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Enabling Technologies

CSMS SCSM
Canadian Society for Mass Spectrometry
Société Canadienne de Spectrométrie de Masse

9th International Symposium on Enabling Technologies for Life Sciences

May 4-5, 2017
Desmarais Building
University of Ottawa, ON, Canada

Chaired by
Lekha Sleno
Université du Québec à Montréal

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

www.etpsymposium.org

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Welcome from the Chair

On behalf of the Organizing and Scientific Advisory Committees, I would like to welcome you to the 9th International Symposium on Enabling Technologies (ETP 2017). Being held in Ottawa, we are coinciding with Canada's 150 years, all the more reason to highlight the great work coming from Canadian labs and encourage our networking. This year also marks a first for ETP since we have collaborated with the Canadian Society for Mass Spectrometry for the organization of the meeting. This year's symposium includes an excellent group of speakers representing a wide range of novel techniques having major influence on life science research. We have encouraged a large student participation this year, and will have two prizes given out for best student presentations. We also have an impressive group of sponsors and exhibitors, and encourage you to visit the exhibition area and attend the sponsored talks to 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 has traditionally emphasized proteomics, however in recent years we have broadened the scope to all recent technological advances applied to life sciences. We welcome your comments and suggestions following the meeting for future topics to cover in future symposia.

Wishing you all a productive and stimulating two days!

Lekha Sleno
Chair, ETP 2017
CSMS President

Organizing Committee

Lekha Sleno, Chair ETP 2017, CSMS President
Janette Champ, President, ETP Symposium Inc.

ETP Symposium Scientific Advisory Board

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Chris Dambrowitz, Northern Alberta Institute of Technology (NAIT)
Norman Dovichi, University of Notre Dame
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Randy Johnston, Dept. of Biochemistry & Molecular Biology, University of Calgary
Pierre Thibault, Université de Montréal

History

The ETP Symposium was initiated as part of a Genome Canada project sponsored by MDS 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.

Historic ETP Symposia, together with full programs, may be found on our website: www.etpsymposium.org

9th International Symposium on Enabling Technologies

Desmarais Building, University of Ottawa
55 Laurier Avenue, Ottawa, Ontario

Subject to Change

Thursday, May 4, 2017	
8:15 - 8:45	***Registration, pick up badges - Lobby Desmarais Building, U Ottawa***
8:55	*Conference Start, Introductions*
Conference Sessions - Room 1160	
Imaging, Ionization and Clinical Applications	
9:00 - 9:40	***PLENARY SPEAKER*** ***Vladimir Baranov, Fluidigm Canada Inc.*** *Imaging of life and its practical challenges*
9:40 - 10:10	***Pierre Chaurand, Universite de Montreal*** *Metal-assisted LDI for high resolution imaging MS of neutral lipids from thin tissue sections*
10:10 - 10:30	***Nidia Lauzon, Universite de Montreal*** ***Winner #1 of the Bill Davidson Graduate Student Travel Award*** *Forensic analysis of latent fingermarks by silver-assisted LDI (AgLDI) imaging MS (IMS) on non-conductive surfaces.* ***SPONSORED BY SCIEX***
10:30 - 10:45	***3 - 5 min Poster Talks*** ***Brandi West, University of Ottawa*** *Quantifying α-MSH peptides in mouse pituitary using MALDI-FTICR imaging* ***Ethan Yang, Université de Montréal*** *Enhancing Ganglioside Species Detection for MALDI Imaging Mass Spectrometry* ***Aafreen Kaur Bagga, York University*** *Alcohol consumption during gestation period alters phospholipid distribution in developing fetal brain*
10:45 - 11:15	*Coffee Break - Lobby Desmarais Building, U Ottawa*
11:15 - 11:45	***Richard Oleschuk, Queen's University*** *"Water Fearing" Materials for Making and Manipulating Droplets*
11:45 - 12:15	***5 - 5 min Poster Talks*** ***Xiaojing Huang, York University*** *Characterizing lipocalin 2 dynamics upon siderophore and iron binding using electrospray ionization mass spectrometry* ***Irina Oganesyan, York University*** *Structural Analysis of Membrane Proteins by Time-Resolved Hydrogen/Deuterium Exchange Mass Spectrometry (TRESI-HDX MS).* ***Reza Pourhaghighi, University of Toronto*** *Protein-Protein Interaction Network of Mammalian Brain Revealed by Large-scale Biochemical-Profiling-Mass Spectrometry (BP/MS)* ***Lisa Szymkowicz, York University*** *Phospholipid-based model membrane system for hydrogen-deuterium exchange mass spectrometry of membrane proteins* ***Michael Cohen, York University, Sanofi Pasteur*** *Characterizing the Immune Response to Novel Vaccine Candidates in a Human Whole Blood Assay Using High-Dimensional Single Cell Proteomics*

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Thursday, May 4, 2017	
12:15 - 1:30	***Lunch Seminar Room 1160 - Desmarais Building, U Ottawa, Sponsored by Bruker*** *Proteomic Profiling for Biomarker Discovery and Validation using a Bruker Impact II Q-TOF,* *Gary H. Kruppa, Ph. D., Vice President 'Omics, Bruker Daltonics Inc., Billerica, MA* *Quallitative Proteomics in Forensics - a case study.* *Leonard Foster, University of British Columbia*
1:30 - 2:00	***Kevin Bateman, MERCK*** *Quantitation of Therapeutic Proteins Using Mass Spectrometry*
2:00 - 2:20	***Timon Geib, Université du Québec à Montréal*** *Absolute Quantitation of Modified Human Serum Albumin for Accurately Monitoring Acetaminophen-Related Hepatotoxicity*
2:20 - 2:40	***Ghazaleh Moghaddam, Université du Québec à Montréal*** *Proteomic analysis of mouse and rat liver after acetaminophen treatment by 2D-LC-HRMS/MS*
2:40 - 3:00	***David Simon, Queen's University*** ***Winner #2 of the Bill Davidson Graduate Student Travel Award*** *Improving Sensitivity and Reducing Duty Cycle with Novel Multi-Electrospray Ionization Emitters* ***SPONSORED BY SCIEX***
3:00 - 3:30	*Coffee Break, Lobby Desmarais Building, U Ottawa*
3:30 - 4:00	***Jeff Agar, Northeastern University*** *A metabolic labeling approach for intact protein half-life determination in any organism*
4:00 - 4:20	***4 - 5 min Poster Talks*** ***Vinod Vashistha, GLA University, Mathura*** *RP-HPLC Enantioseparation of (RS)-Ketamine in human plasma via Chiral Derivatization Based on (S)-Levofloxacin* ***Sean Overton, University of Ottawa*** *Portable FT-IR Spectroscopy; an in-field analysis of substandard antibiotics* ***Mathieu Lavallée-Adam, University of Ottawa*** *TargetSeeker-MS: A Bayesian Inference Approach for Drug Target Discovery using Protein Fractionation Coupled to Mass Spectrometry* ***André LeBlanc, McGill University - Lady Davis Institute*** *Multiplexed MRM-based Protein Quantitation Using Two Different Stable Isotope Labeled Peptides for Calibration*
4:20 - 4:40	***Uros Kuzmanov, Donnelly Centre, University of Toronto*** *Next Generation Cardiac Phosphoproteomics: From Sample Preparation to Data Interpretation in a Mouse Model of Dilated Cardiomyopathy*
4:40 - 5:00	***Irina Slobodchikova, Concordia University*** *LC-MS assay for quantification of 17 mycotoxins in human plasma*
5:00 - 5:30	***Philip Britz-McKibbin, McMaster University*** *The Sweat Metabolome of Screen-Positive Cystic Fibrosis Infants: Revealing Mechanisms Beyond Impaired Chloride Transport*
5:30 - 7:00	***POSTER SESSION & COCKTAIL RECEPTION*** *Lobby Area of Desmarais Building*

Amended April 26, 2017

9th International Symposium on Enabling Technologies

Desmarais Building, University of Ottawa
55 Laurier Avenue, Ottawa, Ontario

Friday, May 5, 2017	
8:15 - 8:45	*Registration & Breakfast Seminar, Sponsored by SCIEX - Room 1160* ***Introducing the New X500B QTOF Mass Spectrometer for a simpler LC-MS solution for routine biomolecular characterization of proteins, lipids and metabolites*** Brigitte Simons, SCIEX, Concord, Ontario, Canada
Lipids, Screening and Structural MS	
9:00 - 9:40	***PLENARY SPEAKER - 2017 Ken Standing Award Winner*** ***Leonard Foster, University of British Columbia*** *Developing and applying proteomics in agriculture and medicine* ***SPONSORED IN PART BY UNIVERSITY OF MANITOBA***
9:40 - 10:00	***Carlos Canez, Carleton Mass Spectrometry Center*** *Recent improvements in TrEnDi to increase the sensitivity and selectivity of plasmalogen PE and plasmalogen PC lipids*
10:00 - 10:20	***Katrin Blank, Carleton University*** *Exploration of glycerophospholipid dynamics during hibernation and freeze tolerance*
10:20:10:50	***Gillian Thomas, Carleton University*** *Optimization of Trimethylation Enhancement using Diazomethane (TrEnDi) derivatization for MS-based analysis of sphingomyelin and cardiolipin*
10:50 - 11:20	*Coffee Break Seminar - Room 1160,* ***Utilization of High Resolution Accurate Mass LCMS for Large Molecule Quantitation*** Keeley Murphy, Senior Marketing Specialist - Pharma/Biopharma, Thermo Fisher Scientific
11:20 - 11:50	***Catherine Costello, Boston University*** *Divide and Conquer Strategies for Glycan Structural Determinations*
11:50 - 12:10	***Prem Kumarathasan, Health Canada*** *Proteomic analyses in environmental particle toxicity testing*
12:10 - 12:30	***Maxime Sansoucy, Université du Québec à Montréal*** *Comparative mussel proteomics for studying proteins associated to byssus filament formation by 2D-LC-MS/MS*
12:30 - 1:30	*LUNCH BREAK - Free Time*
1:30 - 2:00	***Daniel Figeys, University of Ottawa*** *Exvivo screening of human gut microbiome by RapidAIM*
2:00 - 2:20	***Samuel Shields, Carleton University*** *Solution phase charge inversion of methylated phosphatidylcholine for the identification of fatty acyl components via collision induced dissociation*
2:20 - 2:40	***Haidy Matwally, University of Western Ontario*** *Molecular dynamics simulations of the electrospray process, why do crown ethers suppress protein supercharging?*

Amended April 26, 2017

9th International Symposium on Enabling Technologies

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Friday, May 5, 2017	
2:40 - 3:00	*Coffee Break, Lobby Desmarais Building, U Ottawa*
3:00 - 3:30	***Christoph Borchers, UVic Genome BC Proteomics Centre*** *New Developments in Crosslinking-based Structural Proteomics*
3:30 - 4:00	***Derek Wilson, York University*** *Hydrogen Deuterium Exchange in Biopharmaceutical Drug Development*
4:00 - 4:30	***Ann English, Concordia University*** *Factors affecting the protein interactions found in GST-Ccp1 pulldowns from yeast cells*
4:30	***Closing Remarks and Conference End***

Amended April 26, 2017

Symposium Chair

Lekha Sleno
Université du Québec à Montréal

Lekha Sleno is a professor in the chemistry department at UQAM (Université du Québec à Montréal) since 2008. Her research interests focus on studying reactive metabolites covalently binding to proteins and developing new metabolomics-based methods using liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS). Originally from Montreal, she did her BSc (2002) in Biochemistry at Concordia University, followed by a PhD (2006) at Dalhousie (Chemistry, Prof. Dietrich Volmer), focused on bioanalytical mass spectrometry. She then went on to the University of Geneva (with Gerard Hopfgartner) in Switzerland for a first post-doc in pharmaceutical mass spectrometry, working on reactive drug metabolites, followed by a second post-doc at University of Toronto (with Andrew Emili) in proteomics.

Invited Speakers

Jeff Agar
Northeastern University

A metabolic labeling approach for intact protein half-life determination in any organism.

Vladimir Baranov
Fluidigm Canada Inc.

Imaging of life and its practical challenges

Kevin Bateman
MERCK

Quantitation of Therapeutic Proteins Using Mass Spectrometry

Christoph Borchers
UVic Genome BC Proteomic Centre

New Developments in Crosslinking-based Structural Proteomics
In this presentation, we describe several recent advancements in the field of structural proteomics. These fall into three main categories: non-specific short-length crosslinkers, quantitative crosslinking, and the use of crosslinking data as constraints in molecular modeling.

Traditional crosslinking analysis of proteins uses amine-reactive reagents to form covalent bonds between residues that contain amino groups. Short spacer-lengths provide stricter and more useful distance constraints for modeling protein structures. For short-distance crosslinking, however, the availability of amine functional groups is a limiting factor, usually resulting in the formation of only a small number of short-distance crosslinks. Non-selective photo-reactive

reagents can address this problem by increasing the number of functional groups (and amino acids) that can act as targets for the crosslinking reaction. Here we will describe a novel homo-bifunctional photo-reactive reagent, Bis-DiAzirinePyridine (Bis-DAP). This crosslinker has two diazirine groups 4Å apart, which form highly reactive carbene species on photolysis with UV light which can non-selectively target all amino-acid residues. This new crosslinker was first validated on myoglobin, and then used to obtain insights into the structure of α-synuclein. Quantitative cross-linking (qCL) analysis was performed by crosslinking Spy-L32P+/-Im7 using either isotopically "light" Di-SuccinimidylAdipate (DSA)-12C6 or isotopically "heavy" DSA-13C6. Quantitative surface modification (qSM) analysis was performed in the same way, but with "light" PyridineCarboxylicAcidSuccinimide (PCAS)-H4 or "heavy" PCAS-D4 being used instead of DSA. Reactions were quenched, samples containing equal amounts of labelled protein were mixed, digested with either trypsin or pepsin, and LC-MS data collected on an Orbitrap Velos Pro mass spectrometer. qCL analysis revealed 56 crosslinks differentially formed between the unbound and client-bound conformations of SpyL32P.

Finally, we show how pairwise inter-atom distance constraints from crosslinking experimental data can be incorporated into the force field of discrete molecular dynamics (DMD) simulations to form a flexible and efficient method of experimentally-guided de novo structure determination. Models developed using DMD simulations can then be validated using other structural proteomics techniques, such as hydrogen-deuterium exchange, and surface modification. Thus far, protein structures for Myoglobin and FKBP -- models for α-helix and β-sheet rich proteins, with 20 and 24 unique crosslinking constraints, respectively -- were used for proof-of-principle experiments using a combination of experimental structural proteomics data and discrete molecular dynamics simulations. The lowest energy structures obtained using constraints based on short-distance crosslinking were in excellent agreement with the crystal structure, with an RMSD of 5.4Å and 2.7Å respectively. These structures also agree with the results from hydrogen/deuterium exchange, surface modification, and long-range crosslinking`

Contributing Authors
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Catherine E. Costello

Boston University

Divide and Conquer Strategies for Glycan Structural Determinations

Glycans, glycoproteins and glycolipids have a wide range of important biological activities that are dependent on their detailed structures. However, since the molecules of interest generally occur as components of complex mixtures of closely-related structures, often contain branches and labile modifications, and change with time, their determinations present more challenges than biopolymers with linear structures.

Samples of (1) standard glycans and glycoconjugates and (2) biologically-derived components are analyzed using MALDI- and ESI-MS and, using both CID and ExD for dissociation on Q-TOF, Orbitrap and FT instruments, and combined with UPLC and/or IMS, to separate mixtures and generate conformational information.

Pre- and post-ionization methods, alone or in succession, help to resolve the mixtures. In addition, the combination of Ion Mobility with MS/MS generates conformational and structural information. Further details may be gained by dissociation of selected precursor ions. However, CID often results in only partial structure definition, whereas electron-based dissociation methods (ExD) generate peptide, protein and glycan sequence information while preserving labile

modifications, and provide much more extensive glycan linkage information than CID. In this lecture, we will consider how various combinations of these approaches on time-of-flight, Orbitrap and FT-ICR MS instruments can contribute to the success of structural studies, in the context of ongoing biological investigations

Definition of glycan and glycoconjugate structures using combined MS methodologies.

Figeys, Daniel
University of Ottawa

Exvivo screening of human gut micro biome by RapidAIM

Richard Oleschuk
Queen's University

"Water Fearing" Materials for Making and Manipulating Droplets for Mass Spectrometric Analysis

Droplet based microfluidic platforms are attractive methods to move and manipulate discrete samples. The actuation of discrete droplets can be performed by generating electrostatic forces on an array of electrodes coated with an insulating dielectric i.e. electrowetting on dielectric, or alternatively through magnetic actuation. Droplet-based systems rely on a hydrophobic (superhydrophobic) layer to minimize surface interaction/friction between the droplet and surface. As a result droplets can be made to slide across the surface with forces less than a microNewton. We examine the use of fluorinated polymer and fluorinated silica nano particle based coatings to manipulate droplets to carry out droplet actions such as dispensing, splitting, merging and mixing using gravity, magnetic and electrowetting on dielectric actuation methods. Microfabricated and 3-D printed devices are utilized to carry out several proof of principal assays. Furthermore a laser micromachining approach is used to pattern "wettability" enabling the generation of droplet arrays through discontinuous de-wetting.

Oral Presentations

Blank, Katrin
Carleton University

Exploration of glycerophospholipid dynamics during hibernation and freeze tolerance

Hibernation and freeze tolerance allow animals to cope with cold climates. Hibernation is a deep long sleep where animal survives mainly on stored fat. Freeze tolerance is when an animal has the ability to tolerate freezing within their tissues. The interest was to look at glycerophospholipids and see how they change when animals adapt to colder climate changes.
13-lined ground squirrel liver and wood frog leg muscle tissues were chosen as study models for hibernation and freeze tolerance. Lipids were extracted using a modified Bligh and Dyer lipid extraction and liquid chromatography/mass spectrometry was used in the separation, detection and analyses of the lipid species.
It was observed that ground squirrel most likely increases the fluidity of its membranes during hibernation by increasing the degree of lipid unsaturation; this was evident through mass losses of multiple H2 groups. Increasing the unsaturation of the fatty acid chains prevents them from interacting too strongly and thus increases the fluidity and permit continued cellular activity at low temperatures. The preliminary results from wood frog studies did not show that glycerophospholipids go through significant changes during freeze tolerance in leg muscle tissue. It is interesting to look at the glycerophospholipid differences in a vital (liver) and a non-vital (leg muscle) tissues with respect to the climate changes. This work will show lipidomic characteristic fingerprints of hibernation and freeze tolerance versus a normal temperature state.

Contributing Authors
Katrin Blank*, Carlos Canez, Sam Williamson, Gilian Thomas, Hillary Weinert, Kerene Brown, Kenneth Story and Jeffrey C. Smith

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Britz-McKibbin, Philip
McMaster University

The Sweat Metabolome of Screen-Positive Cystic Fibrosis Infants: Revealing Mechanisms Beyond Impaired Chloride Transport

The sweat chloride test remains the gold standard for confirmatory diagnosis of cystic fibrosis (CF) in support of newborn screening programs. However, it provides ambiguous results for intermediate or borderline sweat chloride cases when classifying the complex CF disease spectrum.

Herein we report the first characterization of the sweat metabolome from screen-positive infants and identify metabolites associated with CF status that complement sweat chloride testing when using multiplexed CE-MS technology. Pilocarpine-stimulated sweat specimens were collected independently from two CF clinics, including 50 unaffected infants (e.g., carriers) and 18 confirmed CF cases.

Amino acids, organic acids, amino acid derivatives, dipeptides, purine derivatives and exogenous compounds were detected in sweat, including metabolites associated with affected yet asymptomatic CF infants, such as asparagine and glutamine. Unexpectedly, a metabolite of pilocarpine used to stimulate sweat secretion, pilocarpic acid, and a ubiquitous plasticizer metabolite from environmental exposure, mono(2-ethylhexyl)phthalic acid were present in the sweat of affected CF infants at lower concentrations, indicative of paraoxonase deficiency with reduced arylesterase/lactonase activity. Moreover, repeat sweat samples from a late-diagnosed teenage CF patient with a G551D mutation revealed several metabolites responsive to nutritional and drug therapy using the CFTR potentiator, ivacaftor, corresponding with a notable improvement in growth and/or lung function.

This work sheds new light into underlying mechanisms of CF pathophysiology beyond impaired chloride transport for new advances in precision medicine.

Contributing Authors
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Canez, Carlos
Carleton Mass Spectrometry Center

Recent improvements in TrEnDi to increase the sensitivity and selectivity of plasmalogen PE and plasmalogen PC lipids

TrEnDi derivatization results in significant sensitivity enhancements in tandem mass spectrometry-based analysis for acyl and O-alkyl phosphatidylethanolamine (PE) and phosphatidylcholine (PC) lipids, but the vinyl ether group of plasmalogen PE and PC lipids was cleaved. Two different methodologies were developed to render TrEnDi derivatization compatible with plasmalogen PE and PC species.

Vinyl ether groups were derivatized using iodine and methanol prior to TrEnDi derivatization and mixed with 1 uL of tetrafluoroboric acid and 300 uL of diazomethane before solvent removal via evaporation. Alternatively dilute concentrations of tetrafluoroboric acid were used prior to addition of diazomethane. Derivatized lipids were analyzed via direct infusion ESI-MS or via LC-ESI-MS.

The original trimethylation enhancement via diazomethane (TrEnDi) procedure resulted in complete methylation of phosphate moieties, carboxylic acids and primary amines of phospholipids. But it also provoked vinyl ether cleavage due to the acidic conditions of the reaction. A two-step derivatization process was developed. Iodine and methanol derivatization of O-alkenyl-ether double bonds in plasmalogen PE and PC species prior to TrEnDi derivatization proved effective in preventing plasmalogen cleavage. Alternatively, careful reduction and control of the tetrafluoroboric acid concentration during TrEnDi derivatization allowed for complete derivatization without vinyl ether cleavage. Both methodologies achieved complete methylation of the plasmalogen phospholipid head groups and resulted in significant sensitivity gains for plasmalogen PE species and modest sensitivity gains for PC species.

This is an important modification to reported chemistry that enables unprecedented levels of sensitivity for plasmalogen phospholipids in MS.

Contributing Authors
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Chaurand, Pierre

Universite de Montreal

Metal-assisted LDI for high resolution imaging MS of neutral lipids from thin tissue sections

Lipids play key roles in cellular membranes, signaling and energy storage. In particular, cholesterol, fatty acids and triglycerides play significant roles in the development of life-style associated diseases. Matrix-free metal assisted LDI methods are developed for the imaging MS of targeted lipid compounds with high specificity and high sensitivity.

Tissue sections including brain, kidney, and liver were investigated. For the analysis of cholesterol and fatty acids, a ~20 nm-thick layer of sputtered silver is directly deposited on thin sections. For the analysis of triglycerides, a layer of sodium salt is first deposited followed by ~28 nm-thick layer of sputtered gold is used. High resolution LDI imaging MS is performed on a TOF MS system.

Using silver-assisted LDI IMS we have thus far mapped and identified with certainty several compounds including, cholesterol, arachidonic acid and docosahexaenoic acid. While these compounds show great affinity for silver, other molecules such as triglycerides (TAGs) have strong affinities for other selected cations. For this purpose, the coupling of sodium salts and sputtered gold has been developed to enhance both desorption and ionization yielding a 30-fold increase of TAG signals compared to standard MALDI MS approaches. Although this method targets TAGs, we have also detected and imaged several other compounds including cholesterol esters, which are hard to detect by MALDI MS. These novel metal-assisted imaging MS strategies are being used to study cancer, Alzheimer's and non-alcoholic fatty liver diseases.

Analysis of selected neutral lipid compounds by high resolution LDI imaging MS using UV absorbing metal layers directly deposited on tissue sections.

Contributing Authors
Pierre Chaurand, Martin Dufresne

English, Ann

Department of Chemistry and Biochemistry, Concordia University

Factors affecting the protein interactors found in GST-Ccp1 pulldowns from yeast cells

Cytochrome c peroxidase (Ccp1) functions as a mitochondrial H2O2 sensor and heme donor in yeast (Saccharomyces cerevisiae) cells. To better understand Ccp1's role in cellular redox regulation and heme trafficking we sought to identify its protein binding partners in yeast under various experimental conditions using glutathione-S-transferase (GST) pulldown assays.

GST and its Ccp1 fusion were expressed in E. coli. The fusion protein was purified as heme-free GST-apoCcp1 and we added hemin to generate heme-loaded GST-holoCcp1. We compare pulldowns with both GST-apoCcp1 and GST-holoCcp1 as bait from mitochondria-enriched (P10) and extramitochondria (S10) subcellular fractions obtained on lysis of fermenting one-day and respiring seven-day yeast cells.

In yeast cells, apoCcp1 is targeted to mitochondria where it is heme loaded. Uing high-resolution MS/MS, we identified Ccp1's peroxidase co-substrate, cytochrome c (Cyc1), and 28 novel interactors including mitochondrial Mn superoxide dismutase 2 (Sod2) and mitochondrial/ cytosolic CuZn superoxide dismutase (Sod1), the mitochondrial transporter Pet9 (a putative heme transporter), the three yeast isoforms of glyceraldehyde-3-phosphate dehydrogenase (Tdh3/2/1), heat shock proteins including Hsp90 and Hsp70, and the main peroxiredoxin in yeast (Tsa1) plus its cosubstrate, thioreoxin (Trx1). Identification of these new interactors expand the possible roles of Ccp1 in coordinating the vital and interdependent biological processes of stress response and heme trafficking. We also report on the P10 proteomes and the GST-apoCcp1 interactors detected +/- detergent (N-octylglucoside, CHAPS).

Our results expose the limitations of untargeted large-scale interactome studies that found only four of the numerous Ccp1 interactors isolated here.

Contributing Authors
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Geib, Timon

Université du Québec à Montréal

Absolute Quantitation of Modified Human Serum Albumin for Accurately Monitoring Acetaminophen-Related Hepatotoxicity

We have developed a fast and sensitive assay to accurately quantify modified human serum albumin as a biomarker for acetaminophen-related hepatotoxicity.

This assay uses isotope dilution, a surrogate modified protein standard, solid phase extraction and selective antibody-based enrichment of targeted peptides, coupled to LC-MRM on a QqQ platform. Peptic serum digests were purified by using cysteine-reactive covalent capture tags and TiO2, allowing subsequent robotic liquid handling immunoprecipitation without interference by unmodified peptides.

We then optimized TiO2 resin loading conditions for high specific binding of CysPAT peptides and low binding of the target peptide. The TiO2 resin flow-through and wash were concentrated via SPE on a hydrophilic-lipophilic polymeric resin. Immunoprecipitation with magnetic beads yields highly purified samples for fast LC-MRM analyses. The final method will be applied to evaluate clinical samples from patients exhibiting acetaminophen hepatotoxicity.

This study represents a novel approach to accurately quantify acetaminophen-related covalent binding in human serum.

Contributing Authors
Timon Geib, André LeBlanc, Tze Chieh Shiao, Ghazaleh Moghaddam, Amal Guesmi, René Roy, and Lekha Sleno

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Kumarathasan, Prem

Health Canada

Proteomic analyses in environmental particle toxicity testing

Episodic increases in ambient air particulate matter are associated with increased hospital admissions for cardiorespiratory diseases. Particle exposures are also linked to neurocognitive, reproductive effects and obesity. Mechanistic understanding of particle toxicity is limited. Physicochemical characteristics of ambient air particles can vary and thus can modify particle potencies accordingly.

Human lung epithelial cells (A549) or J774 mouse macrophages were exposed (24 h) in vitro to Ottawa urban air PM and reference particles (TiO2 and SiO2) at doses (0-200 or 300 µg/cm2) doses. Cellular cytotoxicity (CTB, LDH) assays were conducted. Proteomic analyses of fractionated (2D-GE or MW cut off), tryptic digested cell lysates were done using mass spectrometry.

Our results, exhibited that the Ottawa particles at high doses led to frank cytotoxicity. PM exposures altered proteins that were associated with apoptosis, oxidative stress, antioxidant defense and inflammatory responses. Cytotoxicity results were consistent with proteomic changes, and

particle potency rankings based on cytotoxicity data and proteomic information were consistent. Protein changes were observed even at lower doses of particle exposures suggesting altered cellular events preceding frank cytotoxicity.

Toxicoproteomic analyses can be valuable in screening for particle potencies and to gain insight into particle exposure-related toxicity mechanisms.

Contributing Authors
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Kuzmanov, Uros

Donnelly Centre, University of Toronto

Next generation cardiac phosphoproteomics: from sample preparation to data interpretation in a mouse model of dilated cardiomyopathy

Heart failure (HF), a pernicious disorder resulting from a variety of cardiovascular diseases (CVDs), is now the leading cause of mortality in North America. Perturbations in signaling pathways associated with late stage CVD/HF events, such as decreased cardiac contractility, hypertrophic cardiomyopathy, and dilated cardiomyopathy have been identified, but the critical early regulatory perturbations that precede and ultimately result in functional impairment of the heart are not yet known. Differential phosphorylation has been widely implicated as a central regulatory and signaling mechanism in a variety of cellular processes associated with the initiation and progression of CVDs, but systematic global surveys have yet been reported.

To address this gap, we are taking advantage of recent advances in quantitative mass spectrometry (MS)-based techniques to perform large-scale identification and quantification of phosphorylation sites in different cardiac pathologies. This involved: optimized sample preparation and protein extraction protocols, chromatographic separation and enrichment of phosphopeptides, refined data searching and processing, and bioinformatic systems biology-based extraction of meaningful information from the acquired data.

Here, we report the development of an effective proteome-scale quantitative phosphoproteomic pipeline and its application to the analysis of signaling pathway changes in a mouse model of dilated cardiomyopathy (DCM), a primary cause of heart failure, which resulted in the identification and quantification of 7,589 unique putative phosphorylation sites on 1848 cardiac phosphoproteins.

This unprecedented profiling allowed for the bioinformatic/systems biology-based elucidation of hundreds of dysregulated signaling pathways and other biological process in the very earliest stages of DCM, prior to clinical presentation, most of which have not been documented before. Here, we describe critical technical aspects in our phosphoproteomics pipeline required for effective sample preparation, chromatographic separation, phosphopeptide enrichment, mass spectrometry settings, phosphorylation site localization/quantification, and bioinformatic essential for the identification of dysregulated signaling pathways involved in heart failure.

Contributing Authors
Uros Kuzmanov [1,2,3], Hongbo Guo[1], Diana Buchsbaum [2,3], Jake Cosme [2,3], Cynthia Abbasi[2], Ruth Isserlin[1], Parveen Sharma[2], Anthony Gramolini[2,3], and Andrew Emili[1].
1Donnelly Centre for Cellular and Biomolecular Research, University of Toronto, Toronto, Ontario M5S 3E1, Canada [2]Department of Physiology, University of Toronto, Toronto, Ontario M5S 3E1, Canada, [3]Ted Rogers Centre for Heart Research, University of Toronto, Toronto, Ontario, Canada

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Metwally, Haidy

University of Western Ontario

Molecular dynamics simulations of the electrospray process, why do crown ethers suppress protein supercharging?

Molecular dynamics simulations(MD) are capable of providing insights into the formation of gaseous protein ions under native ESI conditions. Addition of supercharging agents produce high charge state ions during ESI-MS. Here we use MD simulations and experiments to examine the effects of crown ethers on the supercharging process.

Haemoglobin was used as a protein model. MD simulations of ESI-droplets were conducted at 370K using Charmm 36 forcefield with TIP4P/2005 water in Gromacs 5 with GPU acceleration and trajectory stitching. Custom designed software was used for droplet assembly. Sulfolane and crown ether(18C6) were parametrized using ParamChem server. Experiments were acquired on a synapt HDMS (Waters).

MD simulations showed that H2O/sulfolane nanodroplets undergo solvent segregation,resulting in an aqueous core that contains the protein and an outer shell of sulfolane. Na+ will stay in the aqueous core. The outer partially non polar therefore impedes charge ejection from droplet via IEM. We Hypothesized that the ability of 18C6 to act as a phase transfer catalyst should facilitate charge carrier penetration through the outer sulfolane layer. Such that IEM will continue. Our data shows that the binding of Na+ to 18C6 allows charge carriers to pass through the nonpolar surface layer, resulting in low charge states.

Ourdata represents the first MD simulations for studying the effect of 18C6 on the behavior of protein containingESI-nanodroplets under supercharging.

Contributing Authors
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Moghaddam, Ghazaleh
Université du Québec à Montréal, Chemistry Department, Montréal (Qc), Canada

Proteomic analyses of mouse and rat liver after acetaminophen treatment by 2D-LC-HRMS/MS

Acetaminophen (APAP) is one of the most commonly used analgesic drugs and is also the main cause of liver injury, liver necrosis and acute liver failure. This hepatotoxicity is associated with glutathione depletion and production of a reactive metabolite, N-acetyl-p-benzoquinoneimine (NAPQI). NAPQI covalently binds to cysteine groups in proteins or peptides mainly in the liver.

The aim of this study was to identify in vivo protein targets of APAP as early indicators of liver injury, in both rat and mouse models and compare the results from both species

Using a bottom-up proteomic approach, sample preparation involved protein extraction and digestion from rat and mouse liver samples. To investigate formation of modified protein, liver extracts were digested separately with trypsin and pepsin. Digests were then subjected to strong cation exchange (SCX) fractionation prior to reversed-phase UHPLC-HRMS/MS. Data processing employed ProteinPilot software to find potential modified peptides, followed by peak integration and verification of absence in control samples to remove any potential false positives.

Several proteins have been identified as modified by NAPQI in mouse, two of which were also found in rat. These proteins are known to be involved in several important biological pathways involved in cell survival during oxidative stress and could potentially be linked to hepatotoxicity. Early diagnosis of modified peptides, as APAP biomarkers, may reduce the development or progression of symptoms associated with the pathological acetaminophen-induced toxicity. Assessment of modified proteins as novel biomarkers of APAP toxicity using proteomics MS-based approaches

Contributing Authors
Makan Golizeh, Lekha Sleno

Pourhaghighi, Reza
University of Toronto

Protein-Protein Interaction Network of Mammalian Brain Revealed by Large-scale Biochemical-Profiling-Mass Spectrometry (BP/MS)

Deciphering the connectivity of neurons is a key challenge in neuroscience. To investigate their basic physical organization, we performed a global analysis of protein complexes and PPIs in mouse brain using a new interactome mapping platform. We identified hundreds of assemblies involved in neuronal function, brain development and neurodegenerative disorders, providing rich mechanistic insights.

To identify stably co-eluting proteins, we performed quantitative tandem mass spectrometry after exhaustive physiochemical fractionation of soluble cerebellar lysates using Isoelectric Focusing followed by orthogonal Ion Exchange Chromatography.

MS analysis of over 500 soluble protein fractions led to the identification of ~6,000 unique proteins in brain. Co-fractionation of stably-associated complex subunits was predicted based on protein profile similarity using machine learning, and evaluated against a reference set (gold standard) of annotated complexes. By integrating the 2D co-fractionation data with complementary functional association information, a large high-confidence brain protein interaction network (B-PIN) was created.

B-PIN is the largest experimentally-derived brain PPI network to date, and will serve as resource for the neuroscience and systems biology researches.

Contributing Authors

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Sansoucy, Maxime
Université du Québec à Montréal

Comparative proteomics of mussel studying proteins associated to byssus filament formation by 2D-LC-MS/MS

Natural protein-based polymers represent an interesting class of biomaterials with unique structural properties and therefore have potential for diverse applications. The mussel's byssus is an interesting collagen-based filament with unique toughness, flexibility and adhesive properties. A 2D-LC-MS/MS methodology was employed to identify proteins uniquely found in mussel's byssal gland (foot).

Tissues were homogenized before protein extraction. Tryptic and peptic digestion were performed on extracts. Sample fractionation based on protein (SEC) and peptide (SCX and reverse phase) level separation was compared. Fractions were analyzed on a LC-QqTOF and proteins identified through protein spectral matching. Further biological and physiochemical study on exclusive foot proteins is ongoing.

Comparison of identified proteins from protein- and peptide-level fractionations revealed that protein solubilisation issues negatively impacted protein-level fractionation. Confident protein identification was supported by using a false discovery rate at 1%. Differential analysis between foot and control (mantle) datasets revealed proteins expressed uniquely in the mussel foot. Strong cation exchange and high pH reverse-phase fractionation were compared along with single-dimension strategy. SCX and RP fractionation have respectively detected 124 and 130 foot proteins whereas the no fractionation approach provided about half of these proteins. Thirty proteins were identified by sequence homology as either part of the mature byssus thread or involved in metabolic and metal binding activities.

Protein identification involved in byssogenesis using multi-dimensional chromatography and high resolution tandem mass spectrometry.

Contributing Authors
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Shields, Samuel
Carleton University

Solution phase charge inversion of methylated phosphatidylcholine for the identification of fatty acyl components via collision induced dissociation

Fatty acid identification of phosphatidylcholine (PC) is commonly performed via CID of PC-acetate (OAc) ion pairs; however, suffers from low sensitivity and incompatibility with TrEnDi-modified lipids. Recently, McLuckey et al. have reported gas phase charge inversion via the addition of a dicarboxylic acid (DCA). Here we optimize charge inversion of PC and TrEnDi-PC in solution with various DCAs.

Numerous DCAs were investigated. Thiodipropionic acid (TDPA), suberic acid (SA), and tricarballylic acid (TCBA) were determined to have optimal compatibility. 100 uM PC was mixed with 10 mM DCA and 1 uM of sodium tetrafluoroborate in a solution of 49.5% MeCN, 49.5% MeOH and 1% NH4OH. The mixture analyzed in negative ion mode via ESI using a hybrid quadrupole TOF mass spectrometer.

Many of the DCA acids were not soluble in the solvent mixture used (MeCN:MeOH:NH4OH), and thus limited the formation of PC ion pair during ESI-MS. However, TDPA was not only soluble in the solvent mixture, but formed ion pairs with methylated PC as well as unmodified PC. Upon CID of methylated and unmodified PC-TDPA ion pair myristate and oleate fatty acyl components could be observed. To compare traditional [PC+OAc] induced charge inversion against [PC+TDPA] and [PC+Me+TDPA], sodium tetrafluoroborate was used as an external standard.
The sensitivity of both [PC+TDPA] and [PC+Me+TDPA] was observed to be dramatically greater than the traditional acetate ion pair. In addition, fatty acyl fragments produced from CID experiments were qualitatively observed to increase in sensitivity. Current experiments are being explored to apply this methodology to biological samples.

This is the first study to systematically explore charge inversion agents for gas phase fatty acid identification of PC in the solution phase.

Contributing Authors
Samuel W. Shields, Stewart T. Bigelow, Jeffrey M. Manthorpe, Jeffrey C. Smith

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Slobodchikova, Irina
Concordia University

LC-MS assay for quantification of 17 mycotoxins in human plasma

Mycotoxins are toxic compounds produced by mold. Human exposure to mycotoxins can occur by eating contaminated food and can give rise to a number of diseases, such as kidney diseases, oesophageal and liver cancer and immunosuppression. It is very important to develop liquid chromatography - mass spectrometry (LC-MS) method to monitor the Canadian population exposure to mycotoxins in human plasma.

The suitable separation of mycotoxins and their metabolites was achieved with pentafluorophenyl column and the mobile phase containing water/ methanol with 0.1 % acetic acid and water/methanol with 0.02 % acetic acid for positive and negative electrospray ionization, respectively. MS analysis was performed at 60,000 resolution on LTQ Orbitrap Velos.

Solvent precipitation with acetonitrile, solid-phase extraction (strong anion exchange, hydrophilic-lipophilic-balanced (Oasis HLB) sorbent and mixed mode sorbent (Bond Elute Certify II)), and liquid-liquid extraction (ethyl acetate versus methyl tert-butyl ether) were compared in terms of analyte recovery, selectivity, and matrix effects. The final choice of sample preparation for this application was 3-step LLE with ethyl acetate. The optimized method was fully validated and LOQs ranging from 0.1 ng/ml to 0.5 ng/ml were obtained with precision less than 20% and accuracy in the range of 80-120%. LC-MS library of 14 mycotoxin metabolites was also constructed using microsomal incubations in order to screen for presence of common mycotoxin metabolites in addition to accurate quantitation of parent molecules.

This is the first multi-mycotoxin LC-MS method in human plasma samples that covers multiple mycotoxin classes with excellent sensitivity.

Contributing Authors
Irina Slobodchikova and Dajana Vuckovic.

Thomas, Gilian
Carlton University

Optimization of Trimethylation Enhancement using Diazomethane (TrEnDi) derivatization for MS-based analysis of sphingomyelin and cardiolipin

TrEnDi applies a fixed positive charge to lipids to increase sensitivity and detection. It is effective on various glycerophospholipids; however, sphingomyelin (SM) lipids exhibit small amounts of peak splitting due to partial hydroxyl moiety methylation. Cardiolipin (CL) also contains a hydroxyl group, thus post-TrEnDi peak splitting is expected. We look at resolving TrEnDi-based signal splitting.

Solvent conditions, acid strength, amount of diazomethane, and incubation times were tested during the methylation reaction on SM (16:0/18:1) to investigate their effects on peak splitting and improved sensitivity. Lipids were derivatized in other ways prior to TrEnDi in order to protect the hydroxyl group from being methylated; a dimethylglycine derivatization was tested on both SM and CL (14:0).

TrEnDi conditions different from what have been previously reported were required in order to drive the reaction to completion and optimize detection sensitivity for SM. It was found that an alcohol is required to mediate the TrEnDi reaction and prevent excess methylation. However, in every case it was evident that the hydroxyl moiety on the sn-1 chain of SM requires protection from methylation by diazomethane. As a result, the ability of a dimethylglycine derivatization reaction to protect the sn-1 hydroxyl moiety was investigated. It was found that the derivatization was successful on SM, and as cardiolipin possesses a similar acidic hydroxyl moiety on its backbone, the dimethylglycine derivatization was also applied with resulting success. However, further studies are currently being pursued to examine the effectiveness of this derivatization in combination with TrEnDi.

First study to optimize TrEnDi chemistry with sphingomyelin and cardiolipin lipids by examining the effect of alcohol and complementary derivatization

Contributing Authors
Gilian T. Thomas, Carlos R. Canez, Samuel W.J. Shields, Jeffrey M. Manthorpe, Jeffrey C. Smith

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Wilson, Derek
York University

Hydrogen Deuterium Exchange in Biopharmaceutical Drug Development

Hydrogen deuterium exchange (HDX) is rapidly becoming an indispensible tool in biopharma. The unique strength of HDX is its ability to provide a detailed picture of conformational transitions in response to drug binding. This presentation will explore several recent applications by our group of HDX to drug development, specifically in neurodegenerative disease and epitope mapping for vaccines.

We have introduced a microfluidics-based approach for automated millisecond Time-Resolved ElectroSpray Ionization mass spectrometry with Hydrogen Deuterium eXchange (TRESI-HDX). Millisecond HDX is exquisitely sensitive to weak hydrogen bonding networks, and is therefore uniquely capable of providing a detailed picture of the biases in 'active', weakly structured regions of proteins.

In an application to neurodegenerative disease, TRESI-HDX was used to determine the structural and dynamic effects induced by a panel of 10 drug candidates targeting the intrinsically disordered Alzheimer's-associated protein Tau. The data indicate a direct relationship between two specific structural effects - binding to hexapeptide II and conformational collapse - and drug efficacy in vitro (i.e., IC50). For vaccine development, we will provide an example of how millisecond TRESI-HDX can suppress allosteric 'interference' in epitope mapping experiments. This was demonstrated using the hemoglobin / anti-hemoglobin interaction as a model system and a novel 'kinetic' workflow. Our results indicate that allosteric effects are suppressed in the first 100 ms of binding and simultanious HDX labeling.

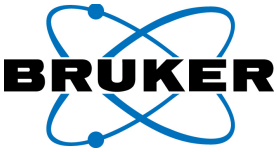
This work details new applications for HDX in drug development, specifically for neurodegenerative disease and epitope mapping.

Contributing Authors
Shaolong Zhu; Bin Deng; Cristina Lento; Kerene Brown and Derek J. Wilson

Tech Talks

Lunch Seminar:

Thursday, May 4, 2017 -
12:15 to 1:30
Room 1160



Proteomic Profiling for Biomarker Discovery and Validation using a Bruker Impact II Q-TOF,
Gary H. Kruppa, Ph. D., Vice President 'Omics, Bruker Daltonics Inc., Billerica, MA

Proteoform profiling is showing great promise as a more efficient tool for biomarker discovery than bottom –up proteomics. Recent advances in proteoform profiling have been enabled by advances in both protein chromatography, and high resolution mass spectrometers. In this talk we will present HPLC-MS workflows using the Bruker Impact II Q-TOF mass spectrometer that result in the detection and label free quantitative comparison of >10,000 proteoforms per sample, in clinical research cohorts.

Qualitative proteomics in forensics - a case study.
Leonard Foster, University of British Columbia

Snakes include some of the most venomous animals on earth. Snake venoms are typically detected by species-specific enzyme-linked immunosorbent assay. A blood sample from a deceased female who might have been exposed to an unknown venomous snake was submitted to a proteomics approach to try to identify any snake venom proteins that might be present. A comprehensive approach using depletion, two types of fractionation, LC-MS/MS in an Impact II QTOF and three database search engines was employed. Between 900 and 1500 proteins were found in four analytical approaches, largely consisting of known blood proteins but also containing several proteins apparently originating from snakes. All snake protein hits with fewer than three unique peptides were rejected. Of the remaining, individual peptides were first checked for uniqueness to snakes – any apparent snake hits where the underlying evidence also matched to human proteins were rejected. Nine snake proteins remained that could not be explained by either spurious matches or complete homology to humans. All nine proteins had unequivocal MS/MS evidence, therefore supporting

the presence of snake venom in the deceased's blood. The sequences of identified proteins suggest that the snake from which this venom originated was one of the North American species of rattlesnake, Crotalus horridus or C. adamanteus. However, because venom proteins are highly variable and many snakes' genome sequences are not yet available for consideration, the species of snake should still be considered uncertain based on these data.

Breakfast Seminar

Friday, May 5, 2017
8:15 to 8:45
Room 1160-

Introducing the New X500B QTOF Mass Spectrometer for a simpler LC-MS solution for routine biomolecular characterization of proteins, lipids and metabolites

Brigitte Simons, SCIEX, Concord, Ontario, Canada

The X500B QTOF System was launched in February and comes to market specifically to deliver ease-of-use for high resolution standardized workflows for biotherapeutic applications, to provide walk-up capability fully characterize biologic products with accurate detail for all critical attributes. The compact and user-friendly MS system includes a new powerful operating system, called SCIEX OS, that enables open access laboratory functionality and simplifies complex data processing. This breakfast seminar will cover details about this new instrument demonstrating workflows for the characterization of proteins and metabolites.

Morning Coffee Break Seminar

Friday, May 5, 2017
10:50 to 11:20
Room 1160

Utilization of High Resolution Accurate Mass LCMS for Large Molecule Quantitation

Keeley Murphy
Senior Marketing Specialist - Pharma/Biopharma, Thermo Fisher Scientific

Bioanalysis is a vital component in many parts of the drug discovery and drug development process, providing quantitative measurements for drugs components, drug metabolites and other biomarkers. Although bioanalysis often brings to mind the quantitation of small molecule targets, in recent years the quantitation of peptides and proteins for the development of biopharmaceuticals has become increasingly visible and important. Furthermore the quantitative analysis of biopharmaceuticals is often being shifted to laboratories traditionally tasked with small molecule analysis. Here we will discuss workflows to meet the needs of large molecule analysis. Simplified and reproducible sample preparation protocols are coupled with UHPLC separation and analyzed utilizing high resolution accurate mass instrumentation to provide complete solutions for large molecule target analytes.

BIO: Keeley Murphy is Senior marketing specialist supporting Pharma and Biopharma screening and quantitative applications. Over the past several years at Thermo, Keeley has provided support for the development and launch of multiple products including the Q Exactive, Exactive Plus, TSQ Quantiva, and Vanquish UHPLC platform. Prior to joining Thermo Fisher Scientific, Keeley was a member of the early ADME profiling group at Novartis Institutes for BioMedical Research (NIBR), Cambridge, MA where he was responsible for utilizing multiple analytical technologies in support of various drug discovery projects.

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

Leonard Foster

University of British Columbia

Dr. Leonard Foster is a Professor in the Department of Biochemistry and Molecular Biology and the Michael Smith Laboratories at the University of British Columbia (UBC). He received his B.Sc from Simon Fraser University and his PhD from the University of Toronto in 2001. From there he went to the University of Southern Denmark to study with Matthias Mann. In 2005 he took up his current position at UBC where his research interests revolve around the application of mass spectrometry-based proteomics to study host-pathogen interactions. He uses proteomics to understand, at a systems biology level, how human pathogens manipulate their host cells and how the cells, in turn, respond to infection. In particular, he has developed potential vaccines for Chlamydia and Salmonella bacteria. He is also known for his research in honey bees, particularly for understanding the mechanisms of disease resistance and using this knowledge to try to guide selective breeding in this important insect. Dr. Foster has published 148 papers and he has trained more than forty undergraduate and graduate students, as well as post-doctoral fellows. He remains very active in outreach and extension and frequently engages the public on various aspects of human health, honey bees and biotechnology.

PRESENTATION:

Developing and applying proteomics in agriculture and medicine

My laboratory has focused on developing and applying quantitative proteomic technologies to a wide range of biological questions, from understanding basic mechanisms to discovering and applying biomarkers. I will discuss two different areas:
a) We correlated protein expression with measured behaviours in 230 honey bee colonies.
Over the following three years we used the markers discovered initially to guide a selective breeding program for hygienic behaviour and benchmarked these selected bees versus bees selected by conventional means or not selected at all. The bees selected using protein biomarkers were as resistant to disease as those selected using the best available conventional methods. This is the first demonstration of successful selective breeding using expression biomarkers that we are aware of
b) We have previously developed Protein Correlation Profiling coupled with SILAC (PCP-SILAC) to quickly, efficiently and accurately map the protein interactome. Recently, we have extended this approach to look at the interaction networks in different mouse tissues. Both SILAC (Lys-6) and normal mice were generated and seven tissues (Brain, Lung, Liver, Heart, Skeletal Muscle, Thymus and Kidney) isolated, subjected to complex extraction and size exclusion chromatography under non-denaturing condition. SILAC labeled samples were then used to generate a global reference mixture that was added to all non-labeled samples to provide a means to compare between and across SEC fractions from different tissues. Using an upgraded analytical pipeline, 9063 protein groups were identified across the seven tissues with 8231 protein groups leading to the generation of unique Gaussian fitted profiles. Using this data, the interactome of each tissue was determined; quality characteristics of these data, e.g., precision, FDR and FNR, were equal to or better then typical tagged based approaches. Interestingly a large proportion of proteins were only observed within a limited range of tissues, suggesting the interactome of each tissue is highly specialized. From the detected Guassians fitted profiles, 31518 protein interactions could be determined with a precision of ~65% based on comparison to the CORUM database..

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.

These award are being presented by SCIEX to

Simon, David
Queen's University

PRESENTATION:

Improving Sensitivity and Reducing Duty Cycle with Novel Multi-Electrospray Ionization Emitters

Since the first commercial Orbitrap was released in 2005 it has become a workhorse for many analytical applications[1]. The speed, sensitivity, resolving power, and mass accuracy have provided us with new techniques and insights into drug discovery, drug metabolism, detection of contaminants, and even a draft map of the human proteome[2]. Recent advances in ion optics, trapping, and detection have been extraordinary in improving mass accuracy and resolution of modern instruments; however new source ionization techniques to enhance the sensitivity of ion generation have been lacking. This discussion will feature the fabrication and utilization of unique nine channel micro-nozzle array for multiple nano-electrospray ionization and its potential impact on 'omic' studies[3]. In preliminary studies we have demonstrated a 35 times signal enhancement for the +3 charge state of the peptide bradykinin over the traditional one channel emitters3. Recent results featuring a more advanced 18-channel emitter will be presented.

(1) Zubarev, R. A., and Makarov, A. (2013) Orbitrap m
(1) Zubarev, R. A., and Makarov, A. (2013) Orbitrap mass spectrometry. Anal. Chem. 85, 5288–5296., (2) Kim, M. S., et al. (2014) A draft map of the human proteome. Nature 509, 575–581., (3) Fu, Y., Morency, S., Bachus, K., Simon, D., Hutama, T., Gibson, G. T. T., Messaddeq, Y., and Oleschuk,
R. D. (2016) A Microstructured Fiber with Defined Borosilicate Regions to Produce a Radial Micronozzle Array for Nanoelectrospray Ionization. Sci. Rep. 6, 1–7.

Contributing Authors
David Simon; Kyle Bachus; Richard Oleschuk; David Zechel

Lauzon, Nidia
University of Montreal

PRESENTATION:

Forensic analysis of latent fingermarks by silver-assisted LDI (AgLDI) imaging MS (IMS) on non-conductive surfaces.

AgLDI IMS is a useful technology to determine and image the molecular composition of latent fingermarks for suspect identification. Here, we demonstrate the capacity of the methodology to be incorporated within standard forensic enhancement techniques (FETs) used by law enforcement agencies when analyzing fingermarks from non-conductive surfaces commonly found during an investigation.

The fingermarks were first revealed by FETs. For IMS analysis, the non-conductive surfaces containing the latent fingermarks were mounted on ITO glass slides using double-sided carbon conductive tape. Silver layers (~14 nm) were then deposited on top of the fingermarks using a sputter coater. Profiling and IMS were performed on a MALDI TOF/TOF mass spectrometer (Bruker Daltonics).

Sputter deposition of a thin layer of silver on fingermarks followed by LDI IMS analysis allows for the detection and imaging of several classes of endogenous as well as exogenous compounds (JASMS, 2015, 26, 878-886). Additionally, the silver layer renders the target surface conductive, which allows the analyses of insulating samples by time-of-flight (TOF) IMS. In this regard, fingermarks deposited on paper, cardboard, plastic bags and lifting tape, were first revealed by the Sûreté du Québec using standard FETs prior to IMS analyses. From these, numerous endogenous compounds including fatty acids, cholesterol, squalene, wax esters, triglycerides, as well as several exogenous substances were detected and imaged. Here we show that AgLDI IMS can associate molecular information to an individual and in some cases, provide visual enhancements of fingerprint patterns (see figure).

AgLDI IMS can be performed after most fingermark FETs used by law enforcement agencies worldwide offering promising perspectives in forensic science.

Contributing Authors
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These award is generously sponsored by

PosterPresentations

Bagga, Aafreen Kaur
York University

Alcohol consumption during gestation period alters phospholipid distribution in developing fetal brain

Fetal Alcohol Spectrum Disorder (FASD) is caused by alcohol abuse during pregnancy. Alcohol can lead to lipid peroxidation and transbilayer migration disrupting lipid bilayer asymmetry and intracellular signaling. The localization and composition of phospholipids in plasma membrane is relevant for physiological nerve cell communication and is investigated in this research using DESI-MS imaging.

The pregnant mice was dosed with 10% alcohol during first trimester to study 28 day old FASD afflicted pups. Wildtype and FASD mouse brains were sectioned into 15µm thin slices using cryostat. The lipid profiles and images were acquired using DESI ion source coupled with LTQ finnigan mass spectrometer (MS). While the identification of lipids was obtained using LTQ orbitrap MS.

ESI-MS analysis of brain tissue in positive ionization mode identified the presence of sodium and potassium adducts of phosphatidylcholine and phosphatidylethanolamine; while, negative ionization mode indicated the presence of phosphatidylserine and phosphatidylinositol. The intensity and distribution of lipids varied significantly between FASD and wildtype brain tissues with majority of the phospholipids being upregulated in FASD as compared to the control in both positive and negative ionization modes. The unique distribution of lipids highlighted FASD specific biomarkers that can be used for disease diagnostics. Moving forward, the research into FASD would open a window of opportunity to identify the role of phospholipids in the pathology of FASD and as a potential aim of a drug discovery process.

This research would enhance the existing knowledge of FASD along with providing a diagnostic tool using DESI-MS imaging.

Contributing Authors
Aafreen Kaur Bagga; Paige Whyte-Fagundes; Georg R. Zoidl; Demian R. Ifa

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York University, Sanofi Pasteur

Characterizing the Immune Response to Novel Vaccine Candidates in a Human Whole Blood Assay Using High-Dimensional Single Cell Proteomics

Monitoring the immuno-modulatory effects of vaccine formulations is critical for novel vaccine development. While animal models have been effective, increasing evidence suggests differences when translating to the human immune response. Hence, the development of new methods to study complex and heterogeneous immune cell populations in a human-based system is of critical importance.

In this study, we will utilize fresh human whole blood as a model to screen vaccine formulations. The immune response will be characterized by high-throughput single cell analysis using mass cytometry. Specifically, this deep proteomic profiling will provide crucial biological information regarding immunophenotyping and the expression of key cytokines and signaling cascades at a single-cell level.

We have conducted a proof of concept analysis on human peripheral blood mononuclear cells to optimize the mass cytometry protocol and devise a panel of target antibodies. Preliminary results revealed optimal immunophenotpying of naive, effector, and memory T-cells, as well as other immune cells including B cells, monocytes, and NK cells. Furthermore, we have established an efficient method for the fresh human whole blood assay by stimulating samples with a range of vaccine formulations and measuring the baseline immune response by ELISA for interferon gamma production. We are currently optimizing the mass cytometry protocol for use with the human whole blood assay.

This unique approach will produce high-dimensional data on adjuvant-modulated, antigen-specific immune responses in a clinically relevant human model.

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Determining protein structure using an electrospray ionization reaction

Determination of structure describes the protein function Two approaches:
- Use of HDX to assess the solvent-accessible portions of the protein. However, when CID is used to assess the location of these modifications, the D-atoms are known to scramble during the activation process.
- Covalently label these solvent –accessible regions so that CID can be use to localize the modifications.

Dissolved the solution of lysozyme into a solution of 95:5 Water: Acetic Acid Part 1- Control - Lysozyme solution mixed with H2O2 solution at different percentages - Each vial run through Waters Synapt G1 Part 2- ESI - Solutions are run through two independent emitters - Side emitter: lysozyme - Top emitter: H2O2 - Three reactions are carried out with different polarities of the top.

As the amount of hydrogen peroxide is increased, there is a higher abundance of the larger charge states. Reaction at positive mode yielded the greatest effect on lysozyme structure. - Lysozyme had a 25% and 21% abundance of the 11+ and 12+ charge states respectively - Similar to an 80:20 lysozyme: hydrogen peroxide sample

Hydrogen peroxide acts as a denaturing agent. The unfolding of the protein exposes more amino acids to the surface of the protein. Allows the protein to be protonated by the acetic acid. - Introduces higher charge states A positive polarity favors the denaturing process by giving cations a higher charge-carrier emission during the final stage of analyte desolvation. Since the reaction occur in a very short period of time in the ion source, the denaturing effect of hydrogen peroxide isn't as evident.

Dual-spray reaction is being used to modify the protein structure of lysozyme within an ion source

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Characterizing lipocalin 2 dynamics upon siderophore and iron binding using electrospray ionization mass spectrometry

Lipocalin 2 is secreted by neutrophil as a bacteriostatic agent that sequesters iron from bacteria. Lipocalin 2 has three binding sites for catecholate-type ferric siderophores at the edge of its beta barrel. Catecholate siderophores are small molecules with high affinity for iron(III), including bacterial 2,3-dihydrobenzoic acid (2,3-DHBA) and mammalian 2,5-dihydrobenzoic acid (2,5-DHBA).

Lipocalin 2 binding specificity to 2,3-DHBA or 2,5-DHBA were assessed by electrospray ionization mass spectrometry (ESI-MS). Both apo and holo lipocalin 2 were intended to unfold by acids (5 v/v% or 10 v/v% acetic acid, and 0.2 v/v% formic acid). Time resolved hydrogen deuterium exchange (HDX) was used to characterize the the dynamic of holo protein in terms of deuterium uptake.

Holo lipocalin 2 expressed in XL-1 blue was co-purified with bacteria siderophore 2,3-DHBA. The protein-siderophore complex was composed with three siderophore and one iron bond to one protein. However, when apo lipocalin was spiked in vitro, only one 2,3-DHBA bound to protein at geometric ratio. For a mammalian analogue 2,5-DHBA, lipocalin 2 will not bind interact with it in vitro, even when protein siderophore mixtures was pre-incubated in PBS(1X). It was consistent among all three acids of choice that apo lipocalin 2 will be unfold to a less extend then holo protein. Additionally, holo lipocalin 2 exhibited a high deuterium uptake around the binding site at the edge of the bate barrel. This may suggest that the ligand binding open up the bate barrel by disrupt the beta sheets at the edge. This distortion of the barrel primed it for a easier unfolding.
Lipocalin 2 can distinguish bacteria and mammalian siderophores. Siderophore-iron bound lipocalin 2 reveals high dynamics around the binding sites.

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TargetSeeker-MS: A Bayesian Inference Approach for Drug Target Discovery using Protein Fractionation Coupled to Mass Spectrometry

Stability-based protein fractionation techniques, when coupled to mass spectrometry (MS), have shown great potential to identify drug targets on a proteome scale. These approaches support the hypothesis that the binding of a drug to a protein will change its stability. Nevertheless, the computational analyses associated with these techniques are rudimentary and tied to the experimental methods.

We propose a novel Bayesian inference method named TargetSeeker-MS to identify drug targets in data produced using stability-based protein fractionation techniques coupled to MS. The algorithm builds quantified fractionation profiles for each protein and evaluates the significance of profile differences between the untreated and drug-treated samples, thereby assessing drug-binding confidence.

We analyzed with TargetSeeker-MS C. elegans protein lysate samples untreated and treated with benomyl, a fungicide. TargetSeeker-MS identified several proteins with fractionation profiles that were significantly altered by benomyl, including aldehyde dehydrogenase, a known target. We demonstrate that TargetSeeker-MS is flexible and that its drug target identifications are reproducible in C. elegans samples that were processed using two different stability-based protein fractionation techniques (thermal shift assay and acetic acid-based separation). We also validate a novel benomyl target by measuring in vitro its enzymatic activity upon drug treatment. TargetSeeker-MS, which is available on the web, allows the confident identification of the targets of a drug on a proteome scale, thereby providing a better understanding of its mechanisms and the evaluation of its clinical viability.

A flexible Bayesian learning approach coupled to mass spectrometry for the confident identification of drug targets on a proteome scale.

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Multiplexed MRM-based Protein Quantitation Using Two Different Stable Isotope Labeled Peptides for Calibration

When measuring protein in plasma, endogenous levels prevent the use of the best calibration strategies since no blank matrix is available. Several alternative calibration strategies are thus employed for targeted bottom-up quantitation by mass spectrometry. We present a new approach using two different stable isotope standard (SIS) peptides, enabling an external calibration curve in human plasma.

Calibration curves (31 peptides, 7 calibration strategies, 5 matrices) were evaluated and compared based on precision and accuracy via quality control samples (n=5, 3 QC levels), using three isotopes (light, heavy, and double labeled). Single point measurement, standard addition, and reverse curve strategies were evaluated. Human pl (also dimethylated pl), chicken pl, PBS+BSA, and PBS were tested.

The calibration approach utilizing two stable isotope labeled peptides, one as the calibrator and the other as the internal standard added uniformly to all samples outperformed single point measurements and reverse or “flipped” curve methodologies. This strategy could replace reverse curves since it does not introduce accuracy bias in the measurement due to ratio flipping, while at the same time it can simplify method development and validation. Tests were performed with 31 peptides of varying hydrophobicities and endogenous concentrations (light, heavy, and double labeled were synthesized in-house). In addition, curves prepared in different matrices were compared. While all matrices performed similarly overall, chicken plasma and dimethylated human plasma were subject to additional interference, while simpler matrices are prone to variability due to adsorption to labware.

The use of two types of labeled stable isotope isotope-containing peptides for improved multiplexed MRM-based peptide quantitation in plasma.

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Structural Analysis of Membrane Proteins by Time-Resolved Hydrogen/Deuterium Exchange Mass Spectrometry (TRESI-HDX MS).

The challenges of studying membrane proteins are countless, but the reward of doing so is big - many of them end up being targets for over 50% of all modern drugs. Here, we were not afraid of any challenges and tried to obtain the structural and dynamic characterization of α-synuclein (intrinsically disordered

membrane protein) in the presence of lipid nanodiscs by a novel approach - TRESI-HDX MS.

Together and on its own purified α-synuclein and assembled nanodiscs are run through the microfluidic device coupled to mass spectrometer, where the rapid mixing of protein and D2O is quenched by the acetic acid at pH 2 and the mix is digested on the protease–activated beads. The result spectrum gives a range of peptides that could be analyzed for deuterium uptake using FORTRAN program.

The HDX data on α-synuclein alone have shown the general increase in deuterium uptake in ten peptides positioned randomly in different regions of the protein. This data is consistent with the intrinsically disorder nature of this protein. The HDX profile on α-synuclein bound to a nanodisc is expected to give a decrease in deuterium uptake especially for the peptides in the N-terminal and central hydrophobic regions due to obtaining alpha helical structure. Additionally, formation of nanodiscs was verified by 1D 31P high resolution NMR. Chemical shift from ~ -16ppm to -0.12 ppm and increase in spin-lattice relaxation time was observed when phospholipids had transitioned to a well-structured nanodisc complex. The 8, 6, and 4 nm nanodisc were formed and verified.

Characterization of α-synuclein dynamics in a native-like lipid bilayer nanodiscs is a key step in drug screening for Parkinson's.

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Portable FT-IR Spectroscopy; an in-field analysis of substandard antibiotics

In recent years, the number of healthcare issues caused by substandard pharmaceuticals has grown around the world. This has made the importance of quality control in this industry paramount to a proper healthcare system. However, established QC methodology often requires the use of costly instrumentation, reagents and infrastructure that developing countries do not have access to or can afford.

With the Agilent 4500 Portable FT-IR, the formulation quality of amoxicillin and ciprofloxacin were quickly and reliably tested to verify that the amount of active ingredient and its excipients conform to the pharmacopoeia regulations. The results were verified by the standard Pharmacopoeia UPLC-UV methods. The Democratic Republic of Congo, Ghana, Haiti, India, and Indonesia where selected as sampling sites and samples were made by local and foreign manufacturers. The results showed that some samples either failed to comply with specifications due to improper powder mixing techniques during the formulation process (50% of the amoxicillin samples and 28% of the ciprofloxacin samples failed) or because of improper filling techniques (18% of amoxicillin samples and 14% of ciprofloxacin samples failed). The FT-IR, when compared to the traditional LC-UV Pharmacopoeia methods, gives similar results with only a 5-10% deviation. However, the Ft-IR can be used in the field and does not require any sample preparation, advanced personnel training or additional infrastructure.

The portable FT-IR is a fast and robust in field alternative to the infrastructure dependent methods traditionally used to identify substandard drugs.

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Protein-Protein Interaction Network of Mammalian Brain Revealed by Large-scale Biochemical-Profiling-Mass Spectrometry (BP/MS)

Deciphering the connectivity of neurons is a key challenge in neuroscience. To investigate their basic physical organization, we performed a global analysis of protein complexes and PPIs in mouse brain using a new interactome mapping platform. We identified hundreds of assemblies involved in neuronal function, brain development and neurodegenerative disorders, providing rich mechanistic insights.

To identify stably co-eluting proteins, we performed quantitative tandem mass spectrometry after exhaustive physiochemical fractionation of soluble cerebellar lysates using Isoelectric Focusing followed by orthogonal Ion Exchange Chromatography.

MS analysis of over 500 soluble protein fractions led to the identification of ~6,000 unique proteins in brain. Co-fractionation of stably-associated complex subunits was predicted based on protein profile similarity using machine learning, and evaluated against a reference set (gold standard) of annotated complexes. By integrating

the 2D co-fractionation data with complementary functional association information, a large high-confidence brain protein interaction network (B-PIN) was created.

B-PIN is the largest experimentally-derived brain PPI network to date, and will serve as resource for the neuroscience and systems biology researches.

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Phospholipid-based model membrane system for hydrogen-deuterium exchange mass spectrometry of membrane proteins

Membrane proteins represent an exciting bioanalytical frontier due to their critical physiology and therapeutic potential as drug targets. Existing structural biology techniques including X-ray crystallography and nuclear magnetic resonance (NMR) are difficult to implement for membrane proteins due to their hydrophobic nature and relatively low expression levels.

This work describes the novel coupling of a phospholipid-based model membrane system with hydrogen-deuterium exchange mass spectrometry (HDX-MS) for studying conformational changes in membrane proteins on a millisecond timescale.

HDX-MS results for the pro-apoptotic Cytochrome C (Cyt C) - membrane interaction revealed global decreases in deuterium uptake in the presence of free membrane phospholipids. Significant decreases in deuterium uptake (>40%) were observed across known heme-binding motifs, providing insight into conformational changes occurring in Cyt C's functional transition from electron carrier to pro-apoptotic catalytic centre. These dynamic results conform to previous biophysical characterization showing disruption of the Met80-heme bond upon phospholipid binding as described in the Extended Lipid Anchorage" model. Regions surrounding the Met-80 bond also experienced large decreases in deuterium uptake providing further insight into conformational changes occurring in the presence of membrane phospholipids."

Together, these results represent the first application of an HDX-MS method for studying the interaction between Cyt C and the mitochondrial membrane.

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RP-HPLC Enantioseparation of (RS)-Ketamine in human plasma via Chiral Derivatization Based on (S)-Levofloxacin

Ketamine [(RS)-KetA, (RS)-1], having systematic chemical name, (RS)-2-(2-Chlorophenyl)-2-(methylamino)cyclohexanone, is a dissociative anesthetic drug used in the treatment of anesthesia, bronchospasm, and complex regional pain syndrome and as an antidepressant. Ketamine is also classified as an N-methyl-D-aspartate (NMDA) receptor antagonist. (S)-1 has twice the anesthetic potency of (RS)-1.

A new chiral reagent was synthesized from (S)-levofloxacin by converting it into a hydrazide derivative taking advantage of the reactivity of its carboxyl group. It made available a reaction site for the ketonic group present in (RS)-ketamine. The diastereomeric hydrazones were separated on a RP C18 column with a mobile phase consisting of 35-65 % MeCN and 0.1 % TFA under gradient elution mode.

The limit of detection was found to be 3.2 and 3.4 ng/mL for first and second eluting diastereomeric hydrazones, respectively. The separation mechanism and elution order of the diastereomeric hydrazones were proposed and supported by developing the geometry optimized 'lowest energy' structures of the two diastereomeric hydrazones using program Gaussian 09 Rev. A.02 at B3LYP/6-31G* level of theory
.
The novelty of work lies in the synthesis of new CDR and its application in the determination and enantioseparation of (RS)-Ketamine

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Quantifying α-MSH peptides in mouse pituitary using MALDI-FTICR imaging

Melanocortin peptides are some of the most important hormones involved in the regulation of body weight, obesity and type 2 diabetes. Despite their pivotal role, very little is known about which of the multiple natural melanocortin peptides are responsible for these physiological functions. This work investigates the most likely group of candidates, α-Melanocyte-stimulating hormone (α-MSH).

Mouse pituitary samples (from wild type and homozugous for the deletion of α-MSH) were collected and analysed using MALDI imaging to locate the region where each peptide of interest. These tissues were then compared with external standards, consisting of mouse brain homogenates which were spiked with a range of standard α-MSH with which a calibration curve was constructed.

Mouse pituitary and mouse brain homogenate were cryosectioned (12μm), and coated with DHB matrix. MALDI IMS data sets were collected using a MALDI-FTICR with resolution of 30-150um. MALDI images were produced using flexImaging software. Accurate mass measurements were collected as additional confirmation of peptide identities. In WT pituitary tissue, α-MSH (m/z 1664.78), desacetyl-α-MSH (m/z 1622.77) and diacetyl-α-MSH (m/z 1706.79) were located in the intermediate lobe and were not detected in the homozygous tissue. In addition, seven other peptides were also positively identified and localised to specific lobes of the pituitary gland. An initial attempt was made to quantify the amount of each α-MSH peptide based on the calibration curve, resulting in quantities ranging from 8-11 ug/g for desacetyl-α-MSH, 8-15ug/g for α-MSH and up to 225-240 ug/g for diacetyl-α-MSH.

Using MALDI-imaging to quantify peptides within specific regions of tissues samples.

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HDX-MS and Molecular Dynamics Simulations Provide Complementary Insights Into The Mechanisms of Allosteric Protein Regulation

Numerous enzymes and regulatory proteins exhibit allosteric behavior, here we focus on this attribute on an important class of signaling proteins. S100A11 is a homo-dimeric complex involved in cancer progression. Its interactions with annexin A2 are allosterically regulated by Ca2+ binding. By applying HDX-MS and molecular dynamics (MD) simulations we gain insights into the mechanistic basis.

Protein samples were deuterated in 90% D2O. After various deuteration time periods aliquots were quenched to pH 2.3 and flash frozen in liquid nitrogen until further analysis. HDX experiments were carried for a total of four sample types: +(-)Ca2+/+(-) annexin A2-derived peptide (PA2). Complementary 1 μs MD simulations were conducted on all four conditions using Gromacs 5.0 at 295 K.

HDX-MS measurements on S100A11 revealed major changes upon addition of Ca2+ and PA2. The largest Ca2+-dependent changes were seen in the EF hand metalation site between helices III/IV. Clearly, unraveling of this segment plays a key role during allosteric regulation. Interestingly, HDX results also suggest the lack of PA2 induces an "energetically frustrated" state on S100A11 with partially exposed hydrophobic surfaces. Our MD simulations were started with the crystal structure of the holo-protein system. Consistent with the HDX-MS results, MD simulation data revealed that Ca2+ removal causes the EF hand Ca2+ binding loops to switch from highly constricted conformations to extensively disordered structures. In summary, allosteric signal transmission in S100A11 involves the rupture of metal coordination bonds, alterations in hydrophobic packing, as well as salt bridge rearrangements.

We uncover how allosteric signals propagate through a signaling protein, from effector binding sites to distant ligand recognition sites.

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Enhancing Ganglioside Species Detection for MALDI Imaging Mass Spectrometry

Gangliosides (GMs) are a family of lipids abundant in the neural cell membrane whose abnormal accumulation has been observed in many neurological diseases, such as Alzheimer's and Hunter's, and in cancers such as neuroblastoma. Despite many published methods, their detection for imaging mass spectrometry (IMS) remains difficult because of their structural complexity and instability in source.

14μm fresh frozen mouse brains were mounted onto ITO slides and desiccated for ≤1h. Three parameters were investigated and optimized: wait time before data acquisition post sample preparation, ammonium formate (AF) washes, and AF spray deposition. Sublimated 1,5-DAN was used as matrix. Data was acquired using a MALDI-TOF/TOF Ultraflextreme in negative reflectron mode and processed in R.

Optimal wait time after sample preparation was found to be 24h (3-fold signal increase). Optimal AF wash was 30s in a 75mM solution (nearly 2-fold signal increase). Optimal AF deposition was 45 passes of 4:6 H2O:ACN 400mM AF solution using the TM-Sprayer (4 to 5 fold signal increase). Combinations of these optimized parameters led to even better results. The best provided both increase in GM MS signal intensity (15-fold) and the number of detected species (from 3 to 15). In detail, this procedure called for an optimized AF wash followed by spray deposition and a 24h wait time prior to IMS analysis. Using this procedure, IMS results obtained at 65μm of spatial resolution not only corroborated visually with the profiling data, but also showed that the histology distribution of the GMs was preserved (see Figure). MS/MS data confirmed the presence of native GM1,2&3 and GD1 species.

Reproducibly detect and image native ganglioside species by MALDI MS at significantly greater signal intensity without affecting spatial resolution.

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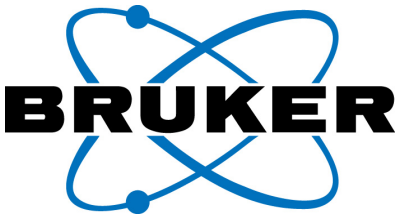

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