Enabling Technologies

7th International Symposium on Enabling Technologies for Life Sciences

April 30 - May 1, 2013
Metro Toronto Convention Centre
255 Front Street West, Toronto, Canada

Chaired by

Dr. Daniel Figeys
University of Ottawa

Bringing Researchers Together

NOTES:

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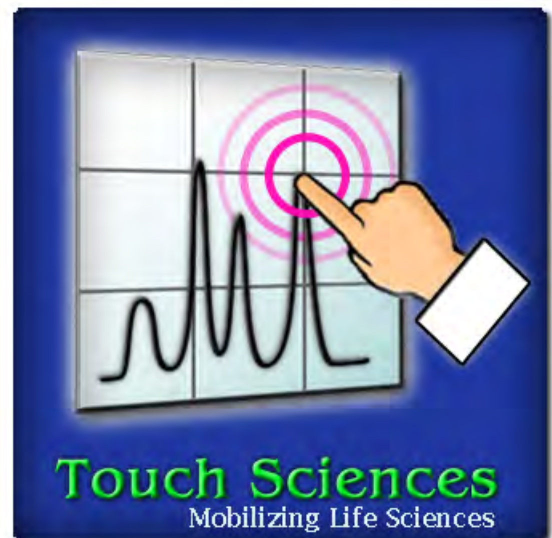

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Welcome

On behalf of the Organizing and Scientific Advisory Committees, I would like to welcome you to the 7th International Symposium on Enabling Technologies (ETP 2013). ETP Symposium has been fortunate, once again, to bring together an excellent slate of speakers representing a wide range of novel techniques that have or will have a major influence on research in the life sciences. We also have an impressive group of sponsors and exhibitors, and I encourage you to visit the exhibition area and find out more about their latest products.

The symposium offers several opportunities for discussions between attendees, speakers and sponsors. We urge everyone to take advantage of these chances to get together with others who share your scientific interests.

The ETP Symposium is held every two years in order to permit our participants to focus on their research and develop new technologies. As of 2011, we deviated from our normal course so that we may alter the emphasis from Proteomics to a more broad scope to meet the most recent advances of technology that impact the life sciences. If you have suggestions for topics to be included in future symposia, please let us know. We welcome all comments and suggestions.

Thank you for supporting the ETP Conferences.

Janette Champ
President
ETP Symposium Inc.

Organizing Committee

Dr. Daniel Figeys, Director, Institute of Systems Biology, University of Ottawa,
Symposium Chair
Dr. Robert Boyd, Researcher Emeritus, National Research Council of Canada
Chair ETP's Scientific Advisory Committee
Janette Champ, President, ETP Symposium Inc.

ETP Symposium Scientific Advisory Board

Ruedi Aebersold, Institute of Molecular Systems Biology, ETH Europe
Robert K. Boyd, Researcher Emeritus, National Research Council of Canada (Committee Chair)
Catherine Costello, Boston University School of Medicine, Mass Spectrometry Resource
Chris Dambrowitz, BioRefining Conversation Network, University of Alberta
Norman Dovichi, University of Notre Dame
Randy Johnston, Dept. of Biochemistry & Molecular Biology, University of Calgary

7th International Symposium on Enabling Technologies (ETP 2013)

AGENDA - Day 1

Tuesday, April 30 **Metro Toronto Convention Centre, Room 206, 255 Front Street West, Toronto**		
8:00 am	*Registration Opens*	
9:00 am - Morning Session Starts		
9:00 am	*Welcome*	Dr. Daniel Figeys, University of Ottawa - SYMPOSIUM CHAIR
9:10 am	*Recent development of technologies and methods for proteome analysis*	Dr. Fangjun Wang, Dalian Institute of Chemical Physics **Sponsored by The China-Ontario Bioanalytical Consortium**
9:40 am	*A novel mammalian split-ubiquitin proteomics approach as a tool for functional investigation of signaling pathways in human cells*	Dr. Bella Groisman, University of Toronto
10:00 am	*Characterization of Cell Membrane N-Glycosylation with Integrated Hydrophilic Interaction Chromatography Solid Phase Extraction and LC-MS/MS*	Dr. Rui Chen, University of Ottawa
10:20 - TECH TALK - Personal Care Products & Pharmaceuticals in Water by LC-TOF - Sponsored by PerkinElmer Health Sciences Canada Inc. **10:20 am Coffee Break - Poster Viewing & Exhibitors**		
10:40 am	*Disposable two-stage spin cartridges for protein purification in a top-down proteomics workflow*	Mr. Andrew Crowell, Dalhousie University
11:00 am	*Exploring bi-directional signaling in pancreatic tumor microenvironment by quantitative proteomics*	Dr. Ruijun Tian, The Samuel Lunenfeld Research Institute
11:20 am	*Antibody colocalization microarray (ACM): A Scalable Cross-reactivity free Nano-ELISA platform for proteomics studies*	Dr. David Juncker, McGill University
11:40 pm - Morning Sessions Ends		
Free Time		
1:00 pm - Afternoon Session Starts		
1:00 pm	*Introduction*	Dr. Daniel Figeys, University of Ottawa - SYMPOSIUM CHAIR
Keynote Lecture		
1:05 pm	*Capillary Electrophoresis for High Throughput Proteomics*	Dr. Norm Dovichi, University of Notre Dame
1:40 pm	*Imaging MALDI MS/MS of Microarrays as a Platform for High Throughput Biomaterials Discovery*	Dr. John Brennan, McMaster University
2:10 pm	*New MS tools for the discovery and characterization of protein-carbohydrate interactions*	Dr. John Klassen, University of Alberta, **Sponsored by Waters Corporation**
2:40 pm	*Trimethylation Enhancement using Diazomethane (TrEnDi): Rapid On-Column Methylation of Peptides and Proteins to Permit Quantitative Analysis Using Tandem Mass Spectrometry*	Dr. Jeffrey Smith, Carleton University
3:00 pm Coffee Break - Sponsored by Thermo Fisher - Poster Viewing & Exhibits		
3:15 pm	*Quantitative proteomics using FAIMS and isotopic labeling*	Dr. Pierre Thibault, Université de Montréal, **Sponsored by Thermo Fisher Scientific**
3:45 pm	*Matrix Assisted Ionization without a Laser, Heat, or Voltage: Identification of Peptides and Proteins Directly from Tissue*	Dr. Sarah Trimpin, Wayne State University
Ken Standing Award Presentation ***Sponsored in part by University of Manitoba***		
4:15 pm	*Electrospray Mass Spectrometry as a Readout of Protein Structure and Function*	Dr. Lars Konermann, University of Western Ontario
The Bill Davidson Graduate Student Travel Award - Presentation ***Sponsored by AB SCIEX*** *Presented by Dr. Bruce Thomson*		
4:45 pm	*Dynamic DNA Assemblies Mediated by Binding-Induced DNA Strand Displacement*	Mr. Feng Li, University of Alberta
GALA RECEPTION ***In Honour of the Late Bill Davidson*** **5:15 - 7:00 pm Reception & Poster Session** ***music provided by Dick Felix Jazz Duo*** **sponsored in part by IONICS**		

7th International Symposium on Enabling Technologies (ETP 2013)

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AGENDA - Day 2

Wednesday, May 1, 2013 **Metro Toronto Convention Centre, Room 206, 255 Front Street West, Toronto**		
8:00 am	Morning Coffee, with Exhibitors	
8:30 am Conference Begins - Day 2		
8:30 am	*New methods for improved determination of metabolic markers using advanced analytical mass spectrometry techniques*	Dr. Dietrich Volmer, Institute for Bioanalytical Chemistry, Universität des Saarlandes
9:00 am	*Resolution of solution structures in the absence of solvent*	Dr. Perdita Barran, The University of Edinburgh
9:30 am	*Platform development to monitor age- and oxidant-related protein post-translational modifications: The case of copper-zinc superoxide dismutase*	Dr. Ann English, Concordia University
10:00 am	*High accuracy mass spectrometry provides direct evidence for site-specific auto-ADP-ribosylation of PARP-1*	Dr. Guy Poirier, Laval University
10:20 am Coffee Break - Poster Viewing & Exhibits		
10:40 am	*Microfluidics-Enabled, Sub-second Hydrogen/Deuterium Exchange Pulse Labeling Reveals Allosteric 'Hotspots' in Enzymes.*	Dr. Derek Wilson, York University
11:00 am	*Multi-Nano ESI, in pursuit of enhanced robustness, stability and sensitivity.*	Dr. Richard Oleschuk, Queen's University
11:30 pm	*Space Charge Effects in Linear Quadrupole Ion Traps with Mass Selective Axial Ejection*	Dr. Donald Douglas, University of British Columbia
12:00 - 1:00 pm - Lunch - Free Time **Lunch Workshop presented by *Bruker (Must be preregistered)*** *Presentation - #1 - Ultrafast Statistical Profiling of myxobacteria natural products for rapid detection and identification of marker compounds* *Presentation - #2 - Top Down Proteomics: A Multi-Platform Approach*		
1:00 pm	*MS-based strategies for the elucidation of nucleic acid – ligand interactions*	Dr. Daniel Fabris, University at Albany
1:30 pm	*DNA-Mediated Binding Assays for Nucleic Acids and Proteins*	Dr. Chris Le, University of Alberta
2:00 pm	*Insights on Isotachophoresis Kinetics from Monitoring Non-uniform Motion of single DNA molecules*	Dr. Hailin Wang, State Key Laboratory of Environmental Chemistry & Ecotoxicology, CAS **Sponsored by The China-Ontario Bioanalytical Consortium**
2:30 pm	*Mass Spectrometry Methods for Characterization and Determination of Disinfection Byproducts in Tap Water and Swimming Pools*	Dr. XingFang Li, University of Alberta
2:50 pm Coffee Break - Exhibits		
3:00 pm	*Automated SDS removal, tryptic digestion and sample cleanup in a disposable two-stage spin cartridge*	Dr. Alan Doucette, Dalhousie University
3:20 pm	*Clearing the Biomarker Barrier: Moving Biomarkers from the Bench to the Bedside*	Dr. David Wishart, University of Alberta
3:50 pm	*False Negatives in Proteomics*	Dr. Ronald Beavis, Beavis Informatics Ltd.
4:20 pm	*Linking protein composition and functionality in biology and medicine*	Dr. John Wilkins, University of Manitoba
Keynote Lecture		
4:50 pm	*Using synthetic biology to study protein signaling networks*	Dr. Anthony Pawson, Mount Sinai Hospital
5:30 pm Conference Ends		

Symposium Chair

Daniel Figeys
Ontario Institute of Systems Biology
University of Ottawa

Daniel Figeys is a professor in the Department of Biochemistry, Microbiology and Immunology at the University of Ottawa. He is also the Director of the Ottawa Institute of Systems Biology, and a Tier-1 Canada Research Chair in proteomics and systems biology. Daniel obtained a B.Sc. and a M.Sc. in chemistry from the Université de Montréal. He obtained a Ph.D. in chemistry from the University of Alberta and did his postdoctoral studies at the University of Washington. His laboratory has published over 110 papers and has been cited over 6000 times.

Research Interest:

Our laboratory focuses on the development and application of proteomics and lipidomics technology. For example, we created a device, called the Proteomic Reactor that allows the concentration and digestion of proteins in 60nl of volume. We are using the technologies developed in our laboratory to study biological processes. For example, we study PCSK9 and its impact on liver cells, the Proteome and lipidome in neuronal diseases, and more recently, we have been working on the proteome of inflammatory bowel diseases.

Invited Speakers

Perdita E. Barran
The University of Edinburgh

BIOGRAPHY:

Perdita E. Barran is currently a Reader in Biophysical Chemistry at the University of Edinburgh. She graduated from Manchester University with a degree in Chemistry with Industrial Experience (1994), and from Sussex University with a PhD in Chemical Physics (1998) under the supervision of Professors Tony Stace and Sir Harry Kroto. She worked as a post-doctoral researcher for Tony Stace, before moving to the University of California Santa Barbara to work with Mike Bowers. She was awarded an EPSRC Advanced Research Fellowship in 2002 which allowed her to commence independent research at Edinburgh University in 2003.

ABSTRACT:

Resolution of solution structures in the absence of solvent

Gentle application of nano-electrospray to proteins buffered in solution to an appropriate pH allows us to use mass spectrometry to interrogate their solution conformations. This can be achieved directly with ion mobility mass spectrometry, where we measure the rotationally averaged temperature dependent collision cross section of mass separated ions, or more indirectly with the use of dissociation methods. Data obtained from both methods provides insight to the structure and stability of the protein, and also detail on its interactions, especially when combined with atomistically resolved data from crystallography and or computational approaches.

We will here illustrate the power (and pitfalls) of using mass spectrometry to analyse protein structure. We will use the metamorphic protein lymphotactin and the transcription factors p53 and c-MYC to illustrate these techniques.

Ronald Beavis
Beavis Informatics Ltd.

BIOGRAPHY:

Ron Beavis has been involved in the application of mass spectrometry to protein chemistry and biology since 1981, when he joined Ken Standing's Lab at the University of Manitoba. He has held various positions in academia and industry. He is currently interested in the practical uses of very large collections of heterogeneous proteomics data and information.

ABSTRACT:

False Negatives in Proteomics

Proteomics experiments using tandem mass spectrometry to identify peptides and proteins are prone to various types of false negative identifications that can affect the utility of the information generated from the experiments, as well as their reproducibility. This talk will discuss the classification of the most common types of false negatives as well as how they can be detected, remedied or avoided.

John D. Brennan
McMaster University

BIOGRAPHY:

John D. Brennan obtained his Ph.D. (1993) in analytical chemistry from the University of Toronto and performed postdoctoral studies in biophysics at the National Research Council of Canada (Institute for Biological Sciences). In 1995 he joined the Department of Chemistry at Brock University in Canada, and moved to his current position at McMaster University in 1998, where he is currently a Professor in the Department of Chemistry & Chemical Biology, Director of the Biointerfaces Institute and holder of the Canada Research Chair in Bioanalytical Chemistry and Biointerfaces. He is an expert in fluorescence spectroscopy, LC/MS, sol-gel chemistry and protein immobilization. Dr. Brennan is an associate editor for Trends. Anal. Chem. and on the editorial advisory boards of Analytical Chemistry and J. Sol-Gel Sci. Technol. His research team develops bioanalytical instrumentation in the area of biosensors, bioaffinity chromatography media, microarrays and mass spectrometry-based drug screening. Dr. Brennan has supervised over 80 trainees, published over 140 peer-reviewed papers, and has several patents in the areas of sol-gel materials, protein immobilization, high throughput screening methods and bioactive paper.

ABSTRACT:

Imaging MALDI MS/MS of Microarrays as a Platform for High Throughput Biomaterials Discovery

Blake J. Helka, Elna D. Luckham and John D. Brennan*

Department of Chemistry & Chemical Biology, McMaster University, Hamilton, ON, L8S 4M1

This presentation will highlight recent work within the Biointerfaces Institute at McMaster University in the area of high throughput screening of biomaterials, with particular emphasis on the use of imaging MALDI for characterization of biomaterial microarrays. Using robotic handling systems and a combination of contact and noncontact microarray printing, we are able to produce several thousand biomaterials per day with a wide range of chemical compositions. Using sol-gel based materials as an example, the presentation will show the workflow utilized to develop new bioactive sol-gel materials for biosensing and small molecule screening applications. This includes methods to produce several thousand materials very rapidly, tools for rapid screening to identify "hits" that show a desired property (high biological activity, low non-specific binding, etc.), and further detailed material analysis using a range of imaging methods based on fluorescence, XPS, MALDI-MS/MS and SPR to fully characterize the properties of biologically active materials. In particular, this presentation will emphasize the unique capabilities of MALDI-MS/MS for evaluating biomaterial properties and the interaction of materials with biological samples. Methods for cataloging, mining and analyzing large datasets within the Database of Biointerface Interactions will also be discussed.

Donald Douglas
University of British Columbia

BIOGRAPHY:

Don Douglas currently holds the NSERC AB Sciex Industrial Research Chair at the University of British Columbia. He received his B. Sc. from McMaster University and his Ph.D. in physical chemistry from the University of Toronto. Following two years as a post-doctoral fellow at the University of California, Berkeley, and a year at the National Research Council in Ottawa, he joined Sciex as a Research Scientist in 1979. At Sciex he was involved in the development of the first commercial triple quadrupole system and had responsibility for developing the first commercial inductively coupled plasma mass spectrometer system. He then developed Sciex's first bench-top triple quadrupole system. In 1995 he moved to the University of British Columbia. At UBC he continued research into new instrumentation for mass spectrometry and also research concerning the properties of gas-phase proteins, particularly noncovalent complexes. Much of his work has centered on new methods of using linear quadrupoles as mass filters and as linear ion traps. He has published over 100 refereed scientific articles and is an inventor on 21 patents and patent disclosures

ABSTRACT:

Space Charge Effects in Linear Quadrupole Ion Traps with Mass Selective Axial Ejection

D. J. Douglas,[1] H, Qiao,[2] C. Gao[1], K. Kim[1] and N. Konenkov[3]

1. Department of Chemistry, University of British Columbia, Vancouver, BC, Canada
2. Current address, Ionics Mass Spectrometry Group, Bolton, ON, Canada
3. Physical and Mathematical Department, Ryazan State University, Ryazan, Russia

Experiments and ion trajectory calculations have been used to better understand and to reduce space charge effects in linear quadrupole ion traps with axial ejection. If too many ions are stored in a linear trap, their mutual repulsion counteracts the trapping forces and the ion oscillation frequencies decrease. Consequently higher trapping radio frequency voltages are required to bring ions into resonance for ejection, and ions appear at higher apparent masses in a spectrum. At the same time the mass resolution decreases. For the experiments of interest here, the mass shifts are about 1 at m/z 609, and the electric field from space charge is a small perturbation to the trapping field. Experiments show that a proper choice of operating conditions can reduce but not eliminate mass shifts. Trajectory calculations of ion motion with various models of the ion cloud provide additional insight, and show that the space charge field is nonlinear and that therefore the ion oscillation frequency depends on oscillation amplitude. Anything that expands the ion cloud produces lower electric fields form space charge, and hence lower mass shifts. Experiments, in progress, show that using broadband excitation, dipole dc, or two-frequency excitation to expand the ion cloud gives modest reductions in mass shifts and improvements in resolution.

PLENARY LECTURE:

Norman Dovichi
University of Notre Dame

BIOGRAPHY:

Norman Dovichi holds the Grace-Rupley Professorship in the department of Chemistry and Biochemistry at the University of Notre Dame. He received his BSc with a dual major in Chemistry and Mathematics from Northern Illinois University and his PhD in Physical Analytical Chemistry from the University of Utah, where he was Joel Harris's first PhD student. He spent two years at Los Alamos Scientific Laboratory with Dick Keller. Since then he has held faculty positions at the Universities of Wyoming, Alberta, and Washington before taking his current position at Notre Dame.

Dovichi has graduated 57 PhD students, has published over 250 papers, holds seven US patents, and has given over 350 invited talks. He has served on the editorial advisory boards of 16 journals and now serves as Associate Editor for Analytical Chemistry. He holds an honorary professorship with the Chinese Academy of Sciences.

Dovichi has primarily focused his research on the use of capillary electrophoresis and ultrasensitive laser-induced fluorescence for analysis of minute amounts of biological molecules. In the 1980s, he introduced the concept of single molecule detection to the chemical literature. In the 1990s, his group employed that technology to measure the activity and activation energy of single enzyme molecules. His group also developed capillary array electrophoresis instruments for high-throughput DNA sequencing. This technology was patented and commercialized as the Applied Biosystems model 3700 DNA sequencer. He was recognized for this work by the journal Science as an "Unsung Hero of the Human Genome Project".

More recently, his group has focused its attention on coupling capillary with tandem mass spectrometry as a tool for high throughput and high sensitivity proteomics. Recent results include detection of 1,250 peptides in a 50-min single-shot separation of the E. coli protoeme, detection of 5,000 peptides in a 350 min analysis of a set of fractions from the E. coli proteome, and quantification of 1,400 protein groups in the 8-plex iTRAQ analysis of the differentiation of PC12 cells following treatment with nerve growth factor.

ABSTRACT:

Capillary Electrophoresis for High Throughput Proteomics

Most protomics studies employ LC-ESI-MS/MS analysis of peptides. We are investigating capillary electrophoresis (CE)-ESI-MS/MS as an alternative technology for bottom-up proteomics. We first developed a rugged and sensitive CE-ESI interface based on electrokinetically-pumped sheath-flow (1). This interface operates in the nanospray domain, produces low-attomole detection limits for capillary electrophoresis separation of peptides, and offers great flexibility in separation buffer conditions (1-2). We then analyzed the secreted protein fraction of M. marinum (3). We pre-fractionated the secretome to produce simpler samples that were better suited to the separation performance of capillary zone electrophoresis (CZE). The results of the analysis of 12 fractions were compared with conventional UPLC analysis of the unfractionated sample. CZE produced slightly more protein identifications in a slightly shorter time period than UPLC. 140 protein groups were identified by CZE-ESI-MS/MS in three hours from this sample. We have recently improved the system. In the single-shot analysis of the E coli proteome, we identified >1,300 peptides and >300 protein groups in a 50-min CZE separation. We have employed this separation system to analyze seven fractions from the E. coli

proteome (4). This system produced 23,706 peptide spectra matches, 4,902 peptide IDs, and 871 protein group IDs in 350 min analysis time. In an alternative separations scheme, we employed capillary isoelectric focusing for the analysis of host-cell proteins in commercial biopharma products; this system identified 37 host cell proteins in a total sample preparation time of 4 hours (6).

1. Wojcik et al. Rapid Commun Mass Spectrom. 2010; 24: 2554-60
2. Wojcik et al. Talanta. 2012;88:324-9
3. Li et al. Anal Chem. 2012; 84: 1617-22
4. Unpublished
5. Li et al. Anal Chem. 2012; 84:

Ann English
University of Concordia

BIOGRAPHY:

Ann English received her BSc from University College Dublin and PhD from McGill University in Montreal followed by a NSERC post-doc at the California Institute of Technology. She has risen through the professorial ranks at Concordia University where she is currently Professor of Chemistry and Biochemistry. She holds a Concordia University Research Chair in Bioinorganic Chemistry and is the founding Director of the Centre for Biological Applications of Mass Spectrometry.

Research in her group combines inorganic chemistry, biochemistry, computational chemistry, mass spectrometry, microscopy, and molecular biology to understand redox processes critical in cell signaling, especially those mediated by metalloproteins and small inorganic hormones such as hydrogen peroxide and the oxides of nitrogen (NOx). Her group is actively investigating the chemical physiology of peroxide and NOx redox signaling in red blood cells and yeast. Use of these physiologically well characterized cells as chemical test tubes is shedding new light on the biochemistry of on vasodilation and peroxide-modified protein function. Their work, which has been published in over 100 peer-reviewed publications and presented in over 80 invited lectures nationally and internationally, has received funding from NSERC, CIHR, and the private sector

ABSTRACT:

Platform development to monitor age- and oxidant-related protein post-translational modifications: The case of copper-zinc superoxide dismutase

Non-enzymatic protein post-translational modifications (PTMs) increase with age and are frequently associated with age-dependent diseases. For example, copper-zinc superoxide dismutase (CuZnSOD) aggregation has been reported in patients with late onset neurodegenerative diseases such as sporadic or familial Amyotrophic Lateral Sclerosis (ALS) and Alzheimer's. Non-enzymatic PTMs in CuZnSOD are linked to its aggregation and hence to the development of ALS and Alzheimer's. Yeast is a widely used model of cellular and organismal aging that can be readily exploited in platforms to monitor age-related PTMs in a cellular environment. Furthermore, yeast and human CuZnSODs share over 90% sequence homology but since yeast age much faster, we are exploring age-related PTMs in the yeast enzyme. On gel filtration of cell lysates we isolated a high-mass inactive form of CuZnSOD, and found by FT-MS that it is oxidized at cysteine 146 (C146) to the sulfonic acid. Importantly, C146 is also found in the sulfonic acid form in CuZnSOD isolated from the brains of Alzheimer's patients(1) and the C146R mutation has been reported in familial ALS.(2) Notably, C146 forms a C146-C55 disulfide bridge that is critical in stabilizing the native CuZnSOD homodimer. In high-mass CuZnSOD, we additionally observe oxidation of histidine 71 (H71) and H120, which ligate the active-site zinc and catalytic copper, respectively. We hypothesize that age-induced oxidation of C146, H71, and H120 contribute to the development of ALS as well as Alzheimer's by releasing the redox-active catalytic copper, which would likely increase oxidative stress and CuZnSOD aggregation. By monitoring the GFP (green fluorescent protein) fluorescence of CuZnSOD-GFP from an isogenic yeast strain expressing this fusion protein, we note that the cellular content of high-mass CuZnSOD-GFP increases ~10-fold from day 3 to day 7. We are currently characterizing CuZnSOD and CuZnSOD-GFP from 15- and 20-day yeast to establish if aggregated CuZnSOD continues to accumulate in old cells. CuZnSOD activity also is known to be inhibited by inflammatory processes in the airway epithelium and C146 oxidation was reported in CuZnSOD purified from the erythrocytes of asthmatic patients.(3) To mimic the inflammatory state of asthma we are examining modification of CuZnSOD after challenging yeast cells with H_2O_2. Our LC-MS platform that tracks modified proteins as yeast age will provide in-depth understanding at the molecular level that is critical towards understanding the functional significance of PTMs in the cellular context.

References:

1) Choi et al. (2005) J Biol Chem 280, 11648-55
Andersen et al. (2003)
2) Amyotroph Lateral Scler Other Motor Neuron Disord 4, 62-73

3) Ghosh et al. (2012) Antioxid Redox Signal, doi: 10.1089/ars.2012.4566

Daniele Fabris
The RNA Institute
University at Albany

BIOGRAPHY:

D. Fabris is Professor of Chemistry and Biological Sciences at the University at Albany, where is also a member of The RNA Institute. His research includes developing MS-based technologies for the investigation of the structure-function relationships of nucleic acids in retroviral systems. In particular, he has developed approaches that combine foot printing and cross linking probes with high-resolution MS detection to solve the 3D structure of noncoding nucleic acids that are not directly amenable to traditional techniques. His interests include also the application of novel top-down strategies for elucidating the interactions of nucleic acids with proteins, small molecule ligands, and metals.

ABSTRACT:

MS-based strategies for the elucidation of nucleic acid – ligand interactions

Ribozymes and riboswitches have keenly demonstrated that the function of nucleic acids is not always linked to the genetic information coded in their sequence, but can also depend on their 3D structure and ability to interact with a variety of species present in the cell. Owing to the versatility afforded by mass spectrometry (MS) in RNA analysis, this platform is rapidly assuming a prominent role in the investigation of structure-function relationships, which is realized by supporting the full characterization of natural and man-made RNAs, as well as the elucidation of specific interactions with cognate nucleic acids, proteins, metals, and small molecule ligands. The talk will illustrate strategies for accessing functional information for nucleic acid complexes and discuss possible hurdles and experimental pitfalls. Examples will be provided in which MS-based techniques are employed to characterize relevant interactions that could represent new therapeutic targets. Other examples will illustrate approaches for elucidating the effects of metals and common nucleic acid ligands on such interactions. The experimental design necessary to assess the strength of binding/ inhibition in either comparative or absolute fashion will be also discussed. The potential afforded by ion mobility spectrometry (IMS) techniques will be evaluated in the investigation of possible conformational effects associated with ligand binding. Owing to the extremely low sample consumption and high speed of analysis, these capabilities will be expected to greatly increase the utilization of MS-based approaches in drug discovery and therapeutics development based on specific nucleic acid interactions.

John Klassen
University of Alberta

BIOGRAPHY:

John S. Klassen received a B.Sc (Honours) in chemistry from Queen's University in 1991. He pursued his doctoral research in the area of gas-phase ion chemistry under the supervision of Paul Kebarle at the University of Alberta and received his PhD in 1996. He spent the following year as a NSERC postdoctoral fellow at the University of California at Berkeley in the lab of Evan Williams. In 1998 he returned to University of Alberta as an Assistant Professor in the Department of Chemistry. He was promoted to Associate Professor in 2004 and to Full Professor in 2008. In September 2004 he became a Principal Investigator in the Alberta Glycomics Centre. His research focusses on the development and application of mass spectrometry methods to characterize non-covalent protein complexes, with an emphasis on protein-carbohydrate interactions. His contributions to the fields of mass spectrometry and analytical chemistry have been recognized with an American Society for Mass Spectrometry Research Award (2000), the Canadian Society for Mass Spectrometry Award (2004), a Petro-Canada Young Innovator Award (2004) and the F.P. Lossing Award for distinguished contributions to mass spectrometry in Canada (2011). In 2012, he was a co-recipient of NSERC's Brockhouse Canada Prize for Interdisciplinary Research in Science and Engineering.

ABSTRACT:

New MS tools for the discovery and characterization of protein-carbohydrate interactions

The direct electrospray ionization mass spectrometry (ESI-MS) assay has emerged as a powerful tool for quantifying protein-ligand interactions in solution. The assay is based on the direct detection and quantification of free and ligand-bound protein ions by ESI-MS for solutions of known initial concentrations of protein and ligand. A brief overview of the ESI-MS assay will be presented, along with recent methodological advances

that overcome the major sources of error in the binding measurements. A high-throughput ESI-MS approach to library screening will also be presented. The catch-and-release (CaR)-ESI-MS assay involves incubating a protein with a library of compounds in solution, detecting the protein-ligand complexes by ESI-MS, activating the complexes to release the ligand. The identities of ligands are determined from measured molecular weights and, if needed, the fragmentation spectra and ion mobility arrival time distributions. The CaR-ESI-MS assay allows for the sensitive, rapid (<1 min analysis time) and direct detection and quantification of specific interactions for libraries containing hundreds of compounds. An overview of the assay will be presented followed by examples highlighting the application of the assay for the discovery of carbohydrate ligands for a variety of bacterial proteins. Finally, the extension of the CaR-ESI-MS assay for the discovery of host cell receptors will be described. The assay involves direct ESI-MS analysis of aqueous solutions of soluble proteins and insoluble receptors, which are incorporated into nanodiscs. The application of this assay for the detection of interactions between bacterial toxins and glycosphingolipids will be presented.

X. Chris Le
Distinguished University Professor
Canada Research Chair in Bio-Analytical Technology and Environmental Health
Director, Division of Analytical and Environmental Toxicology,
University of Alberta

BIOGRAPHY:

Dr. Le holds an inaugural Canada Research Chair in Bio-Analytical Technology and Environmental Health. He is Distinguished University Professor, jointly appointed in the Departments of Laboratory Medicine and Pathology, Chemistry, and Public Health Sciences. He is the Director of Analytical and Environmental Toxicology Division and Vice-Chair (Research) in the Department of Laboratory Medicine and Pathology. He is an elected Fellow of the Royal Society of Canada, Academy of Science.

Dr. Le has published 200 peer-reviewed research articles in the area of bioanalytical chemistry, DNA damage and repair, environmental health sciences, and ultrasensitive assays for biomolecules and environmental contaminants. His achievements have been recognized with numerous teaching and research awards, such as an Award for Excellence in Mentoring (2009) "to recognize outstanding performance in the mentoring of students", the Maxxam Award (2011) to recognize his "distinguished contribution to the field of analytical chemistry", and the Environment Research and Development Award (2011) "for distinguished contributions to research and/or development in the fields of environmental chemistry or environmental chemical engineering".

ABSTRACT:

DNA-Mediated Binding Assays for Nucleic Acids and Proteins.

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The detection of nucleic acids and proteins is fundamental for studying their functions and for the development of molecular diagnostics. Determining these biomolecules in complex systems requires exquisite analytical specificity and sensitivity. To meet these requirements, we have devoted much effort to affinity binding assays that incorporate target recognition, signal transduction, and in-situ amplification. In particular, we focus on homogeneous binding assays, which are carried out in solution, without the need for separation, immobilization, or washing steps. Such homogeneous binding assays can be performed in a single tube/vial or in live cells. They are potentially applicable to point-of-care diagnostics. For example, a binding-induced DNA assembly enables ultrasensitive detection of molecular targets and construction of unique target-dependent nanoreactors. Two DNA motifs that are conjugated to specific affinity probes assemble preferentially only when a specific target triggers a binding event. The binding-induced assembly of the DNA motifs results in the formation of a highly stable hairpin structure, enabling effective differentiation of the target-specific assembly from the background. This strategy pushes the limit of the state-of-art dynamic DNA nanotechnology to include molecules beyond DNA. The ability to generate dynamic DNA assembly by non-DNA molecules opens up opportunities for diverse potential applications, ranging from construction of protein-induced nanodevices, to the in situ detection of proteins, and studies of molecular interactions.

Richard Oleschuk
Queens University

BIOGRAPHY:

Richard Oleschuk obtained his B.Sc.H. (1994) and Ph.D. (1998) from the Department of Chemistry at the University of Manitoba. His doctoral studies with Arthur Chow involved researching both polymer based extraction methods for metal complexes and membrane sample preparation methods for mass spectrometry. In 1998, Richard joined Jed Harrison's laboratory at the University of Alberta as an NSERC Postdoctoral Fellow. In 2000 Richard joined the department of Chemistry at Queen's University where his research has focused on analytical techniques and methodologies that are "stingy with sample", i.e. they utilize a combination of capillary and microfluidic chip based systems mated with high sensitivity detection techniques for chemical analysis. Richard has taken some original and very innovative approaches to tackle problems relevant to the chemical and biochemical analysis community. In particular his laboratory has produced several outstanding examples of microfludics for fast and sensitive protein separations. One of the highlights of his research accomplishments includes the coupling of capillary and microfluidic devices with mass spectrometry utilizing nanoelectrospray ionization. Nanoelectrospray is a very sensitive technique often used by proteomic practitioners who generally have very little sample to work with. Dr. Oleschuk's group has developed novel ways to generate electrospray that circumvent some of the nagging robustness concerns held by those utilizing nanoelectrospray. His group has developed emitters that utilize porous polymeric materials, entrapped microspheres and microstructured optical fibres to generate stable nanoelectrospray from capillary and microfluidic devices which has attracted significant commercial interest. Recently his group has also focused upon the use of microstructured optical fibres in analytical (electrophoresis and chromatography) and materials (templated synthesis) chemistry applications.

ABSTRACT:

Multi-Nano ESI, in pursuit of enhanced robustness, stability and sensitivity.

Electrospray ionization (ESI) as a soft ionization technique, has been critical to mass spectrometry for analyzing biological macromolecules. NanoESI which is operated at lower flow rates (nL/min) exhibits both higher ionization efficiency and sensitivity since the smaller initial electrospray droplets from the emitter afford increased surface charge per analyte molecule. To improve sensitivity and signal without decreasing flow rate, multiple electrosprays have been generated from multiple emitters by splitting one larger flow into smaller flows in the nanoESI regime. Based upon theoretical prediction and experimental results, the measured spray current generated from an emitter producing multiple Taylor cones (Itotal) is enhanced relative to the spray current from a single emitter (Is) at the same overall flow rate by a factor of the square root of the number of emitters (n). We have developed a series of multi-channeled emitters based upon silica microstructured fibers (MSFs). A custom-designed MSF having 9 holes in a radial pattern was fabricated using a "stack and draw" technique, allowing multiple electrosprays from a single fiber. Polymer nozzles have been included in the design to enhance nanoESI performance by first forming a layer of polymer on the inner walls of each channel followed by etching with hydrofluoric acid to expose the nozzles. The protruding divinylbenzene nozzles produce true multi-nanoESI over a wide range of flow rates and mobile phase compositions.

PLENARY LECTURE:

Anthony Pawson
Mount Sinai Hospital

BIOGRAPHY:

Tony Pawson was an undergraduate at the University of Cambridge, England (1970-1973), and obtained his Ph.D. with Dr. Alan Smith at the Imperial Cancer Research Fund, University of London (King's College), working on retroviral gene expression. He undertook postdoctoral work at the University of California at Berkeley with G. Steven Martin (1976-1980), where he identified a variety of retroviral oncogene products, and provided early evidence for the role of tyrosine phosphorylation in malignant transformation. He moved to the University of British Columbia, Vancouver as an Assistant Professor in 1981, and then to the Samuel Lunenfeld Research Institute of Mt. Sinai Hospital, University of Toronto in 1985, where he was Director of Research (2000-2005). Over the last 25 years has explored the mechanisms through which modular protein-protein interactions control intracellular signaling pathways, and provide a general architecture for organizing cellular behaviour, building on his identification of the SH2 domain as the prototypic interaction module.

Tony Pawson is a University Professor of the University of Toronto, a Senior Scientist at the Samuel Lunenfeld Research Institute of Mt. Sinai Hospital, and a Senior Fellow of Massey College. He has received a number of awards, including the Gairdner Foundation International Award, the AACR/Pezcoller International Award for Cancer Research, the Heineken Prize for Biochemistry and Biophysics (Royal Netherlands Academy of Arts and Sciences), the Killam Prize for Health Sciences, the Louisa Gross Horwitz Prize, the Wolf Prize in Medicine, the Royal Medal from the Royal Society of London and the Kyoto Prize in Basic Sciences. He is a Fellow of the Royal Societies of London and Canada, a Foreign Associate of the National Academy of Sciences (US), and an Associate Member of EMBO. He is an Officer of the Order of Canada, and in 2006 was appointed to the Order of Companions of Honour by Queen Elizabeth II.

Lab webpage: http://pawsonlab.mshri.on.ca/index.php

ABSTRACT:

Using synthetic biology to study protein signaling networks

The proteins involved in the transmission of signals within cells are typically complex, in the sense that they often possess multiple interaction and/or catalytic domains, and are directly or indirectly connected to numerous distinct proteins and other biomolecules, such as phospholipids. We have used a variety of synthetic biology and proteomics techniques to investigate the importance of the multiple protein- and lipid-binding domains of the Grb2 adaptor protein and its target, the Ras guanine nucleotide exchange factor Sos1, in controlling a key cell fate decision in early mammalian embryogenesis, namely the differentiation of primitive endoderm tissue. We find that these domains act cooperatively as a coincidence detector to ensure that fibroblast growth factor-dependent signals are transmitted at the right time and in the right place to elicit the appropriate formation of primitive endoderm. We have also developed the use of molecular clamps to restrict the interaction of the Grb2 SH2 domain to specific phosphotyrosine-containing sites, and to define which targets of this SH2 domain are important for the differentiation of embryonic stem cells. The combined use of this technology with SWATH mass spectrometry, and its potential utility in the deconvolution of signaling networks, will be discussed.

Pierre Thibault

McGill University

BIOGRAPHY:

Dr. Pierre Thibault is a renowned bioanalytical chemist specialized in mass spectrometry and proteomics with more than 20 years experience as a principal investigator in academic, government and industry laboratories. Before joining IRIC in 2004, Dr. Thibault was a founding director at Caprion Pharmaceuticals (2001-2004) where he developed an innovative proteomics platform together with bioinformatic tools to identify and quantify proteins differentially expressed in cancer cells as part of immunotherapy programs in partnership with pharmaceutical companies. He was also a Senior Research Officer with the National Research Council of Canada's Institute of Marine Biosciences in Halifax (1990-1996) and Institute of Biological Sciences in Ottawa (1996-2002). He pioneered the use of sensitive high resolution separation methods and microfluidic devices coupled to mass spectrometry and their applications in protein chemistry and cell biology. His current research program in mass spectrometry-based proteomics provides a deeper understanding of molecular mechanisms and post-translational modifications, which regulate the function and translocation of proteins involved in immunity and signalling in cancer cells. His scientific achievements in this area have been recognized by numerous awards and distinctions including the Maxxam award, the National Research Council Outstanding merit award for scientific innovation and the Canada Research Chair in bioanalytical mass spectrometry and proteomics. He is the author of more than 170 publications in scientific journals including Cell, Nature, Nature medicine.

ABSTRACT:

Quantitative proteomics using FAIMS and isotopic labeling

Authors: Eric Bonneil[1], Christina Bell[1,2], Fréderic Lamoliatte[1,2] and Pierre Thibault[1,2,3]

Affiliations: [1] IRIC, Institut de recherche en immunologie et en cancérologie, Université de Montréal, Montréal, Canada; [2] Department of Chemistry, Université de Montréal, Montréal, Canada; [3] Department of Biochemistry, Université de Montréal, Montréal, Canada

Isotopic labeling is a commonly used method in quantitative proteomics to profile changes in protein abundances across different cell conditions. Isotopic labeling can be performed using chemical reagents that

target specific functional group on peptides (MS2 quantitation) or via metabolic labeling (Stable isotope labeling of cell cultures, SILAC) where cells are cultured in media containing in either light or heavy isotopes of amino acids (MS quantitation). The predictable mass differences between peptides (or fragment ions) from two or more experimental conditions enable a direct quantification and provide precise functional information and temporal changes in the proteome. However, the dynamic range and precision of these quantitative measurements can be hampered by the increasing complexity of the mass spectra and the presence of co-eluting isobaric ions. In this context, we evaluated the analytical potentials of high field asymmetric waveform ion mobility spectrometry (FAIMS) in large-scale quantitative proteomics experiments using stable isotope labeling on a LTQ-Orbitrap Elite mass spectrometer. The ability of FAIMS to separate multiply-protonated ions from singly-charged background ions provided a unique advantage to extend the dynamic range of peptide detection while reducing the complexity of LC-MS peptide maps. FAIMS analyses performed on cell extracts derivatized with tandem mass tags (TMT)-6plex isotopic label reagent or grown under SILAC conditions provided improved quantitation and dynamic range compared to conventional nanoelectrospray. Quantitative proteomics analyses performed using FAIMS resulted in a reduction of mixed precursor ions and provided confident reporter ion measurements with increased peptide identification. Examples of application will be presented for monitoring changes in the proteome of cytokine-activated macrophages.

Sarah Trimpin
Wayne State University

BIOGRAPHY:

Sarah Trimpin is an Assistant Professor at Wayne State University. Her current work involves developing new ionization technology in mass spectrometry and its application to biological materials analyses. She holds 3 patents, is co-editor of a book and author or co-author of 6 book chapters, and has published 50 peer-reviewed research papers. She has received a number of awards for her work in mass spectrometry including the Pittsburgh Conference Achievement Award (2013, scheduled March), he WSU Schaap Faculty Scholar Award (2012), the Eli Lilly Young Investigator Award in Analytical Chemistry (2011), the DuPont Young Professor Award (2010), the American Society for Mass Spectrometry Research Award (2010), and the NSF CAREER Award (2009), Her laboratory is highlighted as a Waters Corporation Center of Innovation. Prof. Trimpin received BS and MS degrees (equivalent) from the University of Konstanz, and the PhD (equivalent) degree from the Max-Planck-Institute for Polymer Research with Prof. Mullen before taking a joint Postdoctoral Fellowship between CROET at Oregon Health & Science University and Oregon State University with Profs. Spencer and Deinzer, and a Research Associate position at Indiana University with Prof. Clemmer.

ABSTRACT:

Matrix Assisted Ionization without a Laser, Heat, or Voltage: Identification of Peptides and Proteins Directly from Tissue

A few years ago, we introduced an ionization method for use in mass spectrometry (**MS**) applicable to volatile and nonvolatile compounds which uses laser ablation of a matrix/analyte mixture similar to atmospheric pressure matrix-assisted laser desorption/ionization (**MALDI**), but produces mass spectra nearly identical to electrospray ionization (**ESI**). This new technique called laserspray ionization (**LSI**) has advantages of speed of analysis, high spatial resolution for imaging, mass range extension, and improved fragmentation common with multiply charged ions. LSI using the proper matrix was also shown to be capable of imaging multiply charged ions using atmospheric pressure or vacuum MALDI sources. Crucial for the production of highly charged ions are desolvation conditions to remove matrix from charged matrix/analyte clusters. By using a matrix that readily sublimes, we have now extended matrix assisted ionization by eliminating the need for a laser or even heat to initiate ionization. Ions are spontaneously formed from a solid matrix/analyte material when introduced to a vacuum MALDI source without need of a laser or to a modified ESI source. This matrix assisted ionization *vacuum* (**MAIV**) method and ESI produce similar charge states and drift times of ions as determined by ion mobility spectrometry-MS. Such a simple ionization method, requiring only the vacuum necessary for the proper functioning of the mass spectrometer, may prove useful for analysis of single cells and in clinical applications. How compounds at least as large a bovine serum albumin (67 kDa) become converted to highly charged gas phase ions when exposed in the MAIV matrix to vacuum conditions will be discussed.

Dietrich Volmer
Saarland University

BIOGRAPHY:

Dietrich Volmer is Alfried Krupp Professor and Chair in Analytical Chemistry and Director of the Institute of Bioanalytical Chemistry at Saarland University in Saarbrücken, Germany. He received his BSc in Chemical Engineering from the University of Applied Sciences in Lübeck, Germany, MSc in Chemistry from the University of Osnabrück, Germany, and his PhD from the University of Hannover, Germany. Following postdoctoral research at the National Center for Toxicological Research in Jefferson, AR/USA, he moved to Canada to pursue biological mass spectrometry research at the Institute for Marine Biosciences (IMB) in Halifax, NS, followed by industrial research at Merck in Germany. He returned to Canada in 2001, to become Group Leader in Biological Mass Spectrometry at IMB and Adjunct Professor at Dalhousie University. In 2007, he was appointed Head of Department of Bioanalytical Sciences at the Elsie Widdowson Laboratory in Cambridge, UK. During his tenure in Cambridge, he established and directed the Cambridge Lipidomics Biomarker Research Initiative (CLBRI), a large-scale MS facility for population-based lipidomics research. Dietrich Volmer is a Fellow of the Royal Society of Chemistry (FRSC). He is also the Editor of Rapid Communications in Mass Spectrometry since 2004. His current research program focuses on bioanalytical chemistry, biological mass spectrometry, chemical separations, imaging mass spectrometry, and metabolomics.

ABSTRACT:

New methods for improved determination of metabolic markers using advanced analytical mass spectrometry techniques

New developments in metabolomics and metabolic profiling techniques have led to discovery of metabolic biomarkers indicative of physiological or pathological change, e.g. onset of disease or nutritional effects of a specific diet. Experimental approaches for discovering new biomarkers usually involve proteomics or metabolomics techniques that detect differences between sample sets rather than measuring specific compounds as performed in traditional, hypothesis-driven research. Unfortunately, finding metabolites present in significantly different levels between sample sets is often complicated by experimental variables such as method reproducibility, types of tissue sampling technique, analytical sample preparation technique, ion suppression phenomena, person-to-person or animal-to-animal metabolic variations, isobaric or isomeric noise etc. All these error sources, variations or artefacts can lead to false assignment of metabolites or biomarkers and random and systematic errors during quantification. This presentation will focus on our research on metabolite profiling techniques and sources of analytical errors. It will give examples for applications such as quantitative analysis and fingerprinting of vitamin D metabolites, microcystin toxin biomarkers and lipids. As well, novel technical solutions involving “magnetic” media (fluids and beads), ion mobility spectrometry and chemical labeling techniques are highlighted in this presentation.

Fangjun Wang
Dalian Institute of Chemical Physics

BIOGRAPHY:

Dr. Fangjun Wang was born in 1982 in Sichuan, China. He received his BA degree in 2005 at Zhejiang University with the supervision of Dr. Yuanjiang Pan and received his PhD degree in analytical chemistry at Dalian Institute of Chemical Physics in 2011 with the supervision of Dr. Hanfa Zou. He had studied and worked in University of Ottawa during 2010-2011 with the supervision of Dr. Daniel Figeys. He was selected as “Hundred Talent Young Scientist Program” by Dalian Institute of Chemical Physics at 2011 and worked in CAS Key Lab of Separation Sciences for Analytical Chemistry. His current research is focused on new technologies and methods developing for proteome identification and quantification, and applying these technologies in biomarker discovery. He has 40 original papers published in international journals.

ABSTRACT:

Recent development of technologies and methods for proteome analysis

Fangjun Wang[1], Hanfa Zou[1], Mingliang Ye[1], Daniel Figeys[2]

1 Key Lab of Separation Science for Analytical Chemistry, National Chromatography R&A Center, Dalian Institute of Chemical Physics, the Chinese Academy of Science, Dalian 116023, China
2 Ottawa Institute of Systems Biology, Department of Biochemistry, Microbiology and Immunology, Faculty of Medicine, University of Ottawa, Ottawa, Canada, K1H 8M5

Technologies and methods play the highly important role for proteome analysis. We will present recent development of technologies and methods for proteome analyses in our labs:

(1) Combined technologies and methods for comprehensive glycoproteome and phsophoproteome analysis of human liver, the largest dataset of protein phosphorylation and glycosylation for human liver was achieved.
(2) The solid-phase based technology by integrating all of the digestion, enrichment, deglycosylation together with LC-MS analysis was developed for glycoproteome analysis, and by which both the identification sensitivity and throughput was improved greatly.
(3) The solid phase based labeling approach by integration of glycopeptides and phosphopeptides enrichment and stable isotope labeling on adsorbent beads was developed for relative quantification of protein glycosylation and phosphorylation.
(4) Six-plex stable isotope labeling was achieved by a two stages stable isotope labeling strategy of SILAC and dimethylation labeling, which allows six different protein samples (six-plex) to be reliably labeled and simultaneously quantified at MS1 level. This six-plex isotope labeling strategy was applied to investigate the dynamics of protein turnover, and the 50% turnover time of 1365 proteins were successfully obtained.

Hailin Wang
State Key Laboratory of Environmental Chemistry and Ecotoxicology

BIOGRAPHY:

Dr. Wang obtained B. Sci. degree from Wuhan University (1987-1991), and M. Sci. and Ph.D. degrees from the Dalian Institute of Chemical Physics, Chinese Academy of Sciences (1991-1997). He was recruited as Assistant Professor to Dalian Institute of Chemical Physics in 1997 and promoted to Associate Professor in 1999. He carried out his postdoctoral training in the Department of Public Health Sciences, University of Alberta (2000 – 2004), and then served as a Research Associate in the University of Alberta (2004 - 2005). He was recruited and promoted to Full Professor at the Research Center for Eco-Environmental Sciences under the "Hundreds of Talents" plan of CAS at the end of 2005.

In 2011, he was awarded "Outstanding Young Scientists" by the National Science Foundation of China and "Excellent Nominee" for "Hundreds of Talents" plan by the Chinese Academy of Sciences. He has been awarded "Presidential Special Award for Graduate Student" by the Chinese Academy of Sciences in 1997.

Research topics: 1) Highly sensitive analytical technologies for detection of DNA damages and epigenetic modifications in genomic DNA caused by emerging pollutants; 2) Protein-DNA binding assays for understanding the mechanism of DNA repair; 3) Single molecule detection and imaging.

Recent Publications:
1. Zhao, C.; Yin, R. C.; Yin, J. F.; Wang, H.* Capillary monolithic bioreactor of immobilizing snake phosphodiesterase for mass spectrometry based oligodeoxynucleotide sequencing.
Anal. Chem., 84: 1157-1164 (2012).

2. Zhang, D. P.; Lu, M.; Wang, H.* Fluorescence anisotropy analysis for mapping aptamer-protein interaction at the single nucleotide level.
J. Am. Chem. Soc., 133: 9188-9191 (2011).

3. Wang, H.; Lu, M.; Tang, M.-S.; Van Houten, B.; Ross, J. B. A.; Weinfeld, M.*; Le, X. C*. DNA wrapping is required for DNA damage recognition in the Escherichia coli DNA nucleotide excision repair pathway.
Proc. Natl. Acad. Sci. USA, 106: 12849-12854 (2009).

4. Wang, X. L.; Song, Y. L.; Song, M. Y.; Li, T.; Wang, Z. X.; Wang, H.* Fluorescence polarization combined capillary electrophoresis immunoassay for rapid and sensitive detection of genomic DNA methylation.
Anal. Chem., 81: 7885-7891 (2009). (Accelerated Article, Highlight)

5. Wang, Z. X.; Lu, M.; Wang, X. L.; Yin, R. C.; Song, Y. L.; Le, X. C.; Wang, H.* Quantum dots enhanced ultrasensitive detection of DNA adducts.
Anal. Chem., 81: 10285-10289 (2009). (One of most read article)

ABSTRACT:

Insights on Isotachophoresis Kinetics from Monitoring Non-uniform Motion of single DNA molecules

Hailin Wang, Shengquan Liu, Bailin Zhao, Dapeng Zhang, Cuiping Li

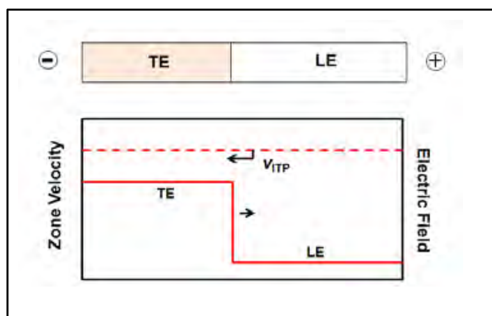
State Key Laboratory of Environmental Chemistry and Eco-toxicology, Research Center for Eco-Environmental Sciences, Chinese Academy of Sciences, Beijing 100085, China

Isotachophoresis (ITP) has wide applications in chemistry and life sciences due to its pre-concentration function. A simplified ITP system consists of a leading electrolyte (LE) and a terminating electrolyte (TE). For LE and TE to migrate at the same velocity (ν), the respective E in each zone must adapt to their electrophoretic mobility (μ) according to Eq. 1.

$$\nu_{TE} = \nu_{LE} = \mu E \qquad (1)$$

The LE zone with a high electrophoretic mobility will have a low E, and the TE zone with a low electrophoretic mobility will have a high E (Scheme 1). Current literature commonly assumes that the adaption of E is fast and is not the rate-limiting step. However, actual adaption kinetics of ITP has not been studied yet. Moreover, it is not clear how electroosmotic flow affects the kinetics of ITP.

By taking advantage of single molecule imaging, here we for the first time examine the changes in electric field and the resulting non-uniform motion of single DNA molecules in capillary isotachophoresis. The individual DNA molecules passing the detection window are consecutively imaged in real time at 50-milisecond intervals. Since the migration velocity of DNA is directly proportional to the applied electric field strength (E), imaging the movement of DNA provides necessary information for understanding the distribution of E throughout the capillary. This approach allows us to gain insights into the kinetics of varying-field ITP and enables us to develop a strategy for focusing and detecting single DNA molecules and DNA damages.



Scheme 1. The distribution of electric field strength in anionic ITP.

John Wilkins
University of Manitoba

BIOGRAPHY:

John received a BSc (Biology and Chemistry) and a MSc (membrane biology) University of Waterloo and a PhD (Immunology) University of Manitoba. He is a Professor in the Departments of Internal Medicine, Biochemistry and Medical Genetics at the University of Manitoba. He is director of the Manitoba Centre for Proteomics and Systems Biology, head of the section of Biomedical Proteomics, Scientific Director of the Rheumatic Diseases Research Laboratory and Director of Research for the Health Sciences Centre.

His research interests focus on the use of high content methods for the analysis of biological and biomedical systems. He uses mass spectrometry extensively in conjunction with immunological, enzyme activity based and functional genomics methods for the development of analytical methods for discovery and validation in a range of clinical systems including renal function and rheumatic diseases. He was a co-recipient of the NSERC Brockhouse Prize for Interdisciplinary Research in Science and Engineering for Biomolecular Mass Spectrometry (2006). He has published more than peer reviewed 100 papers.

He reviews for several granting agencies and is a longstanding member of the College Reviewers for CFI and CRC. He reviews for several journals including Proteomics, Nature Biotechnology, Analytical Chemistry, Arthritis and Rheumatism, and FEBS.

ABSTRACT:

Linking protein composition and functionality in biology and medicine

The repertoire and functional state of proteins expressed by an organism ultimately determines their phenotype. Thus the inclusion of measures of protein activity is an essential step to developing valid models of biological systems in disease and health. While genomics and proteomics provide detailed information on potential and current protein composition, these approaches generally offer limited insight regarding protein functional states. However, many proteins possess catalytic activities that are stringently controlled by mechanisms that restrict substrate accessibility to the active site. This is the central premise of activity based protein profiling.

The use of enzyme activity as a predictor of organ function or health offers several advantages in the clinical environment. The assays can be highly specific because of the structural features of the their substrates. The high substrate turnover numbers of enzymes provides an amplification mechanism that increases assay sensitivity. Activity based profiling offers a means of probing clinical samples for novel enzymatic activities as new reporters. We will present some of our experiences in the application of this approach profiling assessing renal function.

The identification of a protein, even an active one, does not necessarily address the questions of “What does the

protein do?" and "Why is it needed?". These questions can be further complicated by the observations that function(s) are often context specific. We have used integrated proteomic, activity based protein profiling and functional genomics approaches in an effort to examine lymphocyte migration, a process that is central to normal immune function.

David Wishart
University of Alberta

BIOGRAPHY:

Dr. David Wishart (PhD Yale, 1991) is a Professor in the Departments of Biological Sciences and Computing Science at the University of Alberta. He is also a Senior Research Officer and Program Coordinator of the Metabolomic Sensors program at the NRC's National Institute for Nanotechnology (NINT). Dr. Wishart is currently the co-director of The Metabolomics Innovation Centre (TMIC) – Canada's national core facility for metabolomics. Dr. Wishart has active research programs in structural biology, NMR spectroscopy, synthetic biology, prion biology, drug discovery, bioinformatics and metabolomics. From 2006-2009, Dr. Wishart led the "Human Metabolome Project" (HMP), a multi-university, multi-investigator project that catalogued all of the known metabolites in human tissues and biofluids. Using advanced methods in NMR spectroscopy, mass spectrometry, multi-dimensional chromatography and machine learning Dr. Wishart and his colleagues identified or found evidence for more than 8500 endogenous metabolites. This information has been archived on a freely accessible web-resource called the Human Metabolome Database (HMDB). The methods and ideas developed for the HMP have helped lay the foundation for a number of other metabolomic databases (DrugBank, T3DB, SMPDB, FooDB) and metabolomic data analysis packages (MSEA, MetaboAnalyst, MetPA, MetaboMiner).

ABSTRACT:

Clearing the Biomarker Barrier: Moving Biomarkers from the Bench to the Bedside

One of the great promises of the "omics" era was the delivery of a gene or a protein to diagnose every disease. Unfortunately this hasn't quite happened. While our understanding of the etiology of disease has improved tremendously our ability to diagnose or predict disease has not. This has a lot to do with our inability to translate biomarkers from the bench to the bedside. Indeed, after nearly 20 years of work and more than $5 billion in investment, there have been only a few microarray and, as yet, no protein-array or proteomic tests approved by the FDA for diseases diagnosis. On the other hand, dozens of metabolomic biomarkers have successfully made the transition from the lab to the clinic. Why? In this presentation I will discuss some of the secrets to metabolomic's success in clearing the "biomarker barrier". In particular, I will provide some key guidelines for designing, performing and reporting biomarkers in a biomarker study as well as some advice with regard to the process of moving biomarkers from the laboratory to the clinic. I will also provide some examples of how quantitative metabolomics is being used to identify biomarkers for a wide range of diseases and the status of my lab's efforts to try to move some these biomarkers into clinical testing.

The Ken Standing Award

The Ken Standing Award is generously provided by the sponsors of the ETP Symposium and the University of Manitoba.

Professor Kenneth G. Standing is Professor Emeritus, Dept. of Physics & Astronomy, University of Manitoba. He graduated B.Sc. from the University of Manitoba in 1948, and obtained his Ph.D. in Physics from Princeton University in 1955. Dr. Standing was appointed to the faculty of the University of Manitoba in 1953, where he rose through the ranks and eventually became Professor Emeritus in 1995. Along the way he was Director of the Manitoba Cyclotron Laboratory for the period 1959-1974. Starting in the early 1980s he turned his attention to time-of-flight mass spectrometry and its applications to study of biological macromolecules, particularly proteins and peptides, and is widely regarded as the pioneer in this area. He has received many honours during his distinguished career, including the Canadian Society for Mass Spectrometry Award for Distinguished Contributions to Mass Spectrometry in 1998, the Canadian Association of Physicists Medal for Outstanding Achievement in Industrial & Applied Physics in 2003 and the American Chemical Society's Field and Franklin Award for Outstanding Achievement in Mass Spectrometry in 2004. He was elected as a Fellow of the American Physical Society in 2004, and in the same year as a Fellow of the Royal Society of Canada. He has served as Member or Chair of various US National Institutes of Health Special Study Sections. Dr. Standing is regarded by his many colleagues and friends, including several generations of scientists from around the world, as one of the founders of the still-young discipline of proteomics. Never content to only invent and perfect experimental tools he has sought collaborations with biologists, focused on solution of a wide range of difficult biological problems using his own technological innovations as well as those of others. This led to a continuing collaboration with MDS-Sciex in development of mass spectrometers used by proteomics researchers worldwide, recognized in 2000 by the award of an NSERC/Conference Board Synergy Award for University–Industry collaboration to the Manitoba-MDS-Sciex collaboration. In 2007 the Manitoba-MDS-Sciex team was awarded NSERC's Brockhouse Canada Prize for Interdisciplinary Research in Science and Engineering, a prize established to honour the memory of Bertram Brockhouse, Canada's Nobel Laureate in Physics.

Ken Standing's exemplary career continues to provide an inspiration to new generations of scientists in Canada and elsewhere. Despite the recent curtailment of his alpine skiing activities, his energy level in his research laboratory appears to be undiminished. For these reasons the Sponsors of the Enabling Technologies Symposium decided in 2006 to establish the Ken Standing Award to honour the lifetime achievement of this distinguished Canadian scientist. The award consists of a $5000 (CDN) cash grant and a commemorative sculpture, and is presented at the annual ETP Symposium to a young scientist who has made a significant contribution to the development of technology related to the life sciences.

The winner of this year’s Award is presented to

Lars Konermann
Professor and Canada Research Chair
Department of Chemistry and Department of Biochemistry
The University of Western Ontario
London, Ontario, Canada

Lars Konermann holds a Canada Research Chair in Biophysical Protein Mass Spectrometry at The University of Western Ontario (UWO) in London/Ontario, Canada. The work of his laboratory is aimed at gaining insights into protein folding mechanisms, and the relationship between protein function and conformational dynamics. These and other areas are being tackled via a range of mass spectrometry-based approaches that include the use of hydrogen exchange and covalent labeling. Konermann is faculty member at the UWO Department of Chemistry, and he is cross-appointed to the Department of Biochemistry. He completed his Ph.D. in the area of plant photosynthesis in 1996 at the Max Planck Institute in Mulheim (Germany), supervised by A. R. Holzwarth. Konermann was first exposed to the fascinating world of biological mass spectrometry between 1996 and 1998, when he worked as post-doctoral fellow at the University of British Columbia in Vancouver (Canada) with D. J. Douglas. Konermann is recipient of the 2003 CSC Fred Beamish Award, the 2011 UWO Florence Bucke Award, as well as several teaching awards. He was member of the NSERC Chemistry Evaluation group (2009-2011), and he chaired the 2011 Gordon Conference on “Biological Molecules in the Gas Phase and in Solution”.

Presentation:

Electrospray Mass Spectrometry as a Readout of Protein Structure and Function

Proteins are nanomachines that carry out countless tasks in every living organism. To perform these functions, the linear amino acid chain of each protein has to fold into a highly specific three-dimensional

structure. On the other hand, misfolding and aggregation are associated with diseases such as Alzheimer's and Parkinson's. X-ray crystallography remains the gold standard for obtaining high resolution structural information. However, the data generated in this way do not properly reflect the highly dynamic nature of proteins, which is a key prerequisite for biological function. Electrospray mass spectrometry (ESI-MS) provides a number of avenues for exploring protein structure, function, folding, and dynamics. Our laboratory specializes in the use of hydrogen exchange and covalent labeling techniques. This presentation will discuss time-resolved investigations that provide detailed insights into the mechanisms of protein folding. We will also discuss the area of membrane protein structure and function, as well as the use of ESI-MS for probing biological self-assembly processes. In addition, we will highlight recent advances in understanding the physical processes that allow the formation of gaseous biomolecular ions during ESI.

The Bill Davidson Graduate Student Travel Award

Bill Davidson was a PhD graduate from the laboratory of Paul Kebarle at the University of Alberta, where he acquired the expertise in the physical chemistry of ion-molecule reactions that stood him in good stead in his long career. He joined Sciex in 1978 and very quickly became a key part of the early success of that company, contributing to the hardware development and writing some of the first application software (on a PDP8 computer!) for the TAGA (Trace Atmospheric Gas Analyzer). This instrument incorporated an APCI ion source and the first commercially available triple-quadrupole analyzer. Mobile versions mounted on a truck are still in use to monitor air quality, especially in the event of environmental accidents.
Bill's vision and leadership contributed greatly to development of the Aromic System (used as a non-invasive inspection system for air cargo) and in 1989 the API III LC/MS/MS system. The latter pioneered the commercialization of reliable LC/MS/MS instruments for biomedical and other applications, and was the basis of the subsequent commercial success of Sciex.
Bill developed an extensive network of collaborations and interactions with Canadian and inter- national Universities and research institutes that provided the company with exposure to many new scientific advances and technologies. His later role in business and technology development grew from his extensive contacts in the larger scientific community, where his scientific knowledge and judgment were greatly respected.

After retiring from being the Vice-President of Science and Technology at AB SCIEX, Bill co-founded (along with Janette Champ) ETP Symposium Inc. (ETP) to continue to bring researchers together to develop new scientific advances and technologies. ETP is world-renowned as a key conference where international and Canadian researchers get together to collaborate to develop novel technologies to further life sciences research. Bill was an active participant in ETP up until his death in August 2009.

To honour this lifetime achievement, ETP and AB SCIEX have put together an award geared to helping graduate students travel to and participate at the biennial ETP Symposium.

This inaugural award is being presented by
Dr. Bruce Thomson, AB SCIEX to

Feng Li
University of Alberta.

Feng Li received a BSc in Pharmaceutical Sciences from Tianjin University (Tianjin, China) in 2006. He is currently pursuing his Ph.D. degree in Chemistry at the University of Alberta under the supervision of Prof. X. Chris Le. His current research interests include developing ultrasensitive homogeneous assays for point-of-care diagnosis and directing assembled DNA nanostructures using binding-induced DNA strand displacement.

Presentation:

Dynamic DNA Assemblies Mediated by Binding-Induced DNA Strand Displacement

Li F,1 Le XC1,2*
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Dynamic DNA assemblies, including catalytic DNA circuits, DNA nanomachines, molecular translators, and reconfigurable nanostructures, have shown promising potential to regulate cell functions, deliver therapeutic reagents, and amplify detection signals for molecular diagnostics and imaging. However, such applications of dynamic DNA assembly systems have been limited to nucleic acids and a few small molecules, due to the limited approaches to trigger the DNA assemblies. We develop a binding-induced DNA strand displacement strategy that can convert protein binding to the release of a predesigned output DNA at room temperature with high conversion efficiency and low background. This strategy allows us to construct dynamic DNA assembly systems, e.g. catalytic DNA circuit, that are able to respond to specific protein binding, opening an opportunity to expand the existing dynamic DNA nanotechnology to proteins for diverse applications. One such application could be in the area of point-of-care analysis of proteins that could be performed under ambient temperature and without requiring the use of enzymes for signal generation and/or amplification.

This award is generously sponsored by

Lunch Workshop Sponsored by Bruker

PRESENTATION NO. 1

Shannon Cornett
Bruker Daltonics

Ultrafast Statistical Profiling of myxobacteria natural products for rapid detection and identification of marker compounds

Contributing Authors:

Shannon Cornett[1], Daniel Krug[2,3]; Thomas Hoffmann[2,3]; Aiko Barsch[4]; Matthias Witt[4]; Rolf Müller[2,3]

ABSTRACT

Myxobacteria represent an important source of novel natural products that exhibit a wide range of biological activities. Some of these so-called secondary metabolites are investigated as potential leads for novel drugs. Traditional approaches to discovering natural products typically employ bioassays and activity-guided isolation from different myxobacterial isolates, but genomics-based strategies show considerable promise for uncovering novel secondary metabolites from myxobacterial strains, as the number of known compounds identified to date is often significantly lower than expected from genome sequence information.

Current LC-QTOF-MS methods require about 20 min analysis times per sample. Facing several thousand myxobacterial strain isolates as well as numerous genetic knock out mutant strains for the presence of novel secondary metabolites, analysis time becomes a bottleneck. In this study we utilize the high resolving power of FT-ICR to measure metabolite extracts from genetic knock-out mutants at times of 1 min/sample. Principal Component Analysis (PCA) found the spectra clustered according to the bacterial genetic background, i.e. wild type and mutants could be differentiated while the PCA loadings pointed to compounds responsible for this differentiation. Isotopic fine structure revealed at resolving power >750,000 enabled direct "read out the correct elemental composition" for the target compounds.

The established workflow, coined "Ultrafast Statistical Profiling", enables rapid profiling of complex metabolite extracts and identification of relevant marker compounds by making use of the ultrahigh resolution provided by the FT-ICR-MS measurements – thereby addressing two of the major bottleneck in metabolomics, sample throughput and compound identification.

PRESENTATION NO. 2

Matt Willetts
Bruker Daltonics

Top Down Proteomics: A Multi-Platform Approach

ABSTRACT

Although the idea of "top-down" processing intact proteins has been around for some time, only recently have software and hardware advances allowed this thesis to be pursued as a viable analytical workflow. While there are many differing opinions about the most efficient way to gather information from whole proteins, the most conventional misconception is that it requires the use of high performance instrumentation. Indeed, almost any kind of mass spectrometer, especially those capable of interfacing either MALDI or ESI sources, can be used to generate data from intact proteins.
The challenge for the modern mass spectrometrist is to determine what kind of "hammer" to apply to the problem at hand. "Lower end" analyzers such as ion traps that offer lower resolving power and mass accuracy have been given new life by recent advances that combine advanced electron-induced ion fragmentation techniques such as electron transfer dissociation (ETD) and increased analytical performance, and are unquestionably capable of fulfilling top-down quality control (QC) roles. Time-of-flight instrumentation coupled with electrospray ionization (ESI) has recently seen architectural improvements that allow resolving powers in excess of 60,000 allowing charge state and, ergo, mass determination of larger proteins even under fast chromatographic conditions. MALDI-TOF has seen recent methodological advances that allow protein terminal sequencing that rivals standard Edmund sequencing techniques in terms of sequence coverage. Finally, the MS arsenal is completed with Fourier Transform (FTMS) based instruments which remain the most flexible and evaluative form of analyzer for the top-down protein chemists. This discussion will focus on how each of these platforms play an integral role in advancing top-down strategies and how each can be used alone, or in an integrated whole protein strategy to compliment bottom-up workflows.

Oral Abstract Presentations

Rui Chen
OISB
University of Ottawa

Contributing Authors: Rui Chen[1,2], Hanfa Zou[2]*, Daniel Figeys[1,3]*

Characterization of Cell Membrane N-Glycosylation with Integrated Hydrophilic Interaction Chromatography Solid Phase Extraction and LC-MS/MS

Glycosylation of membrane proteins plays important roles in cellular behaviours such as cell-cell interaction, immunology recognition and cell signalling. Despite their importance, the effective extraction of membrane protein, selective isolation of glycopeptides and mass spectrometric characterization of glycosylation are challenging current analytical techniques. In this study, a systematic approach was developed which combined: an integrated hydrophilic interaction chromatography solid phase interaction (HILIC SPE) for simultaneous detergent removal and glycopeptides enrichment, and mass spectrometric identification of both protein N-glycosylation sites and site-specific glycan structure. The HILIC SPE condition was optimized to enable the use of high concentration of strong detergents, such as SDS and Triton X-100, to dissolve highly hydrophobic membrane proteins, thus increasing the yield of membrane protein extraction. We illustrated the performance of this approach for the study of membrane protein glycosylation from human embryonic kidney cell lines (HEK 293T). 200 µg total protein digest was processed using this approach, leading to the identification of 813 N-glycosylation sites from 568 proteins within two experimental replicates. Furthermore, 177 glycopeptides representing 82 N-glycosites with both glycan composition and peptides sequence were identified by high energy collision dissociation.

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Andrew Crowell
Dalhousie University

Contributing Authors: Andrew Crowell, Alan Doucette, and Samantha Rudolph

Disposable two-stage spin cartridges for protein purification in a top-down proteomics workflow

Proper proteome analysis by mass spectrometry depends on upstream sample manipulations, including protein concentration and purification. Cleanup strategies typically rely on chromatography, not only for the relative high recovery and purification efficiency, but also for ease of use through automation. Unfortunately, with chromatographic approaches, sample loss is an expected occurrence, especially for high molecular weight and/or hydrophobic (membrane) proteins. By contrast, organic solvent-assisted protein precipitation is extremely effective at protein purification with minimal sample loss. Quantitative protein recovery is possible, though is highly dependent on the pipetting skills of the individual. It is desired to create a robust protein precipitation protocol by automating the process, thereby maximizing recovery and purity on a high throughput scale.

This presentation introduces a disposable two-stage centrifugal cartridge, tailored to automate the precipitation process. The ProTrap XG comprises an upper filtration cartridge together with a removable reversed phase cartridge, attaching at its base. Protein samples are mixed with organic solvents in the upper filter chamber, which induces precipitation of proteins. The pellet is retained by the filter, while contaminants in the supernatant are discarded to waste through a brief spin in a desktop centrifuge. Multiple devices can be used simultaneously, improving throughput while maintaining consistency between samples. Following precipitation, protein pellets are re-solubilized and subject to final purification through the reverse phase cartridge which is re-attached to the base of the filter. This presentation will discuss effectiveness of the ProTrap XG for recovery and purification of intact proteins in a top-down proteomics workflow.

Alan Doucette
Dalhousie Unversity

Contributing Authors: Alan Doucette, Andrew Crowell, Samantha Rudolph

Automated SDS removal, tryptic digestion and sample cleanup in a disposable two-stage spin cartridge

Prior to MS analysis, bottom up proteomics relies on a significant number of sample manipulations, including protein purification and preconcentration, fractionation, digestion, and subsequent cleanup. Each of these steps comes with a risk of loss of analyte, and adds to the complexity of the proteomics workflow. The GELFrEE fractionation system is a mass-based electrophoretic platform which relies on an SDS-containing sample buffer to isolate protein fractions in solution. Our group has recommended the use of solvent-induced protein precipitation (acetone or chloroform/methanol/water) to eliminate the SDS ahead of LC/MS analysis. However, protein precipitation is a particularly tedious and highly variable technique to recover proteins in high yield. Subsequent resolubilization of the protein pellet is also challenging, and further jeopardizes the success of MS characterization.

To facilitate protein precipitation, we have developed a two-stage filtration and chromatographic extraction cartridge designed to work in a conventional benchtop centrifuge. The device automates the recovery of proteins, ensuring uniform purification on a high throughput scale. Protein samples are precipitated in an upper chamber, with residual contaminants (including SDS) flushed through the bottom filter. At this point, the base filter is capped, trapping proteins in the upper vial for subsequent resolubilization. A combination of urea and trypsin ensure complete digestion of the pellet. The peptides are then subject to reversed phase purification; here we link a chromatographic cartridge to the base of the filter chamber. Key figures of merit of the device, include the purity of SDS removal, protein recovery, digestion efficiency and peptide recover, are demonstrated through standard and complex proteome mixtures, as well as GELFrEE-fractionated samples.

Bella Groisman
University of Toronto

Contributing Authors: Julia Petschnigg, Bella Groisman and Igor Stagljar

A novel mammalian split-ubiquitin proteomics approach as a tool for functional investigation of signaling pathways in human cells

The membrane yeast two-hybrid system (MYTH) is a robust technique for the identification of protein partners of integral membrane proteins. However, its implementation for mammalian proteins is limited, due to differences between mammalian and yeast cells. Here, we present a novel split-ubiquitin-based mammalian membrane two-hybrid (MaMTH) technology that enables investigation of protein-protein interactions (PPIs) in human cells. Briefly, the specific interaction between membrane “bait” and “prey” proteins coupled to ubiquitin halves, allows reconstitution of functional ubiquitin. Subsequently, deubiquitinating enzymes cleave off the transcription factor coupled to the bait, thus activating reporter gene transcription.

Using the MaMTH technology, we successfully confirmed known PPIs of membrane proteins from various cellular compartments. Here, we focus on the ErbB members of receptor tyrosine kinases, mainly on the EGFR wild type (WT) versus the oncogenic kinase-active L858R version - dynamic interactomes and functional analysis. We demonstrate that MaMTH allows sensitive detection of phospho-dependent and drug-inhibited interactions. We show that EGFR WT recruits Shc1 adaptor in serum-dependent manner, while oncogenic L858R shows constitutively stimuli-independent binding. Furthermore, consistent with clinical data EGFR (L858R) interaction with Shc1 was efficiently inhibited by the EGFR small molecule inhibitor erlotinib (tarceva), while the secondary L858RT790M resistant mutant was not affected by the drug. In addition, we created a dynamic interactome of EGFR WT versus L858R with 200 predicted interactors, data which is of high importance for the cancer signaling scientific community.

In summary, here we present a novel powerful proteomics technology, which we are planning to develop as tool for high-throughput proteomic research as well as a drug screening platform.

David Juncker
McGill University

Contributing Authors:

Antibody colocalization microarray (ACM): A Scalable Cross-reactivity free Nano-ELISA platform for proteomics studies

Quantification of proteins with high specificity in blood can be performed using sandwich immunossays (e. g. ELISA). Multiplex sandwich immunoassays (MSI) were developed to measure multiple proteins at once, but cross-reactivity among reagents is known to limit accuracy and reproducibility. Here, we explain the origin of the cross-reactivity in MSI, experimentally show the consequences, and introduce a novel assay format, called the antibody Colocalization Microarray (ACM) that eliminates it.

In MSI, a slide is patterned with an array of capture antibody, incubated with a sample, followed by a mixture of detection antibodies. Here we analyze the combinatorial interactions between detection & capture

antibodies and antigens, and find that it increases quadratically with the number of targets. In an array with 14 targets, we found widespread cross-reactivity.

To overcome the cross-reactivity, we introduce the ACM where all detection antibodies are individually spotted with high accuracy onto their corresponding capture antibody spot. Each spot on the ACM replicates an ELISA, but using on only 1 nl of reagent, constituting a Nano-ELISA. An ACM with 50 targets was shown experimentally to overcome cross-reactivity and to rival the sensitivity of ELISA. The ACM was validated for biomarker studies by measuring proteins in the serum of breast cancer patients and controls, and a candidate biomarker panels was identified. It is currently being used to identify biomarkers for traumatic brain injury.

As the number of targets on the ACM is increased easily, it may become a powerful tool for biomarker discovery and validation.

XingFang Li
University of Alberta

Contributing Authors: Wei Wang, Yichao Qian, Jessica M. Boyd, and Xing-Fang Li

Mass Spectrometry Methods for Characterization and Determination of Disinfection Byproducts in Tap Water and Swimming Pools

Protection of public health requires disinfection of drinking water and recreation pools to effectively prevent water-borne diseases. However, common disinfection treatments unavoidably generate byproducts resulting from reactions between natural organic matter in the source water and disinfectants. The majority (~70%) of disinfection byproducts (DBPs) in treated water remain unidentified. Potential adverse health effects observed in epidemiological studies drive analytical research to identify and determine what DBPs may contribute to these health risks. Here we present the separation and mass spectrometry methods to characterize emerging DBPs of toxicological relevance and investigate the occurrence of these DBPs in swimming pools.

Guy Poirier
Laval University

Contributing Authors: Gagné, J.-P., Chapman, J.D., Goodlett, D.R

High accuracy mass spectrometry provides direct evidence for site-specific auto-ADP-ribosylation of PARP-1

Poly(ADP-ribose) polymerase-1 (PARP-1) is an abundant chromatin-associated protein involved in a variety of pathways such as DNA damage response (DDR), transcription regulation, cell cycle progression and apoptotic cell death. Upon DNA damage induction, DNA-dependent PARPs synthesize an anionic poly(ADP-ribose) (pADPr) polymer to which several proteins bind with the subsequent formation of pADPr-associated multiprotein complexes. PARP hydrolyzes NAD+ in the formation of pADPr on nuclear proteins such as DNA repair enzymes, chromatin remodelers, histones, transcription factors and PARP-1 itself. The covalent attachment of pADPr to a substrate protein alters its physico-chemical characteristics which results in functional consequences on its biological activity.

The exact nature of the ADP-ribose linkage catalyzed by PARP-1 has been the subject of controversy and debate. To date, it is still unclear which of the amino acid side-chains are directly targeted by the ADP-ribose transferase activity of PARP-1. Radiolabeled NAD+ has been a cornerstone in research for tracking and visualizing PARP-catalyzed post-translational protein modification in vitro, but the ability of the negatively charged pADPr to strongly interact with proteins in a non-covalent manner is a cause of confusion when interpreting experimental results. To truly begin to understand the exact nature of the poly(ADP-ribosly)ation reaction, there is a need for more conclusive mass spectrometry evidence of PARP-catalyzed protein modifications. Mapping of these modifications to specific amino acid residues on PARP-1 and other protein substrates of poly(ADP-ribosyl)ation reactions presents significant challenges due to the complex structural heterogeneity of pADPr polymers as well as a loss of amino acid sequencing information that occurs during several mass spectroemetry peptide activation techniques commonly implemented for fragmentation.

Here, we demonstrate a novel method for the identification of PARP-1 auto-poly(ADP-ribosyl)ation sites utilizing high mass accuracy mass spectrometry. By employing an enzymatically driven simplification of the pADPr modifications, we reduce the corresponding complexity of analyzing modified tryptic peptides at the level of the tandem mass spectrum, which in turn enables us to effectively localize exact sites of PARP-1-catalyzed poly(ADP-ribosyl)ation. In conjunction with a phosphopeptide-like enrichment strategy that captures modified peptides, we are able to successfully locate numerous sites of PARP-1 automodification in a high-throughput proteomics screen suitable to universally available mass spectrometry and data analysis pipelines.

Jeffrey Smith
Carleton University

Contributing Authors: Karl Wasslen, Stephen Wood, Jeffrey Manthorpe, Jeffrey C. Smith

Trimethylation Enhancement using Diazomethane (TrEnDi): Rapid On-Column Methylation of Peptides and Proteins to Permit Quantitative Analysis Using Tandem Mass Spectrometry

Trimethylation enhancement using diazomethane (TrEnDi) is an inexpensive and rapid on-column chemical derivatization technique to permit peptide methylation and quantitation. TrEnDi produces trimethylated primary amines that enhances and simplifies label-free quantitation strategies via improved ionization through the formation of fixed quaternary ammonium ions. TrEnDi has been optimized on a microfluidic platform to successfully derivatize peptides from a protein digest with nearly 100% complete N-terminal methylation. Other amino acid side chains with pKa values of approximately 10.5 or less were also methylated. MS/MS analysis confirmed that N-termini trimethylated peptides fragmented to preferentially form the a2 ion, permitting a significant increase in sensitivity when scanning in the MRM mode. This allowed us to perform label free MRM scanning of candidate peptides without prior knowledge of their fragmentation patterns. The trimethylated peptide N-termini were found to be very stable. However, hydrolysis of the newly formed methyl esters occurred over time. Hydrolysis was assisted using base hydrolysis in order to drive the derivatized peptides to homogeneity. To test the analytical sensitivity of derivatized peptides via MRM scanning, several low concentrations of the modified peptides were spiked into a series of more complex peptide solutions and the ability of the MRM method to obtain quantitative data was assessed. Our results have revealed that the trimethylated peptide N-termini produced strong MRM transitions and were identifiable at low concentrations, even in the presence of highly concentrated interferences.

Ruijun Tian
The Samuel Lunenfeld Research Institute

Contributing Authors: Ruijun Tian, Yu Shi, Tony Hunter, Tony Pawson

Exploring bi-directional signaling in pancreatic tumor microenvironment by quantitative proteomics

Pancreatic ductal adenocarcinoma (PDA) is one of the most lethal human cancers with resistance to many chemotherapeutic drugs. This drug resistance effect is believed to be partly caused by stromal-cancer cells interaction in pancreatic tumor microenvironment. Cell-cell interactions are often mediated by protein molecules expressed by one cell that are recognized by specific receptors present on neighboring cells. This intercellular communication activates specific signaling pathways through the induction of dynamic post-translational modifications such as phosphorylation and by protein-protein interactions. Here, we present the development of unbiased quantitative proteomics approaches to characterize a dynamic secretome and critical intracellular signaling in a simulated pancreatic tumor microenviroment in culture. A number of secreted ligand/receptor pairs have been explored which has significant affects to cancer cells proliferation. Our work establishes a generally applicable strategy for studying stromal-cancer cells communication and provides valuable resources for systematically understanding dysfunctional signaling networks in tumor microenvironment in a context closer to physiological conditions than was previously possible.

Derek Wilson
York University

Contributing Authors: Tamanna Rob, Dasantila Golemi-Kotra, Preet Kama-Gill and Derek Wilson

Microfluidics-Enabled, Sub-second Hydrogen/ Deuterium Exchange Pulse Labeling Reveals Allosteric 'Hotspots' in Enzymes.

Regulation of metabolic pathways is achieved by modulation of enzyme activity, commonly via direct interactions at the active site. However, a substantial number of enzymes are also sensitive to changes at locations well removed from the site of catalysis. These 'allosteric' effects are easily detected, but are exceedingly difficult to characterize because they are usually driven by subtle changes in conformation or dynamics. In this work, we use a new microfluidic chip that incorporates a bottom-up workflow with sub-second H/D exchange pulse labeling to probe time-dependent structural and dynamic changes in the ?-lactamase enzyme TEM-1 upon inhibitor binding.

Changes in deuterium uptake were noted from the first time-point after acylation, corresponding to both increases and decreases in labeling ('loosening' and 'tightening' of the structure, respectively). As the acyl-enzyme was allowed to age, time-dependent changes in deuterium uptake were detected, indicating the presence of slow structural changes occurring up to 2 s

after acylation. In general, peptides showing instantaneous changes in uptake were located near the active site, while peptides that exhibited time-dependant changes in deuterium uptake were located at the periphery of the enzyme. In the active site, patterns of increasing and decreasing rigidity explain the roles of Arg244 and Asn276 in the inhibitory mechanism. At the periphery, slow changes in uptake are observed specifically in allosteric 'hotspots' that include residues known from mutational analysis to play a role in TEM-1 catalytic efficiency.

Poster Abstracts

Poster Abstract #1

Janna Anichina
AB SCIEX

Contributing Authors: Janna Anichina, Andre Schreiber, Ron Bonner, and Takeo Sakuma

High-Resolution Tandem Mass Spectrometry Analysis of the Interactions of Oligonucleotides with Selected River Basin Specific Pollutants

Since a great number of organic compounds are annually released into the environment, the necessity to assess in a timely manner the potential risks associated with these chemicals is of high priority. In this study we employed ESI-TOF/MS/MS along with micro LC-ESI-TOF/MS to study interactions of four river basin pollutants (chosen in the context of the European Union Water Framework Directive (EU WFD) with model nucleic acids.

Two AB SCIEX LC-MS systems were used in this study: a hybrid linear ion trap-triple quadrupole system and a hybrid quadrupole-time-of-flight instrument. The AB SCIEX PeakView™ software with a prototype oligonucleotide fragmentation interpretation tool was used for the data analysis.

Interactions of two oligonucleotides (ODNs), d(5'-GCGCATGCGC-3') and d(5'-GCGCGCGCGC-3'), with Diazinon, Diuron, Alachlor and Bis(2-ethylhexyl)phthalate were investigated in the direct infusion mode and with the micro-flow LC separation. LC-MS/MS analysis of the Diuron-ODN incubation mixtures indicated the formation of 1:1 adducts of the single-stranded species at a molar ratio of 10 or higher. Tandem mass spectrometric measurements of the 1:1 adducts of Diuron with both single-stranded ODNs demonstrated that their dissociation proceeds via the loss of a neutral Diuron molecule at a relatively low value of the laboratory frame collision energy. The results of the experiments performed with the studied ODNs and Diazinon were similar to those observed for Diuron-containing species. However, the formation of the 1:1 adducts was observed at a higher excess of Diazinon (50-fold or higher). CID measurements of the Diazinon adducts demonstrated an earlier (compared to Diuron) CID onset which indicates a weaker binding with the ODNs.

No detectable adducts of Alachlor or Bis(2-ethylhexyl)phthalate with either ODNs were formed even at 1000-fold excess of the compound.

Poster Abstract #2

Erica Forsberg
McMaster University

Contributing Authors: Dr. John Brennan

Bio-Solid Phase Extraction and Information Dependent Tandem Mass Spectrometry for the Identification of Enzyme Inhibitors in Complex Mixtures

Identifying novel protein-ligand interactions is a continuing focus in the field of drug discovery, not only to develop novel therapeutics, but also to understand biological processes involved with proteins of unknown function and those involved in disease states. Enzymes are of particular interest since they make up a significant portion of therapeutic targets. There is currently no rapid method available to isolate and identify bioactive compounds from complex mixtures in one simple assay. In this study, enzymes entrapped within bioaffinity columns made by the sol-gel process are used to perform bio-solid-phase extraction (bioSPE), where mixtures are deconvoluted in one experiment.

Bioaffinity columns were prepared using adenosine deaminase (ADA, kcat ~ 10 s-1) and acetylcholinesterase (AChE, kcat > 10,000 s-1). Assay conditions were optimized using an Eksigent nanoLC coupled to a QTrap API 2000 and the known inhibitors erythro-9-(2-hydroxy-3-nonyl)adenine (EHNA, Kd = 6 nM) and huperzine A (Kd = 14 nM). Complex mixtures were infused and equilibrated on the column, subjected to a mild buffer wash to remove unbound compounds, followed by elution of the bound inhibitors with a harsh wash. Elution solvents, including methanol/water, acetonitrile/water and substrates at various concentrations, were assessed for their ability to produce well-resolved peaks. After elution conditions were optimized, data dependent methods were developed to obtain structural information of the

dissociated inhibitor via analysis of MS2 spectra obtained from the fragmentation of peaks above a threshold value.

Poster Abstract #3

Huiyan Li
McGill University

Contributing Authors: Huiyan Li, Sebastien Bergeron, David Juncker

Snap chip 2.0: Double microarray-to-microarray transfer for easy-to-use, high density antibody colocalization microarrays

We introduced the antibody colocalization microarray that allows cross-reactivity free protein profiling of multiple proteins in minute amounts of complex samples and can be used for the discovery and validation of biomarkers. We also introduced the concept of the snap chip that simplifies profiling, but that was limited owing to imprecise alignment. Here, we present the snap chip 2.0 that uses a double transfer of both the capture antibodies and the detection antibodies from slide-to-slide. Thus, following transfer of the capture antibodies, the assay slide with capture antibodies and the intermediate slide with detection antibodies can be stored prior to usage. The alignment accuracy is 63 ?m, and thus over 3000 different antibody spots can be accommodated on a single slide.

To demonstrate the potential of the snap chip, 50 proteins were quantified simultaneously, 40 of which reached pg/ml sensitivity, with EGF reaching 1.1 pg/ml. We then used the snap chip 2.0 to measure the time course of protein levels in the serum of cancer mouse models. Triple-negative human breast cancer cells were injected into 11 mice, along with three controls, and each week blood was collected from each mouse, the concentration of the 50 proteins were quantified, and the size of the tumor was measured. We found 6 proteins whose levels increased continuously and correlated with tumor burden for each of the 11 mice.

To conclude, we have developed the snap chip 2.0 for protein biomarker discovery and validation in blood using serial analysis of minute amounts of sample.

Poster Abstract #4

Peter Liuni
York University

Contributing Authors: Peter Liuni, Derek J. Wilson

Investigating catalysis-linked dynamics in yeast alcohol dehydrogenase by measuring kinetic isotope effects using time-resolved ESI-MS with H/D exchange.

Mass spectrometry combined with millisecond timescale reaction monitoring (TRESI-MS) along with hydrogen/ deuterium exchange (HDX); is emerging as a complementary, straightforward, broad-spectrum alternative to NMR techniques for characterizing dynamics in catalytically active enzyme systems. We use a Synapt G1 equipped with a time-resolved ESI source (Wilson and Konermann, 2003, Anal. Chem. 75) to monitor and initiate yeast alcohol dehydrogenase catalysis (YADH). The YADH system is ideal for examining catalysis-linked dynamic due to the large kinetic isotope effect (KIE) associated with hydride transfer. The extracted ion currents for NADH and NADD at 666 m/z and 667 m/z respectively are monitored as a function of time to obtain KIEs associated with catalysis. Simultaneously, we also monitor the YADH tetramer (5000-10000 m/z) undergoing deuterium exchange as the reaction proceeds. The NAD(H/D) XIC's show a significant observed KIE of VO(NADH)/VO(NADD) = 1.8 ? 0.4. This KIE represents a macroscopic phenomenon which is dominated by the rate determining step - NAD(H/D) release - not hydride transfer. In this case, the apparent specificity constant, KIEspec = (kcat(NADH)/KM(NADH))/(kcat(NADD)/ KM(NADD)) - also written as D(V/K) - is better at defining the KIE, and we observe a D(V/K)= 2.19 ? 0.05. The significant difference between KIEobs and KIEspec suggests a substantial shift in one or more microscopic equilibria upon isotopic substitution. The large (20-fold) excess of co-factor yields mass spectra showing a 4:1 NAD to YADH tetramer stoichiometry as the dominant species, with little to no signal contributing from the other possible stoichiometry's. Presently, we are optimizing the system for H/D exchange experiments, and along with the ion mobility capabilities of the Synapt we may yet reveal intermediate conformations associated with catalytically active states.

Poster Abstract #5

Pik Shan Lo
McGill University

Contributing Authors: V. Laforte, S. Bergeron, P. Lo, J. Marcoux, D. Juncker

Using the Antibody Colocalization Microarray to measure proteins in complex samples from severe traumatic brain injury patients

Introduction

The severely injured brain can develop a hard to predict, complex inflammatory response that leads to generalized swelling of the brain tissue and possibly death. Cerebral microdialysis was developed to sample the brain tissue and monitor several potential biomarkers of tissue injury, but gives samples with small volumes. The antibody colocalization microarray (ACM) is used here to measure the levels of 50 low-abundance inflammatory proteins in small volume, complex samples with high specificity and sensitivity.

Methods

Patients with severe brain injuries undergo surgery where a cerebral microdialysis catheter is inserted into the brain. Hourly microdialysis samples are collected, and samples of cerebrospinal fluid (CSF) and blood are collected every 12 hours for 3 days. 20 ?L of each sample is analyzed using the ACM at without any prior purification step.

Results

Using the ACM, the level of 50 proteins is measured in microdialysate, CSF and blood samples of patients. These proteins have normal levels in the pg/mL range, and are known to be involved in inflammation, brain protection and repair. The levels of several proteins is higher in cerebral microdialysate than CSF and blood, suggesting that they are specifically produced in the brain tissue and may be detected in the blood.

Conclusion

Following this pilot study, additional patients will be recruited. Potential biomarkers to help prognosticate secondary injury in patients suffering from traumatic brain injury will be sought by profiling 100 proteins over time in microdialysate, CSF, and blood, and correlating biomarker profiles with outcome.

Poster Abstract #6

Zhibin Ning
OISB, University of Ottawa

Contributing Authors: Zhibin Ning, Deeptee Seebun, Brett Hawley, Cheng-Kang Chiang, Daniel Figeys

From cells to peptides: "One-stop" integrated proteomic processing using Amphipols.

In proteomics, detergents and chaotropes are indispensable for proteome analysis, not only for protein extraction, but also for protein digestion. In order to increase the protein extraction efficiency, detergents are usually added in the lysis buffer to extract membrane proteins out of membrane structure and to maintain protein in solutions. In general, these detergents need to be removed prior to protein digestion, usually by precipitation or ultrafiltration. Digestion often takes place in the presence of chaotropic reagents, such as urea, which often need to be removed prior to mass spectrometry. The addition and removal of detergents and chaotropes require multiple steps that are time consuming and can cause sample losses. Amphipols (APols) are a different class of detergents that have physical and solubilization properties that are distinct from conventional detergents. They have primarily been used in protein structure analysis for membrane protein trapping and stabilization. Here we demonstrate a simple and rapid protocol for total and membrane proteome preparation using APols. We demonstrate that APols added for cell lysis help maintain the proteome in solution, is compatible with protein digestion using trypsin, and can readily be removed prior to mass spectrometry by a one-step acidification and centrifugation. This protocol is much faster, can be performed in a single tube, and readily replace the conventional detergent/chaotrope approaches for total and membrane proteome analysis.

Poster Abstract #7

Dennis Orton
Dalhousie University

Contributing Authors: Dennis J. Orton, Justin P. Rogers, Alan A. Doucette

A dual capillary nanospray interface for improved throughput in LC-MS

Achieving high confidence in quantitative proteomic studies requires analysis of both biological and technical replicates. Problematically, obtaining quality data from LC-MS analyses is very time-consuming. This often leads to a sacrifice of the total number of replicate runs. Development of technologies to improve LC/MS throughput would therefore improve the quality and confidence of quantitative proteomic results.

This study presents a two-column dual spray system, which employs a single nanoflow "gradient" pump, and an isocratic "loading" pump coupled to an autosampler. The loading pump delivers continuous flow through the autosampler directly to the capillary column at a set solvent composition (i.e. 5% solvent B) for 'loading' samples onto the column. Simultaneously, the nanoflow pump carries out the solvent gradient on the opposing column, eluting analytes for MS detection. A switching valve directs the pump flow to their respective columns, with a high voltage switch to direct voltage to the

operating column. This setup improves throughput by enabling essentially 100% duty cycle (column loading and re-equilibration times are eliminated). This provides up to two times the number of runs to be performed in the same timeframe as traditional LC-MS. Column efficiency and retention time performance are evaluated and controlled using internal standards during each run. The internal standard also corrects for variations in spray efficiency between the two independent columns. Our system provides a cost-effective method for improving throughput of proteomic analyses, providing higher confidence in quantitative data without increasing the time required for analysis.

Poster Abstract #8

Abul Sardar
Institute of Parasitology, McGill University

iTRAQ coupled 2D-LC-MALDI-TOF/TOF analysis reveals proteome changes associated Leishmania donovani promastigote adaptation to oxidative and nitrosative stress

Phagocytic cells produce reactive oxygen and nitrogen species (ROS & RNS) as the most common arsenal to kill intracellular pathogens. Leishmania, an obligate intracellular pathogen also confronts this antimicrobial assault during the early phase of infection but nevertheless is able to survive these attacks and proliferate in macrophage. Adaptation of Leishmania to the toxic effects of ROS and RNS, involves a rapid change in the parasite proteome to combat the host defense response that macrophage mount in combating pathogen. To understand the events associated with combating ROS and RNS species, we performed a proteomic analysis of L. donovani promastigotes treated with sub-lethal doses of menadione (ROS), S-nitroso-N-acetylpenicillamine (RNS) or combination of both compounds. Proteomic changes triggered by these reagents were evaluated by iTRAQ labeling and subsequent LC-MALDI-TOF/TOF-MS analysis. Across the 3 stress conditions, the quantitative analysis identified changes in the proteins which encompass ~20% of the parasite proteome. Major changes were observed in enzymatic machinery of pathways involved in maintaining redox homeostasis, trypanothione metabolism, oxidative phosphorylation, superoxide metabolism, mitochondrial respiration process and other essential metabolic pathways. These observations shed light on how Leishmania promastigotes counter ROS and RNS affects during the initial stage of infection.

Poster Abstract #9

Anne Marie Smith
McMaster University

Contributing Authors: Anne Marie Smith, John D. Brennan

Development of a Multiplexed Kinase Assay via MALDI-MS/MS Detection

One of the most important drug targets today are kinases due to their involvement in a multitude of human diseases and bacterial infections (1). Kinases contain two binding sites; one for substrate and one for ATP. Inhibition of these sites can reduce or prevent enzyme expression and hence diseases and infections (2). Previous work from our group showed the development of a MALDI-MS/MS assay for the detection of small molecule modulators of the aminoglycoside kinase APH3'IIIa (3). Rathore et al. showed the development of a mass spectrometry based assay for detection of inhibitors of a kinase and esterase reaction simultaneously (4). Herein, we demonstrate the development of an enhanced kinase MALDI-MS/MS assay involving concurrent detection of two kinase modification events, amplifying assay throughput. The detection of two kinase reaction products allows for the use of a kinase directed library to be screened with a greater probability of a hit". The assay was validated through generation of a Z'-factor in the presence of high ionic strength (Mg2+). A selection of compounds designed to bind to the ATP binding site of kinases were screened for identification of inhibitory action. Following deconvolutionOne of the most important drug targets today are kinases due to their involvement in a multitude of human diseases and bacterial infections 1. Kinases contain two binding sites; one for substrate and one for ATP. Inhibition of these sites can reduce or prevent enzyme expression and hence diseases and infections 2. Previous work from our group showed the development of a MALDI-MS/MS assay for the detection of small molecule modulators of the aminoglycoside kinase APH3′IIIa 3. Rathore et al. showed the development of a mass spectrometry based assay for detection of inhibitors of a kinase and esterase reaction simultaneously 4. Herein, we demonstrate the development of an enhanced kinase MALDI-MS/MS assay involving concurrent detection of two kinase modification events, amplifying assay throughput. The detection of two kinase reaction products allows for the use of a kinase directed library to be screened with a greater probability of a “hit”. The assay was validated through generation of a Z′-factor in the presence of high ionic strength (Mg2+). A selection of compounds designed to bind to the ATP binding site of kinases were screened for identification of inhibitory action. Following deconvolution, IC50 curves and KI values were generated for those compounds identified as “hits” for either kinase.

1) Cohen, P. Nature Rev. Drug Discov., 2002, 1, 309. 2) Renau, T.E. et al. Annu. Rep. Med. Chem. 1998, 33, 121. 3) Smith, A.M. et al. Anal. Chim. Acta, submitted. 4) Rathore, R. et al. J. Biomol. Screen, 2010, 15, 1001.

Poster Abstract #10

Jun Song
Agriculture and Agri-Food Canada

Contributing Authors: Yang, XiaoTang, Li Li, Jun Song*, Leslie Campbell Palmer, Devanand Pinto, Kenneth Chisholm, Li, XiHong and Zhang, ZhaoQi

OFFGEL peptide pre-fractionation is essential for proteomic approaches employing quantitative stable isotope dimethyl labeling and multiple reaction monitoring (MRM) of fruit proteomic research

OFFGEL isoelectric focussing (IEF) has become a popular tool in proteomics research to fractionate peptides or proteins. We conducted a detailed investigation on fruit proteomics of apple, banana, and strawberry fruit employing OFFGEL-IEF as a crucial step to improve the proteome coverage and quantitative proteomic workflows including isotope dimethylation labeling and multiple reaction monitoring (MRM). We provide the technical details concerning application of OFFGEL-IEF, nano-LC/MS detection and MRM optimization and analysis. Our results demonstrated that the application of OFFGEL is a powerful and effective method for peptide fractionation and increased significantly the number of proteins identified, with more assigned peptides collected. In combination with isotope dimethylation labelling technique as a quantitative proteomic procedure, we identified and quantified more than 500 proteins and reported more than 94 proteins that are in response to ethylene as well as high temperature treatments in banana fruit. Further, we developed a protocol combining OFFGEL and MRM studies to identify and quantitatively investigate monodehydro-ascorbate reductase, a key enzyme in the redox and antioxidant system of apple fruit during fruit ripening. Our results provide direct and comprehensive evidences demonstrating the benefits of OFFGEL and its application for various areas of both shotgun and quantitative proteomics research.

Poster Abstract #11

Chady Stephan
PerkinElmer

Pushing the Frontier in ICP-MS Applications: Counting and Sizing Nanoparticles using Single Particle ICP-MS

Engineered Nanomaterials (ENMs) are synthesized by a manufacturing process that produces and controls ENMs to have at least one dimension in the range of 1 to 100nm in size. ENMs often possess different properties than bulk materials of the same composition, making them of great interest to a broad spectrum of industrial and commercial applications. The widespread use and application of ENMs will inevitably lead to their release into the environment, which raises concern about their potential adverse effects on the ecosystems and, subsequently, human health. To better understand ENMs in the environment, the following ENM characteristics will aid in this assessment: concentration, composition, particle size, shape, and other surface characteristics.

This work describes the theory and application of Single Particle- ICP-MS in analyzing Metal Based Nanoparticles. Single Particle (SP-ICP-MS) allow the differentiation between ionic and particulate signals, quantitate both the ionic and particulate fraction, measures particle concentration (part/mL), particle sizes (if shape is known), and explores agglomeration and size distribution.

SP-ICP-MS is a key analytical instrument in assessing the fate, behavior and distribution of (ENMs) in several types of matrices (environment, food, etc.), evaluating ENMs bioavailability and bioaccumulation in the biota, and improving bio-labeling capabilities and advancements in the medical field.

Poster Abstract #12

Declan Williams
York University

Intracellular signalling network expression profiling by LC-MS with a heavy isotope-labelled QconCAT internal standard

Stefanie Madler[1], Leroi V. DeSouza[1], Ajay Matta[1], and K.W. Michael Siu [1]

Metabolic pathways are coordinated by a complex protein network, the behaviour of which depends on the concentrations of its components. Redundancy and synergy among signal transduction proteins confounds the interpretation of biological consequences arising from expression changes to one or a few pathway members. Here we present a method for profiling the expression of an interaction network employing a single internal standard. Protein expression was standardized using a heavy isotope-labelled QconCAT construct incorporating surrogate tryptic peptides for twenty-nine distinct gene products. Targets of the assay included cell surface receptors and their downstream effectors,

representing pathways controlling cell survival and proliferation. Endogenous peptides and their standards were examined in trypsin-digested cell lysates by targeted nanoflow reversed phase LC-ESI-MS/MS with and without upstream offline strong cation exchange chromatography. The immortal glioblastoma multiforme cell lines U87 and U373 were used to evaluate the performance of the assay. Observed expression levels of several protein targets were consistent with those reported from genomic and other proteomic methods. Assay linearity was evaluated by comparison of the response for mixtures of unlabelled and heavy-labelled versions of the construct. The standardized assay represents a transferrable system for profiling a signalling network which mediates distinct intracellular processes. 1 Centre for Research in Mass Spectrometry, York University, Department of Chemistry, 4700 Keele St. Toronto, ON. M3J 1P3.

Tech Talk

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About ETP Symposium Inc.,

its roots in research.

The ETP Symposium was initiated as part of a Genome Canada project sponsored by MDS Sciex (now AB SCIEX), Genome Alberta and Genome Prairie. Its major theme was to have world-renown scientists present papers on their experiences with novel technologies that have, or will have a major influence on research in the life sciences. The popularity of the original two symposia led to the creation of a not-for-profit entity, ETP Symposium Inc., who now is responsible for organizing the symposium on a biennial basis. The theme remains the same: alerting scientists in the life sciences to advances in new technology that could have a significant impact on their research activities.

The original founders of ETP are Dr. William (Bill) Davidson and Janette Champ.

Since its inception in 2004, ETP has been fortunate to have world-renown scientists present their work as demonstrated below:

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NOTES:

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