Studying protein-small molecule affinities by quantitative proteomics
8:55 – 9:10	**O. Poetz**, NMI Natural Medical and Sciences Institute at the University of Tuebingen, Reutlingen Multiplexed protein quantification using peptide group-specific capture molecules and mass spectrometry
9:10 – 9:25	**M. Przybylski**, Steinbeis Centre Biopolymer Analysis and Biomedical Mass Spectrometry, Rüsselsheim Online Biosensor-Mass Spectrometry Combination: Principles and Application to Elucidation of Antibody Epitopes
9:25 – 9:30	**M. Przybylski**, Steinbeis Centre Biopolymer Analysis and Biomedical Mass Spectrometry, Rüsselsheim Final discussion and farewell

Abstracts of the "Affinity - Mass Spectrometry" workshop at HUPO 2015

Risk Assessment of Preterm Newborns by Affinity Mass Spectrometry

Michael O. Glocker

Proteome Center Rostock, Medical Faculty and Natural Science Faculty, University of Rostock.

Mass spectrometric profiling of severe forms of pregnancy complications has been successful with cord blood serum upon affinity enrichment of serum proteins using a magnetic bead system [1-3]. As of now, plasma or serum is prepared directly in the clinics immediately after blood withdrawal. Yet, one of the remaining challenges for making a mass spectrometry-based profiling assay attractive for clinical use is to bridge the distance between the delivery room and the mass spectrometry laboratory. To overcome hitherto existing limitations we have developed a robust and reliable sample delivery system making use of a novel membrane-based serum / plasma storage device. The key step for success is resolubilization of intact proteins whose relative abundances are individually profiled by MALDI-ToF mass spectrometry.

Serum proteins can be stored intact at room temperature for weeks and shipped by regular mail in a clean envelope without loss of quality when deposited on a "plasma collection disc" (Noviplex ^{TM}). Proteins can be eluted from the "plasma collection disc" using detergent-containing solutions (Rapigest ^{TM}) at pH 8. Transfer of such resolubilized serum proteins onto bead surfaces (ClinProt ^{TM}) is possible by acidification to pH 3, by which the acid-labile detergent is destroyed and the solubilized serum proteins readily adsorb onto the magnetic bead surfaces. Intact serum proteins can be eluted from the hydrophobic bead surfaces into salt-free and volatile buffers that contain organic co-solvents. Proteins from such solutions are subjected to linear MALDI-ToF MS profiling.

We tested investigated cord blood serum samples from newborns. Two groups of premature babies, i.e. small for gestational age (SGA) and intra-uterine growth restricted (IUGR) infants, were analyzed. As babies from both groups are equally small at birth, a molecular pattern of cord blood was determined that allowed to differentiate the two. Multiparametric scoring and biostatistical analyses sort spectra from the IUGR samples into the IUGR group (true positive) and spectra from the control samples into the control group (true negative) with high confidence. The robustness of all involved steps makes this assay attractive to clinics world-wide. Clearly, mass spectrometric profiling of intact serum proteins desorbed from dried serum spots enables reliable differentiation between IUGR and control samples.

[1] M. Wölter, C. Röwer, C. Koy, T. Reimer, W. Rath, U. Pecks and M.O. Glocker (2012). A proteome signature for Intra-Uterine Growth Restriction derived from multifactorial analysis of mass spectrometry-based cord blood serum profiling. **Electrophoresis**, *33*, 1881-1893.

[2] M.O. Glocker, C. Röwer, M. Wölter, C. Koy, T. Reimer and U. Pecks (2014). Multiparametric analysis of mass spectrometry-based proteome profiling in gestation-related diseases. In: **Handbook of Spectroscopy**, 2nd ed., Wiley-VCH GmbH & Co. KgaA. G. Gauglitz and D.S. Moore (eds.). Part IV: Methods 3: Mass Spectrometry, Chapter 12, pages 407–428.

[3] U. Pecks, I. Kirschner, M. Wölter, D. Schlembach, C. Koy, W. Rath, and M.O. Glocker (2014). Mass Spectrometric Profiling of Cord Blood Serum Proteomes to Distinguish Intra-Uterine-Growth-Restricted Infants from Small-for-Gestational-Age and Control Individuals. **Transl. Res.** *164*, 57-69.

A Novel Mass Spectrometry-based Approach in ImmunoDiagnostics

Petr Pompach[1,2], Michael Volný[1], Petr Novák[1,2]

[1] Institute of Microbiology v.v.i., Prague, CZ
[2] AffiPro s.r.o., Mratín, CZ

Modern biochemical tests, used in medicine, veterinary, research and industry, are often based on the interaction of the protein with its biological partner. In case the protein is an antibody and the analyte an appropriate antigen, the test refers to the immunoassay. Mostly, the protein is anchored to a solid surface such as a plastic plate or magnetic beads. It enables manipulation after the antigen has been captured. The major advantage of this procedure is the test specificity and enrichment of the antigen, which significantly helps to improve the limit of detection and quantification. The antigen can be visualized by several different techniques like chemiluminescence, fluorescence and radiation. Since these techniques relays on the specificity of used antibody a novel detection procedure exploring desorption mass spectrometry has been introduced. Currently, there are several possibilities for antibody immobilization from non-covalent adhesion to covalent chemical cross-linking. Unfortunately none of them are perfect. In our pioneering study an extremely effective method has been discovered for preparation of functionalized surfaces, which can be used for both standard chemiluminescence detection and mass spectrometric analysis. The method combines native electrospray and reactive landing. Antibody is electrosprayed from solution to the gas phase in its native state and subsequently transferred using electrostatic lenses to a conductive surface. The potential for the immunodiagnostics is going to be discussed.

Computational Tools for Storing, Scoring and Showing Affinity Proteomics Data

Anne-Laure Gingras

Lunenfeld-Tanenbaum Research Institute, Toronto

tba

iMALDI for quantifying Akt1 and Akt2 expression and phosphorylation in colorectal cancer

Christoph H. Borchers[1,2], Robert Popp[1], Andrew G. Chambers[1], Adriana Aguilar-Mahecha[2], Oliver Pötz[3], Mark Basik[2]

[1]University of Victoria - Genome BC Proteomics Centre, #3101 - 4464 Markham Street, Victoria, BC, Canada, V8Z 7X8
[2]Jewish General Hospital, Segal Cancer Center, Department of Oncology, McGill University, Montreal, QC, Canada
[3]Natural and Medical Sciences Institute (NMI) at the University of Tübingen, Reutlingen, Germany

Targeted treatment of colorectal cancer (CRC) only works in a minority of patients, and reliable methods to quantify signaling pathway activity are lacking. We therefore set out to develop immuno-MALDI (iMALDI) assays combined with on our phosphatase-based phosphopeptide (PPQ) quantitation approach to determine expression levels and stoichiometry of critical phosphorylation sites in Akt1 (P31749) and Akt2 (P31751) in cancer cells and tumors using a Bruker Microflex LRF benchtop MALDI-TOF instrument.

We have developed iMALDI assays to quantify tryptic non-phosphorylated Akt1 and Akt2 peptides (aa 466-480, and aa 468-481, respectively) within a linear range of 0.5 – 10 fmol from 10 µg lysate protein. We were able to quantify expression levels and phosphorylation stoichiometry of Akt1 and Akt2 from 10 µg protein of parental (Akt1: 3.4 ±0.3 fmol; Akt2: 2.3 ±0.04 fmol) and EGF-induced (Akt1: 3.9 ±0.1 fmol ; Akt2: 2.8 ±0.1 fmol) MDA-231 breast cancer cell lysates. As expected, EGF-induced cells showed increased phosphorylation stoichiometry (Akt1: 12.7 ±3.3%; Akt2: 19.8 ±5.2%) than parental cells (Akt1: 3.5 ±8.2%; Akt2: 4.9 ±7.5%). In addition, we showed the applicability of this method to measure cancer tissue lysates by determining expression levels and phosphorylation degree for so far two surgical breast cancer tissue samples.

In conclusion, we have developed iMALDI Akt1 and Akt2 assays for quantitation of expression levels and phosphorylation stoichiometry in cancer tissue samples. Next steps are optimization of precision, including automation of all liquid handling steps on an Agilent Bravo, full assay validation, and extension of biological samples to CRC tissue lysates.

Studying protein-small molecule affinities by quantitative proteomics

H. Christian Eberl

Cellzome GmbH, a GSK Company, Meyerhofstrasse 1, 69117 Heidelberg, Germany

The characterization of small molecule – protein interactions is one of the central challenges in the drug discovery process. This includes the identification of the primary target of a bioactive small molecule but also of off-targets related to adverse drug events. Chemical proteomics technologies enable to quantitatively assess compound selectivity against the proteome under (close to) physiological conditions.

In this course I will present two complementary approaches, affinity enrichment-based chemoproteomics and thermal proteome profiling, which enable unbiased target identification and affinity determination of small molecules to endogenous proteins. In chemoproteomics a small molecule is immobilized on a solid support to enable affinity enrichment of target proteins from cell extracts. When combined with quantitative mass spectrometry, dose-dependent competitive binding experiments with free compound enable the simultaneous determination of potency and selectivity for all detected targets. In thermal proteome profiling, drug-target interactions are inferred from changes in the thermal stability of a protein. These experiments are performed either in lysate or on live cells and thus enable monitoring of drug targets and downstream effectors on a proteome-wide scale.

Although focused on drug discovery, the approaches and principles presented here can be transferred to many other research areas.

Multiplexed protein quantification using peptide group-specific capture molecules and mass spectrometry

Oliver Poetz, Frederik Weiss, Bart van den Berg, Helen Hammer, Hannes Planatscher, Thomas O. Joos

NMI Natural and Medical Sciences Institute at the University of Tuebingen, Markwiesenstr. 55, 72770 Reutlingen, Germany

Mass spectrometry (MS) is an emerging tool for targeted protein quantification. However, usually a biological sample is too complex to be analyzed directly in an MS. Therefore the sample has to be pre-fractionated, which limits the sample through-put.

Within the last few years, various novel approaches that employ a targeted enrichment of a small subset of peptides have been integrated in the proteomics workflow to speed up the process. Sample preparation strategies that reduce the complexity of tryptic digests by using immunoaffinity based methods lead to a substantial increase in throughput and sensitivity (Zhang et al. 2003, Anderson et al. 2004).

One bottleneck in immunoaffinity based approaches is the availability of the appropriate peptide specific capture antibody. Here we present a strategy that uses short terminal specific antibodies – TXP Antibodies – designed for the enrichment of groups of peptides sharing the same terminal sequence (Poetz et al., 2009, Volk et al., 2012, Eisen et al. 2013, Weiss et al., 2015). Using these antibodies as affinity capture reagents subsets of hundreds of peptides with identical termini are enriched and a sensitive mass spectrometry read out allows the detection and quantification of these peptides.

We will present data for the application of such antibodies for the quantification of plasma proteins, membrane receptors and drug transforming proteins.

Online Biosensor-SPR-Mass Spectrometry: New Tool for Simultaneous Detection, Structure Determination and Affinity Quantification of Protein- Ligand Interactions

Michael Przybylski[1,2], Loredana Lupu[1], Stefan Slamnoiu[12], Adrian Moise[2], Camelia Vlad[2], Bastian Hengerer[3] , Mary Murphy[4] and Jeff Bornheim[4]

[1]Steinbeis Centre for Biopolymer Analysis & Biomedical Mass Spectrometry, 65428 Rüsselsheim, Germany
[2]University of Konstanz, Department of Chemistry, 78457 Konstanz, Germany
[3]Boehringer Ingelheim Pharma GmbH, ZNS Research Dept., Biberach, Germany
[4]Ametek-Reichert Technologies, Depew, NY 14043, USA.

Bioaffinity analysis using biosensors such as surface plasmon resonance (SPR) and surface acoustic wave (SAW) has become an established technique for the detection and quantification of biomolecular interactions. However, a principal limitation of biosensors is their lack of providing structure analysis of affinity-bound ligands. We have developed a continuous, automated online biosensor-MS combination with electrospray ionization mass spectrometry (ESI-MS), using both SAW and SPR biosensors. Here we report the first online SPR-MS) combination that enables the simultaneous affinity capture/isolation, chemical structure determination and affinity quantification of protein ligands, dissociated from protein- ligand complexes on a gold chip. Key tool of the SPR-MS combination is a new automated interface that provides sample concentration and in-situ desalting for the MS analysis of the ligand eluate [1]. ESI-MS systems from several MS manufacturers can be coupled, using a in house developed software connection the MS-acquisition and biosensor operation. First applications of the online SPR-MS show broad analytical potential for direct interaction studies from biological material, as diverse as antigen-antibody and lectincarbohydrate complexes, with affinity binding constants (KD) from milli- to nanomolar ranges [2, 3]. Moreover, first applications of online- SPR-MS to the direct analysis of biological samples are shown, such as the "top-down" structural characterization of proteolytic intermediates and oligomers of Parkinson's Disease key protein, alphasynuclein (αSyn) [4] from brain homogenate. The broad application areas amenable with the online SPR-MS include affinity-based biomarker identification, identification of protein and peptide epitopes, precise antibody affinity determinations, and direct label-free antigen quantification.

[1] Slamnoiu, S. et al. (2014) **J.Am. Soc. Mass Spectrom.**, *25*, 1472-1481.

[2] Petre, A, et al. (2012) **J. Am. Soc. Mass Spectrom.**, *23*, 1831-11840.

[3] Moise, A., et al., (2011) **J. Am. Chem. Soc.**, *133*, 14844-14847.

[4] Vlad, C. et al., (2011) **ChemBiochem.**, *12*, 2740-2744.

Short CVs of the speakers of the "Affinity - Mass Spectrometry" workshop at HUPO 2015

Prof. Dr. Christoph Borchers

Director
University of Victoria - Genome BC Proteomics Centre
Vancouver Island Technology Park
3101-4464 Markham St
Victoria, BC, V8Z 7X8
CANADA

The UVic Genome BC Proteomics Centre is a state-of-the-art proteomics research facility located in the Vancouver Island Technical Park in Victoria, British Columbia, Canada. It is supported by a collaborative relationship between Genome BC and the University of Victoria. The Centre is a not-for-profit facility that operates on a cost recovery model. It is the longest operating proteomics core facility in Canada, having been in operation since 1982, and serves clients in academia, industry and government on a fee-for-service and collaborative basis.

The Proteomics Centre aims to develop novel technologies in and push the boundaries of current analytical capabilities. The Centre research activities include Clinical Proteomics, Quantitative Proteomics, Structural Proteomics, Protein Characterization, Metabolomics and Tissue Imaging, and Bioinformatics.

Dr. Hans Christian Eberl

Teamleader Discovery Biochemistry
RD Platform Technology & Science
Cellzome GmbH, a GSK Company
Meyerhofstrasse 1
69117 Heidelberg
Germany

Dr. Eberl studied Biochemistry at the Technical University in Munich, and received his MSc in Biochemistry at the Max-Planck-Institute for Biochemistry. During his PhD at the Max-Planck-Institute for Biochemistry in the group of Matthias Mann, Chris applied quantitative interaction proteomics to epigenetic questions. The application of unbiased proteomics enabled the identification of novel histone trimethyl-lysine readers and their associated complexes. Shortly after his PhD Chris joined the mass spectrometry group at Cellzome in early 2013 and became team leader of a proteomics group in late 2014. His current research focuses on optimization of chemoproteomics workflows and applying those for target deconvolution of novel drug candidates.

Prof. Dr. Anne-Claude Gingras

Director
Lunenfeld-Tanenbaum Research Institute
Mount Sinai Hospital
992A-600 University Ave,
Toronto, ON, M5G 1X5
CANADA

Anne-Claude Gingras received her PhD in 2001 from the Sonenberg lab at McGill University for her studies on signalling pathways regulating translational control. She did her postdoctoral training in the laboratory of Ruedi Aebersold at the Institute for Systems Biology in Seattle where she developed approaches to identify protein-protein interactions amongst signalling molecules using mass spectrometry. She joined the Lunenfeld-Tanenbaum Research Institute and the University of Toronto in 2005 where she is now a Senior Investigator, an Associate Professor, the Canada Research Chair in Functional Proteomics and the Lea Reichmann Chair in Cancer Proteomics. She co-directs the Network Biology Collaborative Centre, a Genome Canada Genomics Innovation Node at the Lunenfeld-Tanenbaum Research Institute.

Her lab focuses on the study of signalling pathways using systematic approaches and the development of quantitative proteomics technologies, both experimental and computational. In collaboration with Alexey Nesvizhskii (U Michigan) and Mike Tyers (IRIC, U Montreal), she has developed a series of software tools to store, track and analyse mass spectrometry data, including powerful tools to identify and visualize protein-protein interactions. Her current research interests include studying dynamic protein-protein interactions using data independent mass spectrometry acquisition and charting the spatial organization of proteins inside human cells.

Prof. Dr. Michael O. Glocker

Director
Proteome Center Rostock
University Rostock Medical Center and Natural Science Faculty
University of Rostock
Schillingallee 69
18059 Rostock
Germany

Prof. Dr. Michael O. Glocker is head of the Proteome Center Rostock which uses all „classical" methods for proteome research (nanoLC-MS/MS, 2-D gel electrophoresis, visualization / imaging methods, spot-picking, and in-gel proteolytic digest as well as MALDI mass spectrometric protein identification and sequencing, data base analyses, etc.) for the characterization of proteome signatures that are extracted from patient materials.

The Proteome Center Rostock is dedicated to perform clinical research investigations on polygenic diseases. Our aim is to elucidate molecular signatures, disease pathways, and structure- / function correlations in protein interaction networks using a comprehensive proteome research approach.

Main disease entities under study encompass autoimmune diseases, cancer, as well as metabolic disorders for which Standard Operation Procedures have been established. State-of-the-art expertise has been developed on Affinity Mass Spectrometry research as well as on the identification of epitopes from (auto)antigens by top-down (starting with the protein of choice) and bottom-up (starting with peptides) approaches.

The Proteome Center Rostock was founded in 1999 as a part of the University of Rostock and provides a modern scientific methodology and technology platform for improving rapidity and sensitivity of clinical proteome analysis - a challenge being set by the complexity of the human genome.

Dr. Petr Novák

Head of Laboratory of Structural Biology and Cell Signaling
Institute of Microbiology of the ASCR, v. v. i.
Videnska 1083
14220 Prague 4
Czech Republic

The Laboratory of Structural Biology and Cell Signaling covers two scientific fields including structural biology and cell signaling. The structural biology group produces recombinant proteins and characterizes the protein or protein-ligand structures and their dynamics utilizing advanced mass spectrometric techniques. On the other hand, the cell signaling group, studies the connections of cellular signaling with the metabolism of cancer cells by conventional molecular biology and biochemistry methods. Through the close collaboration of the two groups a unique research platform is formed, wherein the results obtained by the study of biological systems can also be verified and explained at the molecular level.

Current research interests are focused on characterization of proteins or protein/ligand structures and their dynamics by using advanced mass spectrometry including chemical cross-linking, hydrogen-deuterium exchange, covalent labeling and ion mobility. The influence of the chemical stress on the induction of senescence, apoptosis, necrosis in cancer cells and monitoring of metabolic turnover are

now comprehensively studied using immunochemistry, real-time quantitative PCR, RNA interference, chromatin immunoprecipitation, quantitative proteomics and metabolomics.

Dr. Oliver Poetz

Head of Protein Analytics Department
NMI Natural and Medical Sciences Institute at the University of Tuebingen
Markwiesenstr. 55
72770 Reutlingen
Germany

Dr. Oliver Poetz is biochemist and has been working with the Natural and Medical Sciences Institute at the University of Tuebingen since 2002. His research has focused on the development of methodologies for multiplexed antibody-based protein assays. Since the format of such assays requires antibodies, Dr. Poetz has gained strong expertise in assay development, antibody generation and characterizsation. Over the last ten years he has established more than 50 sandwich immunoassays targeting plasma proteins and cell signaling proteins.

Furthermore, his group is developing a generic strategy for MS-based immunoassays. Immunoaffinity enrichment of proteotypic peptides coupled with mass spectrometry is a highly specific and sensitive approach for determining the absolute concentration of proteins from complex mixtures. However, the "one antibody – one analyte" approach requires a high number of specifically generated binding reagents, thereby limiting the use of this concept for a proteome-wide scale. He addresses this problem by generating antibodies that recognize short c-terminal epitopes. These antibodies are thus capable of simultaneously enriching multiple peptides. This "one antibody – multiple analyte" strategy substantially reduces the number of antibodies required for a proteome scale, while high sensitivity can still be obtained. He was given the GO-Bio award for a research project in which he uses this strategy to develop and commercialize immunoassays capable of detecting toxicologically relevant biomarkers across species.

Prof. Dr. Michael Przybylski

CEO
Steinbeis Centre Biopolymer Analysis and Biomedical Mass Spectrometry
Bahnhofsplatz 1
65428 Rüsselsheim
Germany

The development of efficient "soft ionisation" methods in the last years has provided the basis for the molecular characterisation of biopolymers by mass spectrometry. In contrast to previous limitations in the molecular weight range amenable, electrospray ionisation (ESI-MS) and matrix assisted laser desorption ionisation (MALDI-MS) have provided access to biopolymers >> 100 kDa. The recent development of Fourier transform ion cyclotron resonance (FTICR) mass spectrometry enabled a breakthrough for the ultra-high resolution mass spectrometric analysis of biopolymers using both ESI and MALDI ionisation (A.G. Marshall, Mass Spectrom. Rev. 17, 1, 1998). Present studies in our laboratory on the analytical development of ESI-FTICR mass spectrometry focus on (i) the structure analysis of non-covalent supramolecular biopolymer complexes; (ii) the direct, high resolution mass spectrometry of peptide mixtures in proteome analysis and analysis of combinatorial mixtures; and (iii) the identification of antigen determinant structures of mono- and polyclonal antibodies using the mass spectrometric epitope mapping method developed in our laboratory (M. Przybylski, Adv. Mass Spectrom. 13, 275, 1995). For this new interdisciplinary DFG research programme, a 7 T Bruker FTICR MS instrument with Apollo electrospray and nano electrospray ionisation sources, micro LC-MS and CE-MS facilities, a new MALDI-MS ionisation source with IR-MPD equipment has been installed. New biochemical applications of FTICR-MS are the structure elucidation of cell surface proteins, the epitope elucidation of target antigens in auto-immune diseases and the characterisation of cell-specific proteins for apoptosis. In these studies FTICR-MS is providing new analytical perspectives to biochemical and cell-biological applications of biopolymer structure analysis.