Enabling Technologies

6th International Symposium on Enabling Technologies for Life Sciences

October 7, 2011
Omni Parker House
Boston, MA

Chaired by:
Dr. Catherine Costello
Boston University School of Medicine

Bringing Researchers and Scientists together to develop novel life sciences technologies.

www.etpsymposium.org

NOTES:

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Welcome

On behalf of the Organizing and Scientific Advisory Committees, I would like to welcome you to the ETP Symposium Atlantic Conference. This symposium once again has 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 traditionally has been held every two years. This year we are breaking tradition and organizing not only two meetings, but also expanding to the United States. 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.

We wish to thank Dr. Catherine Costello for Chairing this meeting and for welcoming us to Boston.

Once again, welcome to the Enabling Technologies Symposium, Atlantic Conference.

Janette Champ
President
ETP Symposium Inc.

Organizing Committee

Dr. Catherine Costello, Chair
Dr. Robert Boyd, Researcher Emeritus, National Research Council of Canada
Janette Champ, President, ETP Symposium Inc.

ETP Symposium Scientific Advisory Board

Ruedi Aebersold, Institute of Molecular Systems Biology, ETH Europe
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Chris Dambrowitz, Alberta Innovation
Norm Dovichi, University of Notre Dame
Randy Johnston, Dept. of Biochemistry & Molecular Biology, University of Calgary
Joshua LaBaer, Biodesign Institute, Arizona State University

AGENDA

Friday, October 7, 2011 Press Room, Omni Parker House Hotel, 60 School Street, Boston MA		
7:30 am	Exhibitor & Poster Set Up	
8:15 am	Registration, Coffee with Exhibitors & Sponsors	
9:00 am Conference Begins - Press Room		
9:00 am	Welcome, Opening Remarks	Dr. Catherine Costello Boston University School of Medicine
9:15 am	*Mapping and Measuring Proteomes in Systems Biology*	Dr. Ruedi Aebersold, Institute for Molecular Systems Biology, ETH Zurich
9:45 am	*Interrogating Protein Motions by Hydrogen Exchange Mass Spectrometry*	Dr. Natalie G. Ahn University of Colorado
10:15 am	*Characterizing HIV specific antibodies in sera from individuals who do not progress towards AIDS*	Dr. Brian Chait Rockefeller University
10:45 am Coffee Break - Poster Session & Exhibits - Alcott Room		
11:00 am	*The Impact of Preanalytical Variables on Biomarker Research*	Dr. David Craft, BD Sponsored Tech Talk
11:15 am	*Palaeoproteomics: when archaeology meets mass spectrometry*	Dr. Jane Thomas-Oates, The University of York **Sponsored by:**
11:45 am	*High Resolution Quantitation of Cell Metabolism with Multi-Isotope Imaging Mass Spectrometry*	Dr. Claude Lechene Harvard Medical School
12:15 am	*A New Strategy for Proteomics through Integrated MALDI and ESI (PRIME) Yields a Greater Depth of Information*	Dr. Dale Shannon Cornett, Bruker Daltonics
12:30 to 1:30 pm - Lunch, Poster Session & Exhibits - Alcott Room		
1:30 pm	*Mass Spectrometry on Steroids: Rapid Detection of Small Molecules Relevant to Athletic Doping*	Dr. J. Thomas Brenna Cornell University
2:00 pm	*MS-based approaches the elucidation of Nucleic Acid higher-order structure*	Dr. Daniele Fabris University at Albany
2:30 pm	*Nucleic Acid Sequence and Methylation Enrichment Using SCODA*	Dr. Joel Pel Boreal Genomics
3:00 pm Coffee Break - Poster Session & Exhibits - Alcott Room		
3:15 pm	*Strategies for Increasing Selectivity Towards Peptide Quantification*	Dr. Brigitte Simons, AB SCIEX Sponsored Tech Talk
3:30 pm	*Neuropeptidomics: approaches enabling the discovery of new neuropeptides and the elucidation of their functions*	Dr. Jonathan Sweedler University of Illinios
4:00 pm	*Massively multi-parameter single cell data by Mass Cytometry: the technology of its acquisition and networks for its interpretation*	Dr. Scott Tanner University of Toronto
4:30 pm	*Closing Remarks*	Dr. Catherine Costello Boston University School of Medicine
4:45 pm - Reception, with Posters & Exhibits - Alcott Room		
6:30 pm Conference Ends		

Symposium Chair

Catherine Costello

Boston University School of Medicine

Dr. Costello is a Professor in the Departments of Biochemistry and Biophysics at Boston University School of Medicine and the Department of Chemistry at Boston University, and is the founding Director of both the NIH-NCRR Mass Spectrometry Resource and the NIH-NHLBI Cardiovascular Proteomics Centre at BUSM. She has served as Vice-President and President of the American Society for Mass Spectrometry and is currently President of HUPO and Vice-President (Society) of the International Mass Spectrometry Foundation; she is a Councilor for the American Chemical Society, a member of the boards of the Human Proteome Organization, the Human Disease Glycomics/ Proteome Initiative and the US Human Proteome Organization, and numerous editorial, review and advisory boards.

Dr. Costello is the 2010 winner of the Frank H. Field and Joe L. Franklin Award for Outstanding Achievement in Mass Spectrometry. Dr. Costello's research aims to refine and extend mass spectrometry methods for proteins and glycans and to elucidate structure-activity relationships as they influence or reflect processes related to health, growth and development, and disease.

Invited Speaker Abstracts

Ruedi Aebersold

Institute of Molecular Systems Biology, ETH Zurich and Faculty of Science, University of Zurich

BIOGRAPHY:

Ruedi Aebersold is a Professor of Molecular Systems Biology at ETH Zurich (Federal Institute of Technology) and the University of Zurich, in Zurich, Switzerland. Before moving to ETH he was a co-founder and faculty member at the Institute for Systems Biology, in Seattle, WA and a Professor at the University of Washington in Seattle, WA.

Recognizing that systems biology research is starved for high quality datasets that describe biological systems, the he focus on his research is on the development and the application of new proteomics technologies. These technologies aim at detecting and quantifying the proteins and their interactions at the level of the complete proteome. Specific applications of the technology are directed towards the understanding of biological processes and the detection and validation of protein biomarkers for the early detection, diagnosis and classification of disease.

ABSTRACT:

Mapping and Measuring Proteomes in Systems Biology

The human genome project has taught us that a complete map -in the case of the genome project the complete genomic sequence – along with computational tools to navigate the map - represent invaluable resources for experimental and theoretical biologists. A main consequence of such a complete map is that all the biological processes have to be explainable with the components that constitute the map. Proteomics has not reached the stage that complete maps are available but the urgent need for their generation is now widely recognized.
In this presentation we will discuss experimental and computational challenges related to the generation of complete proteomic maps using mass spectrometry, and instrumentation and methods to use the information contained in proteome maps for targeted proteomic experiments. These measurements are capable of generating complete, reproducible and quantitatively accurate datasets on differentially perturbed systems. These data are therefore ideally suited to support the generation of mathematical models in systems biology research. We will also discuss recent technical advances towards complete proteome analysis and describe software tools and data resources that will transform proteomics from perpetual proteome mapping to the accurate measurement of proteomes in cells at different states.

Natalie G. Ahn
Department of Chemistry and Biochemistry, Howard Hughes Medical Institute, University of Colorado at Boulder, Boulder, CO 80309-0215

BIOGRAPHY:

Natalie Ahn is Professor of Chemistry and Biochemistry and HHMI Investigator at the University of Colorado at Boulder. She received her Ph.D. from the Univ. of California, Berkeley, working with Judith Klinman on the enzymology of hydrogen transfer reactions. She then carried out post-doctoral studies with Christoph de Haen on the purification of annexins, and later with Edwin Krebs on the discovery and characterization of MAP kinase kinases, key regulators of MAP kinase pathways. Dr. Ahn's current research elucidates enzymatic and cellular mechanisms for signaling through MAP kinase, Rho GTPase and Wnt pathways, which she approaches by combining biochemical, cell biological, and molecular biological strategies with proteomics and mass spectrometry technologies.

ABSTRACT:

Interrogating Protein Motions by Hydrogen Exchange Mass Spectrometry

It is often postulated that protein motions underlie conformational and entropic contributions to enzyme catalysis, however relatively little is known about how this really occurs. To study this, we are measuring protein hydrogen/deuterium exchange by mass spectrometry (HX-MS) which reports internal motions of the folded state, where exchange predominantly occurs through low energy fluctuations in protein structure that enable transient solvent accessibility. Such fluctuations, represented by free energy differences (ΔΔG) on the order of 1 kcal/mol, lead to changes in protein function even where no structural differences are observed. HX-MS analysis of the mitogen-activated protein kinase, ERK2, reveals altered conformational mobility within localized regions of the enzyme upon catalytic activation. HX changes can be ascribed to enhanced backbone flexibility at the hinge between the conserved N- and C-terminal domains, predicting that interdomain closure may occur following ERK2 activation. This is corroborated by evidence that ERK2 binds nucleotide in two modes, where the active kinase adopts a closed conformation, whereas inactive ERK2 is constrained in an open conformation. Thus, phosphorylation of ERK2 releases constraints to interdomain closure needed for catalytic turnover, by regulating protein flexibility. Importantly, patterns of activation-induced HX differ between ERK2 and its close paralog ERK1, despite their similarities in overall deuteration. Furthermore, ERK1 does not show constraints to domain closure prior to activation. Therefore, although MAP kinases are closely related with respect to primary sequence and tertiary structure, they utilize distinct mechanisms for dynamic control of enzyme function.

J. Thomas Brenna
Cornell University

BIOGRAPHY:

Tom (J. Thomas) Brenna, PhD, is Professor of Human Nutrition and of Chemistry and Chemical Biology at Cornell University, Ithaca, New York. His PhD was with Professor George Morrison at Cornell in biomedical imaging by Secondary Ion Mass Spectrometry. He and his group are NIH supported for development and application of high precision isotope ratio mass spectrometry (IRMS) for study of polyunsaturated fatty acids in perinatal nutrition. They are currently developing molecular IRMS instrumentation and methodology for detection of doping with endogenous and designer steroids, most recently supported by the Partnership for Clean Competition, a coalition of the US Antidoping Agency, Major League Baseball, the NFL, and other professional and amateur athletics organizations.

ABSTRACT:

Mass Spectrometry on Steroids: Rapid Detection of Small Molecules Relevant to Athletic Doping

Steroids and other small molecules are the most important class of performance-enhancing substances used for improving athletic results. Numbering over 300 on the World Antidoping Agency (WADA) banned substance list, they consist of substances that are (a) endogenously synthesized (e.g. testosterone), (b) ingested in foods (e.g. clenbuterol), and (c) exogenous subtances (e.g. designer steroids). The ever expanding range of small molecules of interest is outstripping the capability to screen for them quantitatively and, importantly, for isotope ratio to reveal exogenous intake of endogenously produced molecules. The nature of the problem will be introduced and discussed from an analytical chemist's perspective. Development of the first high throughput, comprehensive two-dimensional gas chromatography methods coupled to molecular and isotopic mass spectrometry will be shown for urinary steroid analysis.

Brian T. Chait
Camille and Henry Dreyfus Professor,
Rockefeller University

BIOGRAPHY:

Brian T. Chait is currently Camille and Henry Dreyfus Professor at Rockefeller University in New York, where he is Head of the Laboratory for Mass Spectrometry and Gaseous Ion Chemistry. He also directs the NIH-funded National Resource for the Mass Spectrometric Analysis of Biological Macromolecules. Dr. Chait's laboratory specializes in the development and use of mass spectrometry as a tool for investigating a variety of biological and biochemical phenomena. He has co-authored some 327 publications (cited >32,000 times) and has been awarded 23 US patents.

ABSTRACT:

Characterizing HIV specific antibodies in sera from individuals who do not progress towards AIDS

Sequence and structural convergence of broad and potent HIV antibodies that mimic CD4 binding.

A team of researchers at The Rockefeller University and the Howard Hughes Medical Institute has found a way to investigate the broadly neutralizing antibody response against the CD4 binding site of HIV on a monoclonal level. Passive transfer of broadly neutralizing HIV antibodies can prevent infection, which suggests that vaccines that elicit such antibodies would be protective. Thus far, however, few broadly neutralizing HIV antibodies that occur naturally have been characterized. To determine whether these antibodies are part of a larger group of related molecules, we cloned 576 new HIV antibodies from four unrelated individuals. All four individuals produced expanded clones of potent broadly neutralizing CD4–binding site antibodies that mimic binding to CD4. The new antibodies shared a consensus sequence of 68 immunoglobulin H (IgH) chain amino acids and arise independently from two related IgH genes. This led to the identification and characterization of several "highly active anti-CD4 binding site antibodies" (HAADs) and their expanded B cell families (see J.F. Scheid et al., Science, 11 August 2011 [Epub ahead of print]). This presentation will highlight aspects of our mass spectrometric investigation that allowed us to compare for the first time the memory B cell and plasma cell compartments for these highly neutralizing protective antibodies.

Daniele Fabris
University at Albany

BIOGRAPHY:

D. Fabris is Professor of Chemistry and Biological Sciences at the University at Albany. 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 footprinting and crosslinking 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 approaches the elucidation of Nucleic Acid higher-order structure

The observation that less than 1.5% of the human genome codes for actual proteins has lead to the realization that sequence information alone is insufficient to elucidate the function of the vast majority of nucleic acids in living organisms. The recent discovery of riboswitches has keenly reasserted the critical role played by higher-order structure in determining the function of non-coding elements. Beyond sequencing, MS-based approaches can provide direct information about base-pairing and long-range interactions, which respectively define the secondary and tertiary structure of nucleic acids. The ability to observe intact assemblies with other nucleic acid elements and cognate proteins enables the investigation of their quaternary structure. For these reasons, we have been employing electrospray ionization (ESI) with Fourier transform ion cyclotron resonance (FTICR) mass spectrometry to investigate the structure-function relationships of the 5' untranslated region (5'-UTR) of the genome of HIV-1 and its assemblies with the chaperone nucleocapsid (NC) protein. The talk will illustrate the development of solution and gas-phase approaches, which has enabled us to obtain the 3D structure of the psi-RNA region of 5'-UTR. The characterization of the NC binding sites and the ability to detect the ensuing conformational changes have provided the basis for proposing a possible mechanism for the processes of genome recognition, dimerization, and packaging mediated by these specific protein-RNA interactions.

Claude Lechene
Harvard Medical School

BIOGRAPHY:

Dr. Lechene is a professor of Medicine at Harvard Medical School and BWH and director of the National Resource for Imaging Mass Spectrometry (NRIMS) of NIBIB/NIH. He has been Maitre de Conference in Physiology at Paris University, Associate Director of the Basic Sciences Division at Sherbrooke University Medical School. He is a Fellow of the American Association for the Advancement of Science and a Senior Scholar of the Ellison Medical Foundation. He is also a member of a number of professional societies, including the ASCB. He studied Mathematics General Physics and Physical Chemistry at and received an M.D. from the University of Paris.

He has contributed to renal, reproductive, and digestive physiology, cellular biology, the development of ultramicrofluorescence method and electron-probe microanlysis in biology, the conceptual basis of renal concentration mechanism, the biological role of the Na/K-pump, and the role of the gel nature of the cell in maintaining cellular volume. He and his laboratory are presently developing Multi-isotope Imaging Mass Spectrometry (MIMS) which is the combination of ion microscopy-secondary ion mass spectrometer with stable isotope tracer methods and quantitative image analysis.

ABSTRACT:

High Resolution Quantitation of Cell Metabolism with Multi-Isotope Imaging Mass Spectrometry

Claude P. Lechene, MD(1,2), Matthew L. Steinhauser (2), Samuel Senyo(2), Christelle Guillermier(1), Mei Wang(1), Joseph C. Poczatek(1), Todd S. Perlstein(2), Richard T. Lee(2), Andrew Bailey(3), Alex P. Gould(3), Duan-Sun Zhang(4), Valeria Piazza(4,5), David P. Corey(4)

(1)National Resource for Imaging Mass Spectrometry, Division of Genetics, Harvard Medical School and Brigham and Women's Hospital, Cambridge, MA USA
(2)Department of Medicine, Brigham and Women's Hospital and Harvard Medical School, Boston, MA, USA
(3)Division of Developmental Neurobiology, Medical Research Council National Institute for Medical Research, Mill Hill, London, NW7 1AA. U.K.
(4)Department of Neurobiology, Harvard Medical School and Howard Hughes Medical Institute Boston, MA, USA
(5)present address: Georg-August-University, Göttingen, Germany

Multi-isotope Imaging Mass Spectrometry (MIMS) is the combination of an ion microscope-secondary ion mass spectrometer with tracer methods and intensive quantitative image analysis. MIMS allows one to image the distribution and to measure the accumulation of molecules labelled with stable or radioactive isotopes within subcellular domains in volume smaller than 1micron cubed . The lateral resolution is better than 30 nm, and the depth resolution is a few atomic layers. Up to seven quantitative atomic mass images (or tags) can be recorded simultaneously. A whole cell can be studied layer-after-layer and rendered in quantitative 3D.
MIMS can provide unique information in almost all fields of biomedical research, particularly in two broad areas: 1. metabolic turnover (protein, lipids and sugars in sub-cellular domains using as precursors for example 15N-labeled amino-acids or 13C labeled fatty acids) 2. cell lineage tracking individual cells within large populations by labelingDNA. It could also be appplied to drug localization.
Because stable isotopes are integral part of living organisms, they are not toxic. This allows one to label DNA with a stable isotope precursors for period of time that could reach years. And, this allows applications of MIMS to human.
We will show quantitative analysis of metabolites turnover in domains smaller than subcellular organelles using MIMS analysis of either electron microscopy sections (70 nm) or volumetric reconstruction from MIMS analysis of hundreds of planes. We will show generational cell tracking by imaging and measuring nucleotides incorporated into DNA during cell replication.

Joel Pel
Boreal Genomics

BIOGRAPHY:

Joel Pel is the Head of Research and Applications for Boreal Genomics Inc., a 35-person-and-growing start-up company based in Vancouver, B.C, which develops nucleic acid purification and enrichment instruments around its SCODA technology. Pel is also a co-founder of the company, which began in 2007, while he was a graduate student.

Joel has a Bachelor's of Applied Science degree in Engineering Physics, with a specialization in Electrical Engineering, which he obtained from UBC in 2005. During his Bachelors, he began working with Dr. Andre Marziali, developing technologies for life science research, including working with Andre as the first person to demonstrate the SCODA technology. He then went on to do his PhD in Physics with Marziali at UBC to further develop the SCODA technology, while working part time for Boreal Genomics. He completed his PhD in mid 2009, after which he transferred to full time work at Boreal Genomics. Boreal has since released its first nucleic acid purification instrument, the Aurora, and is working towards a second generation system for sequence enrichment.

ABSTRACT:

Nucleic Acid Sequence and Methylation Enrichment Using SCODA

We have previously presented a novel electrophoretic concentration technology, named SCODA (Synchronous Coefficient of Drag Alteration) for efficiently purifying and concentrating nucleic acids. SCODA is able purify DNA from a variety of complex matrices, including samples that contain strong PCR inhibitors. We are also able to recover nucleic acids from extremely dilute samples, with successful concentration from starting DNA concentrations in the zeptomolar range.

More recently we have demonstrated that SCODA can be made specific to the sequence of DNA targets to be concentrated, opening the opportunity for sequence enrichment applications. Recent experiments show that SCODA can enrich for single nucleotide mutations by 10,000 fold compared to the wild type, and that it is capable of separating identical sequences that differ only in degree of methylation.

This presentation will give a brief overview of the SCODA technology with emphasis on recent progress in sequence specific DNA concentration and snapshots to future directions.

Jonathan Sweedler
University of Illinois

BIOGRAPHY:

Jonathan Sweedler received his Ph.D. degree in Chemistry from the University of Arizona in 1988 and spent three years at Stanford before moving to the University of Illinois at Urbana-Champaign. He is currently the Eiszner Family Professor of Chemistry, as well as the director of the Roy J. Carver Biotechnology Center; he is also affiliated with the Institute of Genomic Biology and the Beckman Institute for Advanced Science and Technology. His research emphasizes analytical neurochemistry. Specific areas of technology development include small-volume peptidomics and metabolomics approaches. These involve single cell mass spectrometry, capillary electrophoresis separation methods, laser-based detection methods, nanoliter volume NMR and micro/nanofluidic sampling. The second research theme applies these technologies to the study of the distribution and dynamic release of neuropeptides and classical transmitters, as well as their metabolism, in a cell-specific manner. He is currently an associate editor of Analytical Chemistry. See http://www.scs.illinois.edu/sweedler/

ABSTRACT:

Neuropeptidomics: approaches enabling the discovery of new neuropeptides and the elucidation of their functions

Neuropeptides are critical molecules that modulate the physiological activity of almost every neuronal circuit in the brain. Surprisingly, though, more and more brain peptides are being discovered. Even the rate of brain peptide discovery is accelerating. What do these novel peptides do? Two major areas are addressed here, one technical and one biological. The first area highlights mass spectrometry-based technologies to characterize the brain peptides from samples ranging from defined brain nuclei to single cells. Using these cutting-edge mass spectrometry-based approaches, we generate lists of known and unique peptides from specific brain regions, with these lists reaching to hundreds of peptides. Even for small brain areas, we still detect hundreds of peptides, making follow-up studies daunting. The second area addresses the question of which peptides are worth extensive follow-up studies. Using the suprachiasmatic nucleus (SCN) as an example, we highlight approaches to perform functional studies such as measuring the peptides released from the SCN in an activity dependent manner and at specific times of the day. Other approaches allow us to image peptide distributions without isolating the cells of interest using mass spectrometry imaging, and a modified protocol for high throughput mass spectrometry profiling of neurons. Several additional examples of neuropeptide discovery are described across a range of metazoan life.

Scott Tanner
University of Toronto

BIOGRAPHY:

Scott D. Tanner is President, CEO and Co-Founder of DVS Sciences Inc., a biotechnology company that develops, manufactures and markets analytical instruments and reagents for high throughput, massively multi-parameter single cell analysis. DVS has headquarters in Sunnyvale, California and R&D and manufacturing facilities in Markham, Ontario Canada. He is also a Professor in the Department of Chemistry at the University of Toronto, Canada.

Following receipt of a PhD in physical chemistry from York University (Toronto), he joined Sciex Inc., subsequently MDS Sciex, in 1980 on a Research Fellowship, and stayed for 25 years, eventually becoming Principal Scientist. His interest in space-charge-limited ion flow led him into the development of ion optics for Inductively Coupled Plasma Mass Spectrometry. After developing a string of commercially successful mass spectrometry instruments, Scott and his team began looking at applying the multiplex capability of ICP-MS to bioassays through element-tagging of antibodies.

Scott and his ICP-MS colleagues left Sciex in 2005 for the University of Toronto to take on their new challenge of applying mass spectrometry as a detector for flow cytometry. DVS Sciences spun out of the University to bring the instrumentation and reagents to the market.

Scott received the University of Toronto 2011 Inventor of the Year Award in Biomedical and Life Sciences, the 2011 Thermo Fisher Scientific Spectroscopy Award, the 2003 W.A.E. McBryde Medal for analytical chemistry, the 2001 Manning Innovation Foundation Award of Distinction, and is a Fellow of the Royal Society of Chemistry (UK).

ABSTRACT:

Massively multi-parameter single cell data by Mass Cytometry: the technology of its acquisition and networks for its interpretation

Scott D. Tanner, Dmitry R. Bandura, Olga I. Ornatsky and Vladimir I. Baranov
DVS Sciences Inc., 70 Esna Park Drive, Unit 12, Markham, Ontario L3R 6E7 CANADA

Mass Cytometry brings the power, resolution, sensitivity and quantitative capabilities of atomic mass spectrometry to high throughput single cell analysis in order to address the challenges of multi-parameter, quantitative flow cytometry. Individual cells that have been immunologically stained with stable isotope tags are injected into the analytical instrument that "reads" the tag elements. The cells are vaporized, atomized and ionized in a high temperature plasma, and the atomic composition of each cell – including the metal tags – is measured by time of flight mass spectrometry. Adapted from its long-time use in elemental analysis, the atomic mass spectrometer provides high sensitivity for many (up to 100) independent mass channels and offers the capability for absolute quantification. At present, 35 stable isotopes of the metals are available as tags, and we expect that another 30 will be available in the foreseeable future with the eventual potential for 100. The staining protocol is similar to that of flow cytometry, and the data output is in FCS format for porting into third party flow cytometry analysis software. Because the detection channels are independent, and the sensitivity to each probe is similar, the selection of staining panels is trivial. Accordingly, it is as easy to quantitatively analyze many parameters as a few, facilitated by the absence of need for compensation. A high level introductory tutorial on the technology of element-labeling and analysis will be given. We will use data from our laboratory and that of our collaborators in the Nolan group at Stanford University, notably on determining differential immune and drug responses across a human hematopoietic continuum using 31 simultaneous cell surface and intracellular probes, to assess the current art in multidimensional data analysis.

Jane Thomas-Oates
The University of York

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BIOGRAPHY:

Jane Thomas-Oates obtained her BSc (Hons) degree in Biochemistry from Imperial College of Science and Technology, University of London, UK, in 1981. She carried out her PhD (1981-1984) at Imperial College with Professor Anne Dell, who was pioneering the development and application of the then newly-introduced technique of Fast Atom Bombardment mass spectrometry, for the analysis of glycoconjugates. She went as a post-doctoral researcher and then Senior Research Associate to the group of Professor Peter Albersheim, at the University of Colorado in Boulder, making the move with that group to the University of Georgia, when the group moved to Athens, GA, to establish the Complex Carbohydrate Research Center. Returning to the UK after two years in the US, a further period at Imperial College as a postdoctoral researcher followed. Jane obtained a Beit Memorial Research Fellowship in 1989, which she took up in the then expanding Department of Biochemistry at the University of Dundee, Scotland. Two years in Dundee (1989-1991) were followed by her appointment to a faculty position (Universitair Docent) in the department of Mass Spectrometry at the University of Utrecht's Faculty of Chemistry (1991-1998) and then to a Senior Lectureship in the Departments of Chemistry and Biomolecular Science at UMIST in Manchester (1998-2002), where her group formed part of the Michael Barber Centre for Mass Spectrometry. In 2002 she was appointed to the RSC/EPSRC Chair of Analytical Science at the University of York, UK, where she leads the Analytical Science and Environmental Chemistry Research group, and is Chair of the Centre of Excellence in Mass Spectrometry. The focus of the York Chair of Analytical Science is the development and application of mass spectrometry-based analytical approaches for post-genomic science. Jane's research interests are in biological mass spectrometry, focusing on the development and application of mass spectrometry-based methods for application in the areas of proteomics, metabolomics, and glycomics.

ABSTRACT:

Palaeoproteomics: when archaeology meets mass spectrometry

The persistence of biomolecules, including DNA, lipids and proteins, in the archaeological record allows mass spectrometry (MS) to contribute concrete scientific data to a very wide range of engaging and long-standing archaeological questions. Modern mass spectrometry's excellent limits of detection and mass accuracy make it an outstanding tool for these applications. However, tailored sample handling protocols and innovative approaches to data interpretation are also important in maximizing the contribution of MS to such archaeological investigations.

A wide variety of archaeological problems are accessible to analysis by MS. For example, fossilized bone samples may contain both protein and DNA, the sequences of which can identify the organism from which the bone came, and in the case of extinct organisms, also have the potential to reveal evolutionary links to extant species. In contrast, pottery pieces tend to trap and preserve some components of the materials with which they have been in contact, shedding light on the pottery's use and on aspects of daily life of the original owners.

The talk will present mass spectrometric data obtained during investigations of both ancient proteins and lipids, and will illustrate a range of ways in which MS has contributed to some challenging archaeological questions.

Sponsored Tech Talks

Dale Shannon Cornett
Product Specialist, Bruker, Daltonics

BIOGRAPHY:

Dale Shannon Cornett received a Ph.D. in analytical chemistry from the University of Georgia in 1993, under the mentorship of Jon Amster. Following a post-doc with Terry Lee at City of Hope in Duarte, California, Shannon joined Bruker and spent the next 8 years as MALDI-TOF Applications Scientist, R&D Manager and Product Manager before moving in 2002 to Vanderbilt University as Research Assistant Professor in the Mass Spectrometry Research Facility. There, working with Richard Caprioli, he developed tools and methodologies for tissue imaging and proteomics. In 2009, Shannon rejoined Bruker Daltonics as a Field Product Specialist supporting MALDI imaging and proteomics applications. In the course of his 20-year career in mass spectrometry Shannon has co-authored 25 publications in MALDI instrumentation and applications.

ABSTRACT:

A New Strategy for Proteomics through Integrated MALDI and ESI (PRIME) Yields a Greater Depth of Information

With PRIME, Bottom-up, Top-down and intact-protein analyses as well as in-depth protein characterization merge to illuminate a more accurate picture of the proteome than can be achieved using a single technique. Novel approaches like Glycomics, PTM analysis and MALDI Imaging are embedded to provide the highest level of proteome analysis.

David Craft
Manager, Proteomics Research & Development
BD

BIOGRAPHY:

Dr. David Craft earned his Ph.D. degree in Chemistry from the University of Alberta in Canada where he studied under the mentorship of Professor Liang Li. He has been working in the field of proteomics for over twelve years developing mass spectrometry applications towards the detection of biological molecules. Joining Becton Dickinson as a Scientist in 2006, Dr. Craft focused on enabling protein and peptide biomarker detection in plasma. Dr. Craft has developed novel methods for plasma peptidome analysis enabling a more thorough understanding of protein instability in blood. His work has been applied to the development of specialized blood collection systems. Dr. Craft currently serves as the Manager for Proteomics R&D within BD's Preanalytical Systems business where his group investigates the "starting point" of new diagnostic assays, namely the preanalytical factors affecting accurate quantitation of labile proteins and/or peptides. Dr. Craft and his team own several peer reviewed publications in this field and continue to drive further understanding of protein stabilization and preservation through basic research. Many researches are investigating these biomarkers and their relationship to different disease states and therapeutic treatments.

ABSTRACT:

The Impact of Preanalytical Variables on Biomarker Research

The scientific efforts on biomarker discovery research in the past five years have resulted in numerous potential biomarker candidates. These biomarkers, however, require further investigation by verification and validation in the clinical setting prior to specific application. One major hurdle in the transition from the research lab to the clinical lab is preanalytical variability, most notably, time and temperature, which have significant impact on analyte stability. This webinar will present the potential impact of sample handling on protein and peptide stability and how this variability can be controlled through the use of protease inhibitors.

Specifically, the presentation will discuss the stabilization of Glucagon-like peptide-1 (GLP-1), Gastric inhibitory polypeptide (GIP), Glucagon, and Ghrelin, four plasma peptides of particular interest in metabolic disorder research, especially diabetes drug research. The extremely short half-life of these metabolic peptides in blood provides a challenge for accurate analysis; therefore, preservation of proteomic sample integrity is vital. Some of the methods to minimize instability in these samples will also be examined including:

- The use of time-course mass spectrometry to characterize the kinetic digestion of each incretin peptide caused by active plasma endogenous enzymes;
- Incorporation of a cocktail of protease inhibitors in blood collection tubes (e.g., BD™ P800 blood collection system) to minimize variability/ instability.

Stabilization of the aforementioned peptides enables their use in pharmacokinetic and pharmacodynamic studies. Further, stabilization of proteins and peptides could improve the success rate of transitioning biomarker candidates from discovery research to clinical applications.

Brigitte Simons
Senior Applications Scientist, AB SCIEX

BIOGRAPHY:

Dr. Brigitte Simons is a senior applications scientist at AB SCIEX specializing in the development of accurate mass solutions in academic and biopharmaceutical markets.

ABSTRACT:

Strategies for Increasing Selectivity Towards Peptide Quantification

Interferences are unpredictable and unavoidable in the majority of peptide quantitation assays developed in complex biological matrices, especially in the era of fast chromatographic separations with sample throughput in mind. Techniques for gaining selectivity towards the detection of peptides include: enhanced multiply charge-based separations, ion mobility, and extracted ion chromatographic separations carried out at high resolution. Multiply charged peptides may have differential high field mobility and thus may potentially separate from singly charged noise and other interferences if thoughtfully introduced to optimized chemical environments. The utility of ion mobility is further enhanced if such separations can be carried out in the miliseconds time-scale and could be combined with LC-MRM experiments for a 2nd dimensional selectivity boost towards quantitation. This presentation will demonstrate a new enhancement to selective and sensitive peptide assay development.

Poster Abstracts

Mr Lian Yang
University of Waterloo

Precursor Charge Prediction for Mass Spectrometry Based Peptide Identification

Contributing Authors: Lian Yang, Bin Ma

Electrospray ionization (ESI) is widely used in mass spectrometry analysis of peptides. With ESI, the same peptide can be ionized with different charge states. Precursor ions with different charge states have different intensities, based on which the average charge state can be calculated.

In this research, a novel method is proposed to predict the average precursor charge for mass spectrometry based peptide identification. The model is simple and easy to calculate, while demonstrates impressive predictive power. It can also be used to improve peptide identification results from software engines, by discriminating incorrect sequences from correct ones.

Intuitively, the average charge state is influenced by the number of basic amino acid residues in the peptide sequence, and other amino acids should also have a contribution. In this model, the amino acid composition of a peptide is used to predict the average charge. Each type of residue is associated with a coefficient to be determined by multivariate linear regression on a training dataset. The average charge of a peptide is predicted as the summation of the coefficients of all residues in the sequence plus a constant.

Experiments show that the proposed method for charge prediction is fairly accurate for both CID and ETD data set. Predicted charge state is a potentially very useful feature to improve mass spectrometry based peptide identification. Comparison with the results on decoy sets illustrates some discriminative power for distinguishing the true and false peptide identifications. Tested with PEAKS 5.3 DB Search, the model can be used as a post-process to further increase the number of identified peptides at certain false discovery rates.

Dr Mark McComb
Boston University

Biomarkers of Metabolic Disorder Associated Cardiovascular Disease in a Mouse Model

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Unfavorable metabolic conditions (metabolic disorders) associated with obesity, diabetes, and hyperlipidemia are major causes for cardiovascular disease. The early detection and monitoring of the adverse effects of metabolic disease on the heart and vasculature, although well studied, remain elusive.

Nonspecific changes which occur in plasma protein indicators of inflammation and oxidants may act as evidence of systemic disease. Here we explored label-free proteomics using a mouse model to elucidate potential biomarkers of CVD including protein changes and changes in post-translational modifications (PTMs). Mice with cardiac-directed overexpression of the growth-factor mediating G-protein (Gq) demonstrating both protein oxidation and lipid adducts as well as cardiac failure were used as the model. Plasma was depleted of albumin/IgG. Following digestion, peptides were characterized by LC-MS/MS. Label-free LCMS analysis yielded features with ANOVAs < 0.05. Protein and PTM identifications were obtained using customized search strategies. Hierarchical clustering yielded distinct groups with excellent separation by PCA. Gene Ontology (GO) analysis indicated robustness of the data. In molecular function, catalytic and binding processes were increased while antioxidant activity decreased. Biological processes showed increases in stimulation and regulation. Proteins which changed related to CVD were alpha-1-acid glycoprotein, stabilin, neurofibromin, compliment C3, angiopoietin-related protein 4 and vitamin D receptor and vitamin D binding proteins. PTM changes were those associated with an increase in oxidative stress. Development of a CVD-specific protein/PTM panel obtained from mouse models will afford the first step in biomarker panel development such that disease diagnosis and

progression may be performed directly at the molecular level.

This research is supported by NIH-NHLBI contract N01 HV00239 and NIH-NCRR grants P41 RR10888, S10 RR15942 and S10 RR20946

Dr Xiaobin Xu
Boston University

Qualitative and Quantitative Analysis of Novel Synphilin-1 Interactors Involved in Aggresome Formation and Parkinson Disease Pathogenesis Using Tandem Affinity Purification and Mass Spectrometry

Contributing Authors: Xiaobin Xu; Anatoli B. Meriin, Nancy Leymarie, Mark E. McComb, Michael Y. Sherman, Catherine E. Costello

Abnormal protein aggregates can cause cell toxicity leading to various pathologies, including Parkinson disease (PD). Synphilin-1 is one of the major components of these inclusions, and is implicated in the pathogenesis of PD. Expression of Synphilin-1 in mammalian cells led to cytoplasmic aggregates, which were transported to form aggresomes upon proteasome inhibition. Capturing aggregates of abnormal proteins in aggresome(s) relieves their toxicity, and therefore clarifying mechanisms of aggresome formation is essential for understanding the origin of many protein misfolding disorders, including PD. Deletion of Synphilin-1 functional domains revealed that unknown signaling proteins must be associated with Synphilin-1 to trigger aggresome formation. In this presentation, we report identification and quantification of these unknown protein candidates using tandem affinity purification and LC-MS/MS, and investigation of their relevant functions and pathways.
This research is supported by NIH grants P41 RR10888, S10 RR15942, S10 RR20946 and R01 GM86890, and NIH-NHLBI contract N01 HV00239.

Dr Yanyan LU
Boston University, School of Medicine

Tandem MS Analysis of Protein Deposits in Human Subcutaneous Fat Tissues of a Patient with Immunoglobulin (Ig) Light Chain Amyloidosis

Extracellular amyloid deposits, composed of insoluble protein aggregates rich in ?-sheet structure, can disrupt normal cellular and tissue function and often culminate in the patient's death. Immunoglobulin (Ig) light chains (LC) are associated with a systemic form of amyloidosis. We have found that deposited LCs are extensively processed, leading to fragment patterns that differ from patient to patient [F Lavatelli, et al., Mol. Cell. Proteomics, 2008, 7, 1570-1583; LH Connors et al., Biochemistry, 2007, 46, 14259-14271]. We are presently analyzing and comparing LC fragments in amyloid deposits from fat biopsies to expand our knowledge of this disease.

The fat biopsy described herein showed deposition of Ig ?LC proteins (3+ score, Congo red stain). Tryptic peptides derived from the biopsy sample were analyzed with 1) a Reflex IVT MALDI-TOF MS (Bruker), 2) an ultrafleXtremeT MALDI-TOF/TOF MS (Bruker) and 3) an LTQ-OrbitrapT MS (ThermoFisher) with an Acuity nanoLC (Waters) and TriVersa NanoMateT robot (Advion). Data was analyzed with MASCOTT; assignments were verified manually.

Even without access to the cDNA of the patient, it was still possible to determine the amino acid sequence and PTMs of the Ig light chain using de novo sequencing with TOF/TOF MS/MS and with HCD fragmentation followed by detection in the Orbitrap. The MS data indicated that LC proteins had undergone extensive C-terminal truncation.

Acknowledgements
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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:

Ruedi Aebersold, Institute for Molecular Systems Biology, ETH Zurich
Leigh Anderson, Plasma Proteome Institute
Cheryl Arrowsmith, Ontario Cancer Institute
Alison Ashcroft, University of Leeds
Ron Beavis, Beavis Informatics Ltd.
Christoph Borchers, University of Victoria
Richard Caprioli, Vanderbilt University
Steven Carr, Broad Institute of Harvard & MIT
Brian Chait, Rockefeller University
Thomas Conrads, University of Pittsburgh
Catherine E. Costello, Boston University School of Medicine
Norm Dovichi, University of Notre Dame
Andrew Emili, University of Toronto
Ger van den Engh, Institute for Systems Biology
Werner Ens, University of Manitoba
Simon Gaskell, UMIST, Manchester UK
Jed Harrison, University of Alberta
Pierre Thibault, Universite de Montreal
Gerard Hopfgartner, Universite de Geneve
Donald Hunt, University of Virginia
Michael Karas, Johann Wolfgang Goethe University
Juergen Kast, University of British Columbia
Lars Konermann, University of Western Ontario
Andrew Krutchinsky, Rockefeller University
Joshua LaBaer, Harvard Institute of Proteomics
Liang Li, University of Alberta
Bin Ma, University of Waterloo
Alan Marshall, Florida State University
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Scott McLuckey, Purdue University
Garry Nolan, Stanford University
Richard Oleschuk, Queens' University at Kingston
Tony Pawson, SLRI, Mount Sinai Hospital
Emanuel Petricoin, George Manson University
Kevin Rosenblatt, UT Southwestern Medical Centre
Michael Roukes, California Institute of Technology
David Schriemer, University of Calgary
Jochen Schwenk, School of Biotechnology, KTH
Andrej Shevchenko, Max Planck Institute
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David Wishart, University of Alberta
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