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Enabling Technologies

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

TRI-CONFERENCE

1st EASTERN CANADA MASS SPECTROMETRY CONFERENCE

10th INTERNATIONAL SYMPOSIUM ON ENABLING TECHNOLOGIES

THE 35th TRENT CONFERENCE ON MASS SPECTROMETRY

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

It is my pleasure to welcome you to the beautiful Bishop's University campus for the 2019 TRI-Conference, encompassing the 1st Eastern Canada Mass Spectrometry Conference, the 10th International Symposium on Enabling Technologies and the 35th Trent Conference.

We have a very exciting program to offer you. The opening session on Sunday evening includes two invited talks by Dajana Vuckovic, our 2019 CSMS young investigator award winner, and Christiane Auray-Blais, a leader in clinical metabolomics and director of the newborn screening program for hereditary metabolic diseases in Quebec. We then invite you to our welcome reception where food and drinks will be served in the poster hall. We have a full schedule on Monday and Tuesday, mostly filled with great student talks. We are very pleased to have Kristina Hakansson as our invited speaker on Monday afternoon. She will be covering the exciting area of electron-based tandem mass spectrometry. Our distinguished Ken Standing award winner, Emma Lundberg, will present her plenary lecture on Tuesday just prior to our special BBQ reception, at the TD Terrace at the John H. Price Sports Centre. Wednesday is our « Training day » with career talks and informative Mass Spectrometry tutorials on five different topics at Bishop's new Library.

I would like to take the time here to thank our generous sponsors, who have helped keep registration fees at a reasonable level. We have tried to elicit the most student participation as possible and have everything included in one registration price. Please take the time to visit the vendor booths we have on site during the coffee breaks and poster receptions. Special thanks to MS-ESE, an NSERC-funded training network headed by Derek Wilson from York University, for sponsoring an invited speaker and the conference itself as well as enabling many students to travel to the conference.

I would like to also acknowledge the hard work that Janette Champ has invested in helping organize a successful conference. She will be available throughout the conference if you have any questions on logistics, etc.

To facilitate and encourage networking, all meals are included, either at the cafeteria or special reception events. Boxed lunches will be served in the new Library space on Wednesday. We have tried as much as possible to create a « retreat » feel to this conference. We hope you enjoy the program and please do not hesitate to give us your impressions to keep in mind for future meetings.

Wishing you all a great meeting!

~ Lekha Sleno, CSMS President

PROMOTING MASS SPECTROMETRY ACROSS CANADA

Join Canadian Society for Mass Spectrometry (CSMS) in our effort of stimulating interest and collaborations in the Canadian mass spectrometry community! Plus as a member receive preferential discounted rates at conferences (including ETP symposium, Lake Louise Tandem Mass Spectrometry Workshop), apply for or get nominated for CSMS numerous awards, access to online "members-only" employment center and discussion forum plus keep up to date from our subscription to CSMS info emails.

Organizing Committee

Lekha Sleno, Conference Chair, CSMS President, Professor (UQAM chemistry)
Janette Champ, President, ETP Symposium Inc

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History of ETP

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-renowned 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 non-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

History of the Trent Conference

"The Trent Conference" started in 1977, with organizers Diethard Bohme (York University) and Raymond March (Trent University), and has historically emphasized Canadian research in gas phase ion chemistry. The format of the conference has traditionally been to have oral presentations almost exclusively by graduate students or post-doctoral fellows. Past meetings have been held for many years at Trent University (1977/80/86/88-2000), with a few other venues including U. Laval, U Ottawa, Queen's, Waterloo, Leslie Frost Centre (2001-2003), in conjunction with IMSC 2016 in Toronto and for several years at the YMCA Geneva Park (between 2004-2017). This year we are very happy to incorporate the 35th Trent conference within the 2019 Tri-Conference on Mass Spectrometry.

Symposium Chair

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. As of 2019, she is a Full Professor and holds an UQAM Strategic Chair in Bioanalytical Chemistry. She is also currently CSMS President (2017-2020).

General Information

Accommodation Information

If you requested accommodation, we will have information and room keys together with meal plan cards for you at Registration. All accommodations will be located in the Patterson Residences or the Abbott Residences.

Signage and Maps will be available for attendees.

Residence Check-In

Please come to the Registration Desk, located at Cleghorn Room, McGreer Building (B12). Here you will receive your registration package, which includes room keys, meal plan cards, and conference materials.

A $50 deposit for the use of the Meal Plan Cards and Room Keys are required. Pursuant to the Terms set out at the time of Registration. Upon return of both these key cards, your deposit will be returned to you. The deposit may be through Credit Card information, cheque or cash.

Residence Check-Out

To check-out, please go to the Registration Desk which will be located at the Library Agora for the last day of the Conference.

Please ensure that you have vacated your room prior to noon on your last day at the conference.

Upon receipt of your **room key and meal plan card**, your deposit will be returned.

Poster / Exhibit Area – Cleghorn Room, Greer Building

Poster Set-Up

Posters are to be placed prior to 19:00 on Sunday, August 4 and are to be removed no later than 15:30 on Tuesday, August 6.

Exhibit Set-Up/Take-Down

Exhibition is to be set up by no later than 19:00 on Sunday, August 4 and are to be removed no later than 15:30 on Tuesday, August 6.

AGENDA

Sunday, August 4, 2019	
16:00 - 17:30	***Residence check-in and Event Registration*** *Cleghorn Room, McGreer Building (B12)*
	Conference Start, Introductions, *Bishop Williams Hall*
	Opening Session (Bishop William Hall)
17:30 - 18:15	**CSMS Award Winner - Young Investigator Award** ***Dajana Vuckovic, Concordia University*** *Improving lipid coverage and data quality for untargeted lipidomics and oxylipin profiling*
18:15 - 19:00	**Invited Speaker** ***Christine Auray-Blais, Université de Sherbrooke*** *A Metabolomic Approach for Biomarker Discovery and Precision Medicine*
19:00 - 22:00	***POSTERS& RECEPTION, (Cleghorn Room)***

Monday, August 5, 2019	
8:00 - 9:00	***Breakfast, (Dewhurst Dinning Hall, Residence Complex Building D2)*** *(Cafeteria is open for Breakfast from 7:00 to 8:30)*
9:00 - 10:35	***Pharma, Clinical Session, Bishop William Hall***
9:00 - 9:20	***Wondewossen Gebeyehu, Carleton University*** *Method development and implementation of rapid, on-site drug checking in a supervised injection site using portable mass spectrometry*
9:20 - 9:40	***Liang Hu, York University*** *The Prospects of Mass Spectrometry in Direct Quantitative Analysis of Multiple microRNAs (DQAMmiR)*
9:40 - 10:00	***JoAnn Chen, University of Toronto - MS-ESE York University*** *Investigating Conformational Effects of DNA-drug Interactions by Gas-phase Förster Resonance Energy Transfer*
10:00 - 10:15	***TBA, PerkinElmer*** *TECH TALK - Dual Source LCMSMS-improved functionality*
10:15 - 10:40	***Coffee Break, with posters & exhibits*** *Cleghorn Room, McGreer Building (B12)*
10:40 - 12:20	***Environmental - Small Molecule, Bishop William Hall***
10:40 - 11:00	***Sean Overton, University of Ottawa*** *Unimolecular reactions of NPAHs*
11:00 - 11:20	***Malick Diedhiou, University of Ottawa*** *Ion Dissociation Dynamics of 1,2,3,4-Tetrahydronaphthalene (Tetralin) As A Simple Test Case For Hydrogenated PAHs*
11:20 - 11:40	***Jessica Clothier, York University*** *New Mass Spectrometry Analysis for Perfluorinated Alkyl Substances (PFAS)*
11:40 - 12:00	***Lisa Szymkowicz, York University*** *Development of a targeted nanoLC-MS/MS method for quantitation of residual toxins from Bordetella pertussis*
12:00 - 12:20	***Thanh Ngan Thang, Université de Montreal*** *Applying Design of Experiments to the optimization of elution gradients in LC-MS*
12:20 - 14:00	***LUNCH BREAK - with Free Time*** *Dewhurst Dinning Hall, Building D2* *(Cafeteria is open for lunch from 12:00 to 13:30)*

Monday, August 5, 2019	
14:00 - 14:25	**Invited Speaker** ***Kristina Håkansson, University of Michigan*** *Electron-based tandem mass spectrometry for biomolecular cations and anions*
14:45 - 15:00	***Coffee Break, with posters & exhibits*** *Cleghorn Room, McGreer Building (B12)*
15:00 - 16:20	***Imaging, Bishop William Hall***
15:00 - 15:20	***Nidia Lauzon, RI-MUHC*** *Metabolomics by MALDI TOF Imaging MS: Biomedical Applications and Challenges.*
15:20 - 15:40	***Consuelo Perez, York University*** *Internal Standard Application Strategies in Mass Spectrometry Imaging by Desorption Electrospray Ionization*
15:40 - 16:00	***Ethan Yang, Université de Montréal*** *Silver spray deposition for AgLDI imaging MS of cholesterol, a fast alternative to sputter deposition*
16:00 - 16:20	***Maryam Yousefi-Taemeh, York University*** *Direct analysis of Cannabis derivatives from Cannabis-infused edible products by DESI-MS*
16:20 - 19:00	***Free Time & Dinner Break, Bishop's Cafeteria*** *Dewhurst Dinning Hall, Building D2* *(Cafeteria is open for Dinner - 17:00 to 18:30)*
19:00 - 22:00	***POSTER SESSION & SOCIAL*** *Refreshments will be served - Gleghorn Room / Posters & Exhibits*

Tuesday, August 6, 2019	
8:00 - 9:00	***Breakfast, (Dewhurst Dinning Hall, Residence Complex Building D2)*** *(Cafeteria is open for Breakfast from 7:00 to 8:30)*
9:00 - 10:35	***HDX Proteomics, Bishop William Hall***
9:00 - 9:20	***Chris Thibodeaux, McGill University*** *Advancing Biosynthetic Enzymology with Structural Mass Spectrometry*
9:20 - 9:40	***Banafsheh Mehrazma, York University*** *The unfolding of lipocalin 2 in the presence of siderophores*
9:40 - 10:00	***Shaolong Zhu, York University - Sanofi*** *Epitope Mapping of Diphtheria Toxin by Hydrogen Deuterium Exchange Mass Spectrometry (HDXMS)*
10:00 - 10:15	***Gaëlle Bridon, Agilent*** *Agilent LC/Q-TOF MS, Accelerating Capabilities for High Resolution, Accurate Mass MS analyses*
10:15- 10:40	***Coffee Break, with posters & exhibits*** *Cleghorn Room, McGreer Building (B12)*

Tuesday, August 6, 2019	
10:40 - 12:00	***Proteomics, Bishop William Hall***
10:40- 11:00	***Guy Poirier, Université Laval*** *Proteomics analysis the role of PARP in DNA homologous recombination*
11:00 - 11:20	**Bill Davidson Student Award Winner** ***Timon Geib, Université de Québec a Montréal*** *Reactive Metabolite formation and protein binding by LC-MS/MS*
11:20 - 11:40	***Marie-Pier Ouellet, Université de Montréal*** *Applying An Experimental Design To Improve The Use Of Insoluble Crosslinked Proteolytic Enzymes For Better Sample Preparation In Microscale Proteomics*
11:40- 12:00	***Maxime Sansoucy, Université de Québec a Montréal*** *Post-translational modifications in biological materials with unique properties*
12:00- 13:30	***LUNCH BREAK*** *Dewhurst Dinning Hall, Building D2* *(Cafeteria is open for lunch from 12:00 to 13:30)*

13:40 - 14:40	***Metabolites / Metabolomics, Bishop William Hall***
13:40 - 14:00	***Anca Baesu, McGill University*** *Application of non-targeted analysis to study the fate of malachite and leucomalachite green in shrimp following thermal treatment*
14:00- 14:20	**Bill Davidson Student Award Winner** ***Irina Slobodchikova, Concordia University*** *Characterization of phase I and II metabolites of 17 mycotoxins using liquid chromatography high-resolution mass spectrometry*
14:20 - 14:40	***Ben Warnes, Carleton University*** *Diazomethylation Enhancement of Phosphatidic Acid for Application in Mass Spectrometry*
14:40 - 15:00	***Ons Ousji, Université de Québec a Montréal*** *Studying the metabolism of BHT in vitro by LC-HRMS/MS*
15:00 - 17:00	***Coffee Break, with posters & exhibits + Free Time*** *Cleghorn Room, McGreer Building (B12)*
17:00 - 21:30	***The Ken Standing Award Presentation - Bishop William Hall & Reception with Special BBQ Dinner***
17:00 - 18:00	**The Ken Standing Award Winner** ***Emma Lundberg, Visiting Associate Professor, Genetics, Stanford University*** *Single cell proteome variability in human cells*
18:00 - 21:30	***Special Reception & BBQ Dinner,*** *TD Terrace, Roof Top of Sports Complex Building C6*

Wednesday, August 7, 2019	
8:00 - 9:00	***Breakfast, (Dewhurst Dinning Hall, Residence Complex Building D2)*** *(Cafeteria is open for Breakfast from 7:00 to 8:30)*
9:00 - 10:35	***Tutorials - Library Agora***
9:00 - 9:20	***Peter Liuni, York University*** *The Nuts and Bolts of a Mass Spectrometry Core Facility*
9:20 - 9:40	***Sydney Wellman, York University*** *Getting Out and Getting In (To Toxicology)*
9:40 - 10:20	***Derek Wilson, York University*** *Hydrogen Deuterium Exchange Mass Spectrometry: Up Close and Personal*
10:20 - 1040	***Coffee Break,*** *Library Agoura, Building B3*
10:40 - 11:20	***Pierre Chaurand, Université de Montréal*** *Imaging Tutorial Talk*
11:20 - 12:00	***Jeff Smith, Carlton University*** *Mass spectrometry-based lipidomics: fundamentals, opportunities and perspectives*
12:00 - 13:00	***Lunch,*** *Box Lunch - Library Agoura, Building B3*
13:00 - 13:40	***Karen Waldron, Université de Montréal*** *Capillary Electrophoresis - Mass Spectrometry: Recent Developments and Applications*
13:40 - 14:20	***Paul Mayer, University of Ottawa*** *Gas Phase Ion Chemistry*
14:20	***Conference Ends***

Invited Speakers

Christiane Auray-Blais, Université de Sherbrooke

Christiane Auray-Blais is the Director of Provincial Neonatal Urine Screening Program for hereditary metabolic disorders in Sherbrooke, Quebec. More than 3 400 000 newborn babies were screened in the Province of Quebec for disorders of amino acids and organic acids. She holds a Ph.D. in radiobiology from the Faculty of Medicine and Health Sciences (FMHS) at the Université de Sherbrooke and postdoctoral studies from Duke University Medical Center in North Carolina, US. She has a master's degree in Health Law from the Faculty of Law at the Université de Sherbrooke and a bachelor's degree in biochemistry. She is the author of more than 280 publications, book chapters, abstracts and articles. She is a full professor in the Medical Genetics Division in the Department of Pediatrics at the FMHS and a researcher at the Clinical Research Centre in Sherbrooke, and in the Mother-Child Axis. She is the Scientific Director of the Waters-CHUS Expertise Centre in Clinical Mass Spectrometry and director of the Waters Centre of Innovation. She is the principal investigator and co-investigator in numerous research grants. She has received awards for her involvement and expertise in preventive genetic medicine.

A Metabolomic Approach for Biomarker Discovery and Precision Medicine

Fabry Disease is a multisystemic, X-linked lysosomal storage disorder caused by a decreased alpha-galactosidase A enzyme activity which is involved in the recycling of cellular sphingolipids. Although it is an X-linked disease a large proportion of heterozygous females are also affected. Renal, cerebral, and cardiovascular manifestations often result in premature death of patients. There is a marked variability in the genotype (+1000 mutations reported) and phenotype in Fabry disease patients leading to major differences in biomarker profiles. In fact, patients with late-onset cardiac variant mutations often present normal values for biomarkers usually detected in classical Fabry disease patients, such as globotriaosylceramide (Gb_3). In order to find a solution to this major issue, and to better understand the biochemistry and pathophysiology of the disease in adults, as well as in the pediatric population, we have performed time of flight mass spectrometry metabolomic studies for biomarker discovery for Fabry disease patients. We detected novel analogues of a glycosphingolipid, globotriaosylsphingosine (lyso-Gb_3), which were found to be increased in Fabry patients presenting the cardiac variant mutation of the disease. Robust quantitative tandem mass spectrometry methods were devised and validated for both urine and plasma lyso-Gb_3 and related analogues. Normal reference values were established. Longitudinal biomarker studies on the effect of treatment were evaluated. In the era of translational research and precision medicine, it is strongly recommended to offer complete novel biomarker evaluation strategies for early detection, monitoring and follow up of patients having different genotypes.

Kristina Håkansson, University of Michigan

Kristina Håkansson received M.Sc. and Ph.D. degrees in Molecular Biotechnology from Uppsala University, Sweden. She was a Swedish Foundation for International Cooperation in Research and Higher Education (STINT) postdoctoral fellow with Professor Alan G. Marshall at the National High Magnetic Field Laboratory, Florida State University. In 2003, she joined the faculty in Chemistry at the University of Michigan. Dr. Håkansson received a Searle Scholar Award (2004), an American Society for Mass Spectrometry Research Award (2005), an Eli Lilly Analytical Chemistry Award (2005), and an NSF Career Award (2006). In 2009, she was promoted to Associate Professor, with tenure and, in 2013, to Professor. In 2016 she received the Biemann Medal from the American Society for Mass Spectrometry and, in 2018, an Agilent Thought Leader award. Her research interests are in Analytical Chemistry and Chemical Biology with a focus on biomolecular mass spectrometry, including carbohydrate structural determination and functional studies of natural product biosynthetic enzymes

Electron-based tandem mass spectrometry for biomolecular cations and anions

Tandem mass spectrometry (MS/MS) can provide structural information for a variety of biomolecules, including peptides, lipids, and carbohydrates. However, the conventional MS/MS activation method, collision induced dissociation (CID), is only suitable for certain applications due to gas-phase cleavage of only the most labile chemical bonds. We employ free electrons at a range of energies for both cation and anion activation. Such activation methods require a magnetic field for electron confinement. We mostly utilize FT-ICR MS for this purpose but are currently expanding to an ion mobility/time-of-flight set-up with an added, commercially available, electron capture dissociation (ECD) cell.

We pioneered multivalent metal ion assisted ECD and negative ion ECD (niECD). The former strategy allows the analytically valuable gas-phase radical ion chemistry of ECD (and the related technique electron transfer dissociation (ETD)) to be applied to analytes that do not typically yield multiply charged cations (required for ECD/ETD). The latter technique involves electron capture by low charge state anions, a novel physical phenomenon with analytical outcomes paralleling conventional ECD but with the advantage of superior performance for acidic species and analytes with low cation stability. We are also exploring negative ion free radical initiated peptide sequencing (nFRIPS) as an alternative to niECD.

Contributing Authors Isaac Agyekum, Nicholas B. Borotto, Eunju Jang, Hye Kyong Kweon, Nhat H. V. Le, Carson W. Szot, Kristina Håkansson, *Dept Chemistry, University of Michigan, Ann Arbor, MI, USA*

Sydney Wellman, York University

Born in a small hamlet in southern Ontario, Sydney moved to the “big city” of Peterborough to attend Trent University where she completed a joint major in Forensic Science and Chemistry. At the suggestion of one of her professors, she applied for graduate school in the “real big city” of Toronto and was accepted into the University of Toronto’s Chemistry graduate program in 2012. The focus of her PhD was primarily concerned with combining mass spectrometry with laser spectroscopy to study porphyrins and other small chromophores. However, through her participation in the Mass Spectrometry-Enabled Science and Engineering (MS-ESE) program, she also completed a one year internship with Waters and the Ontario Centre of Forensic Sciences (CFS). During her internship, she developed a quantitative QTOF method for novel psychoactive substances. Her graduating year of 2017 was one thing after another starting with an interview at CFS, acceptance of a position as a Forensic Toxicologist, the start of work in May, graduation in August, and a baby in November. In April 2019, she opted to transition to be a full-time stay-at-home mom and is now expecting a Christmas baby for 2019.

Getting Out and Getting In (To Toxicology)

It’s possible to be a great PhD student but not have any idea on how not be a PhD student. But part of doing a PhD is getting yourself to the finish line and starting a career on the other side. Surprisingly, finishing may be less about how many papers you’ve published and more about how good your communication and negotiation skills are. This talk will cover aspects of your PhD you may not be taking seriously yet such as developing your character, building relationships outside your institution, and getting your boss on board to help you (actually) finish your PhD. Finally, having worked for the Ontario government as a Forensic Toxicologist, some of the key differences between working for the private and public sector will be discussed, along with some specifics on what the process is like to get in to the Centre of Forensic Sciences.

Peter Liuni, York University

Peter Liuni graduated from York University with an Honors B.Sc in Chemistry specializing in Pharmaceutical and Biological Chemistry. He earned his Ph.D. in Chemistry at York University in 2015 under the supervision of Professor Derek Wilson where he developed time-resolved ESI methods to study the conformational dynamics of enzymes. In 2016 Peter moved on to a MITACS Post-Doctoral Fellowship at Sanofi Pasteur where he established HDX-MS methods for epitope mapping under the supervision of Andrew James, and later transitioned into a contract Scientist position developing lipid and oligonucleotide LC-MS quantitative methods for clinical vaccine projects. Peter entered his current role in 2017 as a Mass Spectrometry Specialist in the YSciCore – York University’s Core Analytical Research Facility, where he serves over 20 internal and external clients, driving multiple projects and developments in proteomics, peptide mapping, small molecule, and DNA characterization

The Nuts and Bolts of a Mass Spectrometry Core Facility

Core Facilities function at the intersection between academic science, small business, and education; offering an attractive path for Scientists at any stage in their career. At any given point, your role in the core can shift from developing methods as an application scientist, to marketing and sales to promote your services, to repairing and maintaining instruments as a service engineer; cores by their very nature are multidisciplinary and “small business-like”. Often times these aspects are not apparent to anyone outside the core, and in many cases core facilities operate like black boxes. The goal of this presentation will be to illuminate the black box, and provide a ground-level perspective on how to start-up, operate, and manage a mass spectrometry core facility. We will draw from real world examples spanning 2 years at York University’s Mass Spectrometry facility - the YSciCore, and provide strategies for handling the most commonly encountered problems.

Award Winners

CSMS Award Winner—Young Investigator Award

The Young Investigator award is a recent addition to the CSMS awards (1st YIA was given to Jeff Smith from Carleton U in 2018). This year the second award will be presented to **Dajana Vuckovic** (from Concordia University) for her work in clinical metabolomics. This award is meant to highlight the outstanding contribution of early-to-mid career researchers (15 years from PhD) to Canadian mass spectrometry research. Nominations for next year's YIA are due in May 2020.

Dajana Vuckovic, Concordia University

Dajana Vuckovic received Honours BSc in Chemistry from the University of Toronto in 2002 and PhD in 2010 at the University of Waterloo under the supervision of Janusz Pawliszyn. Following an NSERC PDF with Andrew Emili at the Donnelly Centre of the University of Toronto, Dajana joined the Department of Chemistry and Biochemistry at Concordia University in 2012, where she is currently Associate Professor and Concordia University Research Chair (New Scholar) in Clinical Metabolomics, Biomarkers and Preventative Health. Dajana is the recipient of the 2018 Dean's Excellence in Scholarship Award, 2016 FRQS Chercheur Boursier Junior 1 and 2014 Petro Canada Young Innovator Award. She has published 39 peer-reviewed papers, 11 book chapters and has delivered 30 invited talks to date.

The Vuckovic Group develops new analytical methods and devices to improve metabolite coverage and data quality in metabolomics. This includes improving the detection of low-abundance and unstable metabolites, with the over-reaching goal of discovering and validating biomarkers to facilitate personalized medicine. Current research interests include sequential-extraction methods, new extraction materials, *in vivo* solid-phase microextraction, lipidomics, hormone peptide assays, metabolite identification using high-resolution mass spectrometry, as well as the fundamentals of extraction selectivity and electrospray ionization.

Improving lipid coverage and data quality for untargeted lipidomics and oxylipin profiling

In this talk, I will briefly discuss our current strategies for in-depth untargeted lipidomics and targeted oxylipin profiling and describe the critical roles of chromatographic separation and the use of acetic acid as a mobile phase additive in negative electrospray mode to ensure sensitive, selective and accurate measurements of oxylipins and other lipids. I will then present the first *in vivo* solid-phase microextraction sampling of oxylipins from the brains of moving, awake rats. More than 50 oxylipins could be measured using 15-min *in vivo* sampling, thus confirming microextraction as an exciting new tool for *in situ* elucidation of lipid mediator activity with unprecedented temporal resolution and oxylipin coverage. Moreover, full untargeted lipidomic profile could also be obtained from the single sampling without requiring any tissue removal. Finally, the advantages and disadvantages of this new approach including the detailed comparison to *ex vivo* tissue extraction will be discussed.

Contributing Authors
Alexander Napylov, Ankita Gupta, Shama Naz, Cian Monnin, Nathaly Reyes Garces, German Gomez-Rios, Mariola Olkowicz, Sofia Lendor, Barbara Bojko, Clement Hamani, Janusz Pawliszyn, Dajana Vuckovic

Ken Standing Award

The Ken Standing Award is provided by the sponsors of this conference and the University of Manitoba.

Kenneth G. Standing, alumnus and professor from the department of Physics & Astronomy at the University of Manitoba, recently passed away on March 21, 2019 at the age of 93 following a world-wide recognized career. 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 from 1959 to 1974. Starting in the early 1980s he turned his attention to time-of-flight mass spectrometry and its applications to study 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 (Fred P. Lossing Award) 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 served as Member or Chair of various US National Institutes of Health Special Study Sections. Dr. Standing has been 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 reserach to the Manitoba-MDSSciex 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 provides an inspiration to new generations of scientists in Canada and elsewhere. For these reasons, the Sponsors of the ETP 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 each 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:

Emma Lundberg, Stanford University/KTH Royal Institute of Technology

Dr. Lundberg is Associate Professor in cell biology proteomics at KTH Royal Institute of Technology, Sweden, and Director of the Cell Atlas, part of the Human Protein Atlas program. For this work, she was recently top 10 under 40; future leaders in biopharma. Dr. Lundberg is currently spending two sabbatical years as visiting Associate Professor at Stanford School of Medicine and the Chan-Zuckerberg Biohub. In the interface between bioimaging, proteomics and artificial intelligence her research aims to define the spatiotemporal organization of the human proteome at a subcellular level, with the goal to understand how variations and deviations in protein expression patterns can contribute to cellular function and disease. Dr. Lundberg has a keen interest in citizen science, and has engaged over 300,000 gamers to help her research through the first ever MMO citizen science computer game.

Single cell proteome variability in human cells

Partitioning of biological reactions in time and space is an important mechanism to allow multiple cellular reactions to occur in parallel. Thus, resolving the spatiotemporal cellular distribution of the human proteome would greatly increase our understanding of human biology and disease.
Using high-content microscopy we have generated a high-resolution map of the subcellular distribution of the human proteome as part of the open access Human Protein Atlas database (www.proteinatlas.org). We have shown that as much as half of all proteins localize to multiple compartments. Such proteins may

have context specific functions and 'moonlight' in different parts of the cell, thus increasing the functionality of the proteome and the complexity of the cell from a systems perspective. Recently we turned to single cell analysis to identify proteins with temporal variability in expression. We identify 17% of the human proteome to display cell-to-cell variability, of which we could attribute 25% as correlated to cell cycle progression and present the first evidence of cell cycle association for 258 proteins. Single cell sequencing data further demonstrates that only 18 % of these proteins are temporally regulated at the transcript level, indicating that the majority of the novel cell cycle proteins are subjected to translational or post-translational temporal regulation.

All of this work is critically dependent on computational image classification, and I will discuss both citizen science and machine learning approaches for this. In summary, I will demonstrate the importance of integration of spatial proteomics data for improved single cell biology.

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 expertise in the physical chemistry of ion-molecule reactions. 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 on the first commercially available triple-quadrupole analyzer. Mobile versions mounted on a truck are still used 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 international 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. to continue to bring researchers together to develop new scientific advances and technologies. ETP is known as a key conference where international and Canadian researchers get together to develop ideas for novel technologies for life sciences research. Bill was an active participant in ETP up until his death in August 2009.

To honour this lifetime achievement, ETP and AB SCIEX have put together an award geared to helping graduate students travel to and participate at the biennial ETP Symposium. This year two awards are being presented by SCIEX to:

Timon Geib, UQAM

Irina Slobodchikova, Concordia University

Tech Talks

TBA, *PerkinElmer*

Dual Source LCMSMS-improved functionality

Gaëlle Bridon, Agilent Technologies

Agilent LC/Q-TOF MS, Accelerating Capabilities for High Resolution, Accurate Mass MS analyses

What about getting both high resolution and extended dynamic range in a single untargeted run? The 6546 LC/Q-TOF enables you to simultaneously obtain high speed, high resolution, and wide dynamic range without data quality sacrifices, unlike other HRMS technologies. Introduction of Data Independent Analysis Q-RAI (Quadrupole-Resolved All-Ions) and the improved performance and combination of recent enhancements to MassHunter software suite makes it a perfect tool for small molecules analysis. Expertly curated accurate mass databases and MS/MS libraries provide a shortcut to results as we offer Personal Compound Database and Libraries (PCDL) including metabolomics, pesticides, vet drugs, water contaminants, mycotoxins, and extractables and leachables. The 6546 LC/Q-TOF coupled with the 1290 Infinity II LC System is the centerpiece of multiple workflows, allowing uncompromised data quality in qualitative and quantitative analyses.

Oral Presentations

Anca Baesu, McGill University Department of Food Science and Agricultural Chemistry

Application of non-targeted analysis to study the fate of malachite and leucomalachite green in shrimp following thermal treatment

Veterinary drug use in food production has mostly been associated with traditional agricultural activities like poultry farming. Recently, an increase in drug use in seafood has been observed in response to an increased consumer demand. One veterinary drug that is frequently detected in seafood, despite its ban due to possible carcinogenicity, is malachite green. Risk assessments have not yet fully taken into consideration the risk associated with cooked malachite green contaminated seafood. Reduction in residues has been observed, but degradation products have not been characterized and must be further studied as they may show higher toxicity. In such cases, a non-targeted approach can be applied as it is based on identification of unknown compounds. In this study, the fate of malachite and leucomalachite green in shrimp muscle was studied. QuECHERS based non-targeted extraction was used and quantification was achieved using liquid chromatography coupled to high regulation mass spectrometry (LC-QTOF-MS). Non-targeted data treatment workflow for the identification of degradation products will be discussed.

Contributing Authors Anca, Baescu, Stéphane Bayen

JoAnn Chen, University of Toronto

Investigating Conformational Effects of DNA-drug Interactions by Gas-phase Förster Resonance Energy Transfer

Mass spectrometry is commonly employed for rapid characterisation of DNA-drug complexes. To understand how the conformation of DNA is affected by interactions with small drug molecules in the gas phase, we probe Förster Resonance Energy Transfer (FRET) of mass-selected gaseous DNA and DNA-drug complex ions. FRET, a widely-used spectroscopic technique in solution, serves as a “molecular ruler” through nonradiative energy transfer from an electronically excited donor chromophore to an acceptor chromophore via dipole-dipole interaction, where its efficiency is inversely proportional to donor-acceptor distance raised to the sixth power (shorter distances increase FRET efficiency, and vice versa). The donor (BODIPY FL) and acceptor (BODIPY 576) chromophores are covalently bonded to the 5’- ends of double-stranded (ds) DNA. We have shown that charge state and electrospray source conditions (“harsh” vs “gentle”) influence the FRET efficiency, indicating a conformational change in the gas phase. Anti-tumour drugs of various known binding modes are added to form dsDNA-drug complexes to understand the effects of drug binding on dsDNA conformation, and to provide a baseline for using mass spectrometry as a structural biology tool.

Contributing Authors JoAnn C Chen, Stephen V. Sciuto, Rebecca A. Jockusch

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Jessica Clothier, York University

New Mass Spectrometry Analysis for Perfluorinated Alkyl Substances (PFAS)

Perfluoroalkyl substances (PFAS) are widely used in many consumer and industrial products including stain repellents, non-stick food paper or pans, and fire fighting foams. Perfluoroalkyl carboxylic acids (PFCAs) are resistant to degradation and have adverse effects to the environment and human health. PFCAs’ long lifetimes in the environment is due to the chemical and thermal stability of the carbon-fluorine bond. Long chain PFCAs are known to be toxic in the environment, bioaccumulate, and magnify in the food chain. Short-chain PFCAs (scPFCAs) are PFCAs with four or fewer carbons and are less studied with their toxicity poorly known; they have been observed to accumulate in different plant tissues, including crops. While the toxicity of scPFCAs are not well known, their long lifetimes and persistence in the environment indicate a risk to long term exposure that would adversely affect environmental and

human health. PFCAs have been observed in the High Arctic indicating that they participate in long-range atmospheric transportation. An additional major source for PFCAs is from the Montreal Protocol-mandated chlorofluorocarbon (CFC) replacements that have known degradation pathways to scPFCAs. Atmospheric scPFCAs have not been widely measured, which is an area of research that is lacking with currently no real time or quasi-real time measurements of scPFCAs. Therefore, I am developing new analytical methods to measure real atmospheric PFAS using GCMS.

Contributing Authors Jessica Clouthier, Cora Young

Malick Diedhiou, University of Ottawa

Ion Dissociation Dynamics of 1,2,3,4-Tetrahydronaphthalene (Tetralin) As A Simple Test Case For Hydrogenated PAHs

The dissociation of the tetralin (1,2,3,4-tetrahydronaphthalene, THN) radical cation has been studied using two different methods: imaging photoelectron photoion coincidence spectrometry (iPEPCO) and atmospheric pressure chemical ionization−collision induced dissociation mass spectrometry (APCI-CID). The protonated parent (m/z 132), yielded fragment ions at m/z 104 (C2H4 loss), m/z 131 (H loss), m/z 117 (CH3 loss) and m/z 91 (C3H5 loss). Possible decomposition pathways are investigated and four reactions were investigated: (R1) $C10H12^{\bullet+} \rightarrow C10H11^{+} + H$ (m/z 131); (R2) $C10H12^{\bullet+} \rightarrow C9H9^{\bullet+} + CH3$ (m/z 117); (R3) $C10H12^{\bullet+} \rightarrow C8H8^{\bullet+} + C2H4$ (m/z 104); (R4) $C10H12^{\bullet+} \rightarrow C7H7^{\bullet+} + C3H5$ (m/z 91). iPEPICO was used to obtain ion dissociation data in threshold photoionization as a function of photon energy, which were modeled with RRKM theory to extract kinetic parameters for these reactions.

Contributing Authors Malick Diedhiou, Brandi J. West, Jordy Bouwman and Paul M Mayer

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Wondewossen Gebeyehu, Carleton University

Method development and implementation of rapid, on-site drug checking in a supervised injection site using portable mass spectrometry

A collaboration between University of Ottawa, Carleton University and Sandy Hill Community Health Center has led to the installation and operation of a linear ion-trap mass spectrometer with electrospray ionization in a supervised injection site in the SHCHC. Clients of consumption sites can administer drugs under the supervision of harm reduction staff. With the installation of a mass spectrometer, users now have the option of having their drugs tested for adulterants that may trigger overdose. Results on the detection of common illicit drugs are available within 3 minutes of sample acquisition. Further spectral interrogation may provide detailed information on the complete composition of the sample. Analysis results are delivered orally. User drug-checking results have been limited to qualitative information thus far. However, with the optimization of standard operating procedures and the use of an isotopically-labelled fentanyl internal standard, the method has produced a linear calibration curve for fentanyl ($R^2 = 0.9704$) confirming the fully-quantitative capabilities of this methodology. Current work by the Carleton Mass Spectrometry Centre is focused on systematic expansion of calibrated drug compounds and the implementation of quantification in drug-checking procedure.

Contributing Authors Wondewossen Gebeyehu, Karl V. Wasslen, Jeffrey C. Smith

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Timon Geib, UQAM

Reactive Metabolite formation and protein binding by LC-MS/MS

Drug-induced liver injury is often related to the metabolism of xenobiotics. Two examples include acetaminophen (APAP) and clozapine (CLZ), both of which are known to form reactive metabolites, and covalently bind to hepatic proteins. We have investigated these two compounds and their reactive metabolite binding to glutathione and specific proteins in vitro. Covalent binding of APAP and CLZ was

studied using liver microsomes or purified CYPs (Supersomes), as well as chemically using hypochlorite solutions. We have developed several LC-MS/MS strategies and compared them for identification of adducts. For protein binding, traditional shotgun proteomics (DDA) was employed on a QqTOF system with database searching. A targeted MRM method was also optimized for modified peptides on a QqLIT was to increase sensitivity of site identification with higher throughput of analysis.

Contributing Authors Timon Geib, Lekha Sleno

Liang Hu, York University

The Prospects of Mass Spectrometry in Direct Quantitative Analysis of Multiple microRNAs (DQAMmiR)

DQAMmiR is a hybridization-based approach aiming at direct, quantitative analysis of multiple microRNAs (miRNAs), which are proposed as potential biomarkers for disease diagnosis. In this approach, complementary hybridization probes, labeled for detection are taken in excess to miRNAs, bind miRNAs sequence-specifically. The probe-miRNA hybrids are separated from each other and from the unreacted probes simultaneously by capillary electrophoresis (CE) with strong electroosmotic flow (EOF). The EOF propels the probes and the hybrids toward the detector at the end of the capillary; quantitative detection of the probes and the hybrids facilitates absolute quantitation of the miRNA targets without calibration curves. The feasibility of this approach has been proved in multiple studies with commercial available CE setup with laser-induced fluorescence (LIF) detector by solving the challenging problems in electrophoretic separations, resulting in two different versions of DQAMmiR with different strategies. One of major remaining problems is that the limit of detection (LOD) of the detectors in the commercially available setup is not low enough for detecting all miRNAs in clinical samples. Mass spectrometry (MS) is one of the most sensitive detectors used to detect and quantitate molecules, and it has been coupled with varies of separation patterns as sensitive on-line detectors. Here, we will discuss the prospective strategies of using MS in DQAMmiR.

Contributing Authors Liang Hu, Sergey N. Krylov

Banafsheh Mehrazma, York University

The unfolding of lipocalin 2 in the presence of siderophores

Lipocalin 2 (Lcn2) or siderocalin protein levels are increased in chronic inflammatory conditions such as obesity and diabetes pathogenesis. Lcn2 acts as an antibacterial agent in immune system and it plays a crucial role in isolating bacterial siderophores chelated to iron ions. Bacterial siderophores such as enterobactin and 2,3-DHBA are engaged in cellular iron transport, and hence are essential in bacterial survival. To study the mechanism of this process, we have explored the binding features of Lcn2 to enterobactin and 2,3-DHBA by using isothermal titration calorimetry (ITC) and hydrogen-deuterium exchange (HDX), in the presence and the absence of iron. To further investigate the observed changes in dynamics of this protein, molecular dynamics (MD) simulations on Lcn2 with and without enterobactin-iron complex are carried out.

Contributing Authors Xiaojing Huang, Sladjana Slavkovic, Banafsheh Mehrazma, Cristina Lento, Philip Johnson, Gary Sweeney, Derek J. Wilson

Marie-Pier Ouellet, University of Montreal

Applying ab experimental design to improve the use of insoluble crosslinked proteolytic enzymes for better sample preparation in microscale proteomics

Enzymatic digestion for bottom-up proteomics can be achieved with insoluble proteolytic enzymes, which offer many advantages and use in a microfluidic format. Our group is using trypsin and chymotrypsin,

crosslinked with glutaraldehyde (GA), to make immobilized enzymes without the need for a solid support like silica particles [1]. Previous studies showed that GA-chymotrypsin could only be used for two consecutive digestions [2]. An apparent physical or chemical degradation of the enzyme resulting in loss of activity was suspected to be caused by the multiple washing steps. Design of Experiments (DOE) was used to optimize the number of washes of enzyme needed to achieve high activity, as evaluated by CE-based peptide mapping of lysozyme digests with migration time correction [3]. LC-MS was used to determine the trace amounts of enzyme in the wash solutions and identify where trypsin losses might be occurring. The goal is to improve robustness of GA-enzymes in batch and microreactor formats.

Contributing Authors Marie-Pier Ouellet, Guillaume Lasnier, Coralie L. Dubois, Karen Waldron

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Ons Ousji, UQAM

Studying the metabolism of BHT by LC-MS/MS

Environmental contamination by xenobiotics is a worldwide phenomenon as a result of human activities such as the industrial production of plastics, additives in food, the pharmaceutical industry, etc. The exposure to these environmental xenobiotics can induce adverse effects on human health. BHT is one of the most widely used preservatives in food, cosmetics and pharmaceutical industry to inhibit lipid autoxidation.

The metabolism of BHT, and several of its analogs, has been studied in vitro to investigate the formation of stable and reactive metabolites. Metabolites were formed using in vitro incubations with microsomes and S9 fractions,and profiled using a newly developed LC-HRMS/MS method. Positive and negative mode data were processed to probe the formation of oxidative metabolites, as well as glutathione, glucuronide and sulfate conjugates. High resolution MS/MS data was employed to elucidate fragmentation pathways and for the structural elucidation of metabolites.

Contributing Authors Ons Ousji and Lekha Sleno

Sean Overton, University of Ottawa

Unimolecular reactions of NPAHs

Carbon-based macromolecules are an important constituent of interstellar material, comprising 10-20% of the carbon in the galaxy. They play an important role in the physical and chemical evolution of the photodissociation regions of the galaxy due to ultraviolet emissions from stars. These macromolecules can take the form of polyaromatic hydrocarbons (PAHs) or other, aromatic-based molecular systems but their complete characterization remains elusive. Over the past 25 years of the so-called PAH hypothesis, these molecules have been argued to be involved in shielding organic reactions in the interstellar medium (ISM) and playing an active role catalyzing these reactions, especially H2 formation. We have been exploring the dissociation dynamics of ionized PAHs by a combination of tandem mass spectrometry (collision induced dissociation) and imaging photoelectron photoion coincidence spectroscopy. This presentation will examine the unimolecular reactions of so-called NPAHs, PAH ions containing nitrogen in a ring, as studied by CID. The goal is to establish trends in the reactivity as a function of structure and size, with the aim of predicting the reactions that would occur in interstellar environments.

Contributing Authors Sean Overton, Paul M. Mayer

Consuelo Perez, York University

Internal Standard Application Strategies in Mass Spectrometry Imaging by DESI

Ambient mass spectrometry imaging has become an important analytical tool to investigate intact molecules from biological tissues in their native environments with minimal sample preparation. Desorption electrospray ionization mass spectrometry imaging (DESI-MSI) is a qualitative surface

analysis technique that can elucidate the spatial distribution of molecules directly from surfaces and biological tissue sections and visualized as 2D and 3D images. Currently, research in quantitative MSI has been for the most part underexplored and there is a significant need to develop quantitative imaging approaches to simultaneously characterize and map the spatial distribution of chemical compounds with their respective concentrations from biological tissues. An important aspect in quantitation is the incorporation of internal standard to report accurate concentrations from complex biological matrices. In this study, we investigated quantitatively and qualitatively three methods to incorporate the internal standard into the DESI-MSI analysis. We developed an invertebrate tissue model with the invasive freshwater golden apple snail (pomacea diffusa) to quantify the psycholeptic drug, phenobarbital using the isotopically labelled internal standard, phenobarbital-d5. DESI-MSI selected ion monitoring monitored phenobarbital (m/z 231) and phenobarbital-d5 (m/z 236) directly from snail head tissues.

Contributing Authors Consuelo Perez and Demian R. Ifa

Guy Poirier, *Université Laval*

Proteomics analysis the role of PARP in DNA homologous recombination

We will describe the characteristics of covalent versus non-covalent modification interaction with nuclear protein especially the ones involved in DNA repair. Various approaches will be used to identify the amino acids involved in covalent modifications. Finally, the role of PARP inhibitors in homologous recombination proficient and deficient cells will be discussed.

Maxime Sansoucy, UQAM

Post-translational modifications in biological materials with unique properties

Some biological materials with very interesting properties have unique protein modifications associated to these traits. Mussel byssus is a secreted fibre having very high strength and flexibility to secure the animals to solid surfaces in turbulent waters. Crosslinking in proteins, via tyrosine hydroxylation to dopa, is very important for underwater adhesion and fiber sclerotization (or tanning). We have used LC-MS/MS to probe which crosslinks are prevalent in these samples. Another example is velvet worm slime used for hunting purposes, having characteristic properties argued to be a result of rare PTMs. This slime turns solid when a minimal mechanical shock is applied. This very unique material has also been studied by LC-MS/MS, with emphasis on looking for PTMs and crosslinks.

Contributing Authors Maxime Sansoucy, Tobias Primel, Alexander Baer, Matt Harrington, Lekha Sleno

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Irina Slobodchikova, Concordia University

Characterization of phase I/II metabolites of 17 mycotoxins using LC-HRMS

Filamentous fungi produce variety of toxic secondary metabolites, called mycotoxins. Human exposure to mycotoxins can occur via the intake of contaminated food, dermal contact or inhalation. The majority of methods for biomonitoring of mycotoxin exposure do not include their metabolites, thus leading to underestimation of their contamination. Metabolism studies of 17 mycotoxins: aflatoxins B1, B2, G1 and G2, zearalenone, 7-α-hydroxy-zearalenol, 7-β-hydroxy-zearalenol, zearalanone, 7-α-hydroxy-zearalanol, 7-β-hydroxy-zearalanol, T-2 toxin, HT-2 toxin, deoxynivalenol, nivalenol, 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol and fusarenon X were performed using human liver microsomes to generate phase metabolites. The analysis of microsomal incubation samples was performed with liquid chromatography-high resolution mass spectrometry instrumentation (LTQ Orbitrap Velos). The resolution at 60000 and 2 ppm accuracy reduced the determination the number of possible elemental formulas for a measured m/z value. Data-dependent acquisition in combination with collision-induced dissociation or higher energy collisional dissociation was used to ensure adequate fragmentation and study the structure of the

mycotoxin metabolites. In total, 47 metabolites were characterized and used to build an extensive LC-MS library. This library will be used during exposure studies to prioritize which metabolites should be used routinely during human biomonitoring of urine and blood specimens.

Contributing Authors I Slobodchikova, S Rahman, R Sivakumar, C Mihai, D Vuckovic

Lisa Szymkowicz, York University

Development of a targeted nanoLC-MS/MS method for quantitation of residual toxins from Bordetella pertussis

Pertussis (i.e. whooping cough) is a highly contagious respiratory disease caused by infection by Bordetella pertussis (B. pertussis). B. pertussis pathogenesis is driven by cell-surface adhesion proteins and secreted toxins; some of which have been harnessed as purified components in acellular vaccines. Three of these virulence factors, tracheal cytotoxin (TCT), adenylate cyclase toxin (ACT) and dermoneucrotic toxin (DNT), are toxins with potential for co-purification and thus must be monitored as process-related impurities. This work describes the development of a targeted parallel reaction monitoring (PRM) method for sensitive and selective detection the three residual toxins in a single nanoLC-MS analysis. The outlined standard-driven approach to surrogate peptide selection can be broadly applied to accelerate quantitative LC-MS method development across multiple sample matrices.

Contributing Authors Lisa Szymkowicz, Derek Wilson, Andrew James

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Thanh Ngan Thang, Université de Montréal

Applying DofE to the optimization of elution gradients in LC-MS

The CO2 produced by combustion of fossil fuels can be captured using regenerable aqueous amine solvents. However, these amines are subject to degradation which can not only reduce the efficiency of CO2 capture but also form potentially toxic products. To determine these compounds, our group is using hydrophilic interaction liquid chromatography coupled to mass spectrometry (HILIC-MS) since these species are very polar and lack chromophores. Previous studies using two stationary phases (BEH Amide and Luna CN), a mobile phase composed of 10 mM ammonium formate/0.05% formic acid/5% EtOH (eluent A) and acetonitrile/5% EtOH (eluent B), and optimized MS parameters still resulted in poor resolution (R) of some of the degradation products despite several gradients tested. Our goal was to achieve R > 1.5 for 4 pairs of peaks that were the most difficult to separate. Therefore, a more systematic approach was applied using Design of Experiments (DoE) in which fewer experiments can be used to predict the optimal HILIC parameters that give the best response. DoE experiments for each HILIC column involved changing parameters like the percentage of eluent A at a given time or the segment duration in the non-linear gradient in order to improve R—our response for the DoE method—of the 4 peak pairs in a single run. This presentation will show the DoE principles, our choice of parameters, and the peak resolutions obtained.

Contributing Authors Thanh Ngan Thang, Stéphanie Gallant, Marie-Pier Ouellet, Alexandra Furtos, Karen C. Waldron

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Christopher Thibodeaux, McGill University

Advancing Biosynthetic Enzymology with Structural Mass Spectrometry

Natural products have proven to be a reliable source of biomedically relevant compounds. Understanding the enzymatic mechanisms of natural product biosynthesis is critical for studies aimed at engineering or tailoring the structures of these useful molecules, which often remain refractory to total chemical synthesis. My lab focuses on elucidating the biosynthetic mechanisms of an interesting class of genetically encoded peptide natural products that are modified in multistep enzymatic reactions. Relying heavily on a suite of emerging biomolecular mass spectrometry-based techniques, such as hydrogen-deuterium exchange and native mass spectrometry coupled to ion mobility, our lab is investigating the

chemical and kinetic mechanisms of these peptide biosynthetic enzymes, and we are defining the under appreciated role of conformational changes and biophysical interactions in guiding the multistep peptide maturation process. This information will guide the rational engineering of these biomedically important enzyme systems.

Contributing Authors Christopher J. Thibodeaux, Yeganeh Habibi, Kevin Uggowitzer, Hassan Issak

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Ben Warnes, Carleton Mass Spectrometry Centre (CMSC)

Diazomethylation Enhancement of Phosphatidic Acid for Application in Mass Spectrometry

Phosphatidic acid (PA), a cellular membrane phospholipid consisting of a phosphate head group with two fatty acyl chains linked to a glycerol unit. PA serves specific roles in glycerolipid metabolism, membrane biogenesis, and regulation of cellular processes.In recent development, it has been shown that PA regulates numerous processes pertinent to cellular function by acting as a signalling lipid, which regulates a protein in a corresponding cellular process which is dependent on the concentration of the lipid in the membrane bilayer. As a result of the dependence of the effectiveness of cellular regulation and concentration of PA, quantification of the concentration of this phospholipid is crucial. Previously, our group has shown increased sensitivity of ESI-MS by chemical derivatization in-situ by diazomethylation (iTrEnDi) of phospholipid subclasses PE, PS, and PC, respectively. With continued derivatization using iTrEnDi, the sensitivity difference of ESI-MS analysis of derivatized and non-derivatized PA will be evaluated.

Contributing Authors Ben Warnes, Karl V. Wasslen, Samuel W.J. Shields, Christian Rosales, Jeffrey C. Smith, Jeffrey M. Manthorpe

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Ethan Yang, University of Montreal

Silver spray deposition for AgLDI imaging MS of cholesterol, a fast alternative to sputter deposition

Silver-assisted laser desorption ionization (AgLDI) imaging mass spectrometry (IMS) is a powerful technique selective for olefin species specifically cholesterol. To date, this approach has been demonstrated to be useful for analysis of cholesterols and fatty acids thin tissue sections, such as the brain, as well as endogenous and exogenous compounds in fingermarks. Because no solvent is introduced during the sample preparation step, the original approach of silver deposition by sputtering allows for an extremely high spatial resolution down to 5 µm. However, despite its high performance, this method has yet to be widely adopted by the IMS community, possibly due to the lack of easy access to such a device. To increase the appeal and accessibility of AgLDI IMS, we have developed a fast and reliable method that employs an automatic spray system typically used for matrix deposition. Specifically, for cholesterol, we have optimized a silver nitrate spray deposition approach onto thin tissue sections that yielded comparable results to that of sputtered silver.

Contributing Authors Ethan Yang, Frédéric Fournelle and Pierre Chaurand

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Maryam Yousefi-Taemeh, York University

Direct analysis of Cannabis derivatives from Cannabis-infused edible products by DESI-MS

Recently in Canada and parts of the US, Marijuana has become fully legalized and regulated, for both medical and recreation cannabis products such as edibles even more popular al purposes. This fact is going to make Can than ever before. Therefore, it is assumed that there will be a high demand for analytical methods accurate and sensitive enough to be used in different forensic and pharmaceutical Cannabis related applications. Here, we demonstrate two different brands of cannabis infused chocolates

analysis to confirm the ability of Desorption Electrospray Ionization Mass Spectrometry to detect cannabinoids in complex matrices in a short time with no sample preparation requirements. DESI involves the surface to be sprayed with ESI spray and extracted ions go into the mass spectrometer. In this ambient technique, the sample can be analyzed directly, under open-air in its native state with little or no pre-treatments. The analyst is able to perform a direct, rapid, real-time, and high-throughput analysis for a broad range of materials. The performed experiments confirm the clear presence of the cannabinoid peaks in the spectra. Cannabis and cannabinoid detection is an important area of research nowadays. Thus, here we demonstrate the use of DESI-MS towards rapid detection of these compounds.

Contributing Authors Maryam Yousefi-Taemeh, Demian R Ifa

Shaolong Zhu, York University - Sanofi

Epitope Mapping of Diphtheria Toxin by Hydrogen Deuterium Exchange Mass Spectrometry (HDXMS)

Hydrogen Deuterium Exchange Mass Spectrometry (HDXMS) has emerged as a critical tool for characterizing protein conformation, protein-protein and protein-ligand interactions. This technology has been adopted in biopharmaceutical settings for evaluating protein stability, structure, and for analysis of antigen/antibody epitopes. In this study, HDXMS was implemented to conduct epitope mapping of diphtheria toxin (DTx) against two monoclonal antibodies (mAbs) in an effort to better understand mAb/antigen interactions during development of next-generation antigenicity/potency assays. DTx is composed of three domains: 1) Receptor (R) domain for entry into the cell through receptor-mediated endocytosis. 2) catalytic (C) domain for inhibiting protein synthesis through ADP ribosylation of elongation factor 2. 3) translocation (T) domain for insertion and creation of pore into the endosome facilitating the transfer of C domain into the cytosol Waters Synapt G2-S coupled to a nanoAcquity UPLC system with HDX module was used to perform all the HDX time course experiments. 10μM of free DTx and 10μM:20μM (DTx:mAb) of the complex was allowed to mix with deuterated buffers (90%) at pH/pD 7.5 for times ranging from 2 min to an hour. The reaction was quenched at pH 2.5 with subsequent digestion of deuterated protein in pepsin/protease XIII (1:1) column. HDXMS profiles comparing free DTx, and two DTx-mAbs complexes, yielded approximately 90% sequence coverage.

Contributing Authors Shaolong Zhu, Peter Liuni, Derek Wilson, Andrew James

Tutorials

Paul Mayer, University of Ottawa

Paul has spent his career in mass spectrometry probing a multitude of ion chemistry questions, ranging from questions of ion activation to the chemistry of interstellar molecules. After his PhD in Gas Phase Ion Chemistry from the University of Ottawa, he expanded his experience with postdocs in kinetics (UNC Chapel Hill) and computational chemistry (ANU, Canberra, Australia). Along the way he has dabbled in several analytical mass spectrometry pursuits, proving that he truly belongs only in Physical Chemistry.

Gas phase ion chemistry

Central to mass spectrometry is the chemistry of ions in the gas phase. A mass spectrum is simply the result of competing unimolecular reactions of activated ions. The observations we make with mass spectrometry are influenced by this chemistry, the various rate constants, the ion internal energy distribution and instrumental timescales. I will give a brief overview of the common mass spectrometry-based experiments used to probe ion chemistry, notably ion structure and reaction mechanism elucidation.

Pierre Chaurand, Université de Montréal

Pierre Chaurand received his Ph.D. in physical chemistry from the Université Paris Sud, (Orsay, France) in 1994. After a postdoctoral period at the Heinrich-Heine Universität, (Düsseldorf, Germany), he integrated in 1998 the laboratory of Prof. Richard Caprioli as Research Faculty at Vanderbilt University School of Medicine (Nashville TN, USA). Since 2009, he is Professor in the Department of Chemistry at the Université de Montréal (Montreal, Canada). His primary background is in biochemistry with significant analytical and organic chemistry components. His current expertise's are in fundamental and analytical mass spectrometry. With over 20 years of experience in the field, he is one of the pioneers of the imaging mass spectrometry (IMS) technology. His current research interests are focused on the development of new strategies and methods to improve the specificity and sensitivity of tissue based IMS with applications in fundamental and clinical biology.

Imaging mass spectrometry, everything you need to know to get started

Twenty years ago, Richard Caprioli and colleagues demonstrated that using MALDI MS, it was possible to obtain spatially resolved molecular information from thinly cut tissue sections in perfect correlation with the underlying histology. It took a few years for imaging MS to migrate from a mass spectrometry curiosity to an accepted technology offering detailed insights into local molecular processes for a wide range of applications. Today, imaging MS is performed at cellular scale using state-of-the-art MS instrumentation at very high speed. I will present here the necessary instrumentation and protocols to successfully acquire and mine imaging MS data and highlight some of the mainstream applications of the technology.

Jeff Smith, Carleton University

Dr. Jeff Smith is a Professor in the Department of Chemistry and the Institute of Biochemistry at Carleton University. He received his BSc from Trent University in 2000 and earned his PhD at York University in 2005. After three years at the Ottawa Institute of Systems Biology at the University of Ottawa, he joined Carleton in 2008, and is currently the Director of the Carleton Mass Spectrometry Centre. His research focusses on the use of mass spectrometry to investigate the biomolecular mechanisms of cellular life.

Mass spectrometry-based lipidomics: fundamentals, opportunities and perspectives

As more information is collected on the natures of different types of cellular lipids, it has become increasingly apparent that they are not merely structural or energy storage molecules but play intrinsic roles in cellular signalling and homeostasis. As such, it is imperative that the contributions of lipids be considered when studying biology as a system, placing an increasing demand for more accurate and insightful tools with which to study them. Over the past decade, mass spectrometry has become increasingly useful for to advance the field of lipidomics. This tutorial will highlight the mechanisms by which mass spectrometry may be employed to study lipids including different ionization modes and instrumental parameters that are useful in the field. Separation schemes will be discussed including analyses on the relative strengths and weaknesses of chromatography versus direct infusion. Different perspectives on significant advancements in the field will be described as well as areas that require further attention and development. The tutorial will be offered in an informal manner with an open invitation to questions and discussion throughout.

Karen Waldron, Université de Montréal

Karen C. Waldron completed her B.Sc. at Queen's U. in 1985 followed by a 4-month internship at the Nuclear Research Center (KFA) in Jülich, Germany. She obtained her M.Sc. from Queen's in physical chemistry in 1987 then worked as a lecturer at UNB-Saint John during 1987-88. She then went to U. of Alberta for her doctorate in analytical chemistry under the supervision of Norman J. Dovichi, working on new detection and analysis methods coupled to capillary electrophoresis for protein sequencing applications. After obtaining her Ph.D. 1993, Dr. Waldron completed an 11-month NSERC Industrial Postdoctoral fellowship with SCIEX. She joined the Department of Chemistry at U. de Montréal in November 1994 where her research since has focused on the development of microscale analytical instrumentation and methods for biomolecule characterization and peptide mapping. She was a visiting professor at the ESPCI (Paris, France) in 2002-03, studying biological MS with the group of Jean Rossier. Dr. Waldron is currently a full Professor of Chemistry at U. de Montréal where she has mentored approximately 90 students and postdocs in analytical chemistry, and has taught 14 different courses within the Faculties of Arts & Sciences, Medicine, and Pharmacy. Outside the university Dr. Waldron has been involved in several chemical societies, notably as vice president, then president, of the Division of Analytical Chemistry of the Canadian Institute for Chemistry from 2001 to 2007. She is currently on the Board of Directors of the Society for Microscale Separations and Bioanalysis.

Capillary Electrophoresis - Mass Spectrometry: Recent Developments and Applications

The coupling of MS detection to capillary electrophoresis (CE) was first reported in 1987 and in the twenty years that followed several CE-MS interface designs were reported in the literature. Sensitivity and signal stability were less than ideal for a long time but in the last decade CE-MS has seen resurgence with new commercialized interfaces available and analysis problems unsolved by LC-MS. The basic principles of CE-MS will be described in this tutorial, including interfaces that can be "lab-built" and those available on commercial CE instruments. Applications of CE-MS to bioanalysis, biopharmaceuticals, proteomics, glycomics and metabolomics will be discussed.

Derek Wilson, York University

Derek Wilson was born on a cool, stormy night in Richmond Hill about 41 years ago using paddles. Since then he has shown moderate competence in most things with the exception of the violin at which he was exceptionally good, but not terribly interested. He completed his undergraduate degree at Trent university where he did his first research protein folding project under the gentle tutelage of Steven P. Rafferty. He then went to do his PhD at Western with Lars Konermann, who is very tall and also very German. From there he went to storied Cambridge university for a post-doc where he spent most of his post-doc in the

basement trying to figure out how the hell NMR works, with little success. Then he took up a professorship at York university, where he has got some grants and done some stuff, most of it vaguely related to Hydrogen Deuterium Exchange mass spectrometry.

Hydrogen Deuterium Exchange Mass Spectrometry: Up Close and Personal

The tools of structural biology, X-ray Crystallography and NMR, provide exquisitely detailed snapshots of protein structure. But 'structure' is only half the picture. In order to achieve biological function, proteins must also undergo transient, thermally driven excursions to higher energy structures, known as 'conformational dynamics'. Hydrogen Deuterium Exchange (HDX) is a technique that allows qualitative and semi-quantitative measurements of protein conformational dynamics, based on the rate at which amide hydrogens from the peptide backbone exchange with solvent deuterium. In this tutorial, we'll look at HDX from the ground up - how we make the measurements, how we interpret them to give us a picture of protein dynamics, and how this can be useful in the context of understanding protein function, from understanding the machinations of giant 'protein machines' to drug design in cancer and degenerative disease.

Poster Presentations

David Barnett, Atlantic Cancer Research Institute

Mass spectrometric proteomic analysis of plasma-derived extracellular vesicles from pancreatic cancer patients and healthy controls.

The successful identification of protein biomarkers in plasma is severely hampered by the presence of a few background proteins of high abundance, most notably human serum albumin (HSA). While blood has an advantage of providing a comprehensive sample, the dilution of biomarkers originating from a distal tumour presents an analytical challenge. Extracellular vesicles (EVs) are known to contain DNA, RNA and protein that could be useful markers of a pathology. It has also been confirmed that EVs are released at elevated levels from disease or tumour sites. At the Atlantic Cancer Research Institute, a novel affinity based peptide reagent for EV isolation called Vn96 has been developed. This peptide binds with heat shock proteins (HSPs) expressed on the surface of these vesicles and causes them to precipitate from biological fluids including plasma, urine and saliva. These precipitated vesicles can be thoroughly washed to remove any proteins that exhibit non-covalent association with the vesicle surface.

Contributing Authors DA Barnett, N-N Mai-Thi, JW Roy, RJ Ouellette, AP Joy

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Frédéric Fournelle, Université de Montréal

Understanding visceral fat delocalisation in imaging mass spectrometry

Lipid delocalization in tissue sections is a major problem in imaging mass spectrometry (IMS). It can also lead to significant ion suppression resulting in gross misinterpretation of lipid distributions. To understand and minimize lipid delocalization on- and off-tissue, we analyzed fatty mouse kidney tissue sections mounted on ITO-coated glass slides by IMS using different sample preparations approaches. We employed CBS-AuLDI IMS to characterize the delocalisation of triacylglycerols (TAGs) present in the visceral fat of the kidney and classic 1,5-DAN and 2,5-DHB matrix application by sublimation to characterize phospholipid distributions. Our results show significant delocalization of various TAGs as well as various ion suppression patterns for phospholipids induced by TAG delocalization. To date, aluminium oxide slides have shown the greatest potential over ITO slides to minimize analyte migration reducing by 18-fold TAG delocalization. Work is ongoing to examine new surfaces to minimize migration of TAGs.

Contributing Authors Frédéric Fournelle, Ethan Yang, Martin Dufresnes, Pierre Chaurand

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Amal Guesmi, UQÀM

Triclosan metabolism studied by LC-HRMS/MS

Triclosan is an antibacterial and antifungal agent found in many hygiene products. However, this molecule can induce harmful effects on human health and the environment. We have studied the metabolism of triclosan using liver fractions, to evaluate the formation of oxidative metabolites as well as glutathione, glucuronide and sulfate conjugates using LC-HRMS/MS. It was found through this work that cleavage of triclosan is implicated in the formation of a reactive metabolite. We also detected several glucuronide and sulfated conjugates with triclosan and its cleaved product. This project is aimed at understanding the routes of detoxification of this xenobiotic and could be used to better assess human and environmental exposures of triclosan.

Contributing Authors Amal Guesmi, Lekha Sleno

Yeganeh Habibi, McGill University

Characterizing Conformational Changes in the Haloduracin Lanthipeptide Synthase (HalM2) by HDX-MS

Lanthipeptides belong to the family of ribosomally synthesized and post translationally modified peptide (RiPP) natural products. Lanthipeptides are genetically encoded precursor peptides that undergo multistep modification catalyzed by lanthipeptide synthetases. These enzymes function iteratively during the stepwise modification of the precursor peptide into a structurally complex peptide macrocycle, which often exhibits antibiotic activity. The relaxed substrate specificity of lanthipeptide (and other RiPP) synthetases likely results, in part, from conformational changes of lanthipeptide synthases in presence of their substrate(s). In this study, we have utilized hydrogen-deuterium exchange mass spectrometry (HDX-MS) to investigate the involvement of conformational changes in the model class II lanthipeptide synthetase, HalM2. Consistent with our hypothesis, we find HalM2 to be a highly dynamic enzyme, and we have uncovered changes in the dynamic properties of the enzyme that have enabled us to locate structural elements involved in precursor peptide binding as well as allosteric communication between the two active sites of the enzyme. The functional assignments for these structural elements is supported by biochemical and kinetic studies of a panel of mutant enzymes. This work has revealed mechanistic paradigms that will likely be shared with other RiPP biosynthetic enzymes.

Contributing Authors Yeganeh Habibi, Christopher J. Thibodeaux

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Alaa Huwaidi, Université de Sherbrooke

Gold nanoparticles enhance X-ray-induced DNA damage from emission of low energy electrons from gold surface

Most low energy electrons (LEEs) produced by high-energy ionizing radiation have energies less than ~30 eV, with most probable energy of ~10 eV. LEEs induce DNA damage including base and sugar modifications as well as single and double-strand breaks. The presence of gold nanoparticles (AuNPs) during irradiation enhances DNA damage by increasing the local photon absorption. Here, we show that AuNPs enhance LEE-induced radiation damage to DNA. DNA-AuNPs complexes were prepared by combining aqueous solutions of DNA and AuNPs. The samples were irradiated in the solid phase with x-

rays that favor the absorption of ionizing radiation by AuNPs. Irradiated samples were enzymatically digested to nucleosides and subjected to LC-MS/MS. Previously, we reported that the reaction of LEEs with nucleic acids leads to a specific reaction involving cleavage of the C3'-O and C5'-O bonds and formation of stable 2',3' and 2',5'-dideoxyribonucleosides, respectively. Our results show that the irradiation of DNA when attached to AuNPs considerably increases the formation of dideoxy-ribonucleosides in DNA, a signature of LEE-induced reactions. In contrast, no such damage was observed in the absence of AuNPs. The above studies support the proposed mechanism of radiosensitization by AuNPs involving LEEs and permits further studies to probe the potential application of AuNPs as a radiotherapeutic agent.

Contributing Authors Alaa Huwaidi, Gabriel Robert, Pierre Cloutier, Brigitte Guérin, Léon Sanche and J. Richard Wagner

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Andrew Joy, Atlantic Cancer Research Institute

A multiparametric extraction method for the molecular characterization of Vn96-captured plasma extracellular vesicles

Extracellular vesicles (EVs) have been shown as a source of diagnostic markers of disease, however isolation and subsequent characterization of EVs has been challenging. The development of a solution for this technical challenge has been paramount in the future development of a clinically amenable assay for liquid biopsy. We have designed a synthetic peptide, known as Vn96 (ME™ kit), which efficiently captures EVs from multiple biofluids without the use of specialized lab equipment and in a short timeframe. In the current study we utilize Vn96 with a limited plasma sample to collect EVs following which we have developed a multiparametric extraction protocol for the sequential isolation of DNA, RNA, and protein. From the isolation and multiparametric protocol, we are able to obtain sufficient amounts of all biologically relevant materials for analysis. In particular, the protein isolation provided strong evidence of EV markers and lists of unique proteins isolated from biological samples demonstrating the potential biological variability in plasma EV analysis. Together, our analyses of DNA, RNA, and protein isolated from Vn96-captured EVs using our multiparametric extraction method demonstrate that this material is amenable to downstream biomarker analysis using methods such as ddPCR, small RNA sequencing, and MS.

Contributing Authors Roy JW, Joy AP,Taylor CA, Beauregard AP, Ayre DC, Fry S, Chacko S, Wajnberg G, Mai-Thi NN, Crapoulet N, Barnett DA, Ghosh A, Lewis SM, and Ouellette RJ.

Maggy Lépine, UQAM

Determining isocyanate exposure in human urine by LC-MRM

4,4'-methylene diphenyl diisocyanate (MDI), toluene diisocyanate (2,4-TDI and 2,6-TDI), and 1,6'-hexametylene diisocyanate (HDI) are isocyanates used in polyurethane production. Workers exposed to these types of products may develop acute and chronic effects, with the primary manifestation being occupational asthma. After absorption in the body, these molecules get metabolized through acetylation or macromolecule conjugation reactions before their elimination into urine. The hydrolysis of urine samples releases free amine compounds as biomarkers of exposure: 4,4'-methylenedianiline (MDA), toluenediamine (2,4-TDA and 2,6-TDA) and hexamethylenediamine (HDA). Modern analytical methods don't allow simultaneous determination of these four isocyanate biomarkers to monitor occupational exposure based upon recommended threshold values. A new quantitative assay has been developed in human urine using a simple solid phase extraction (SPE) followed by LC-MRM analysis.

Contributing Authors Maggy Lépine , Sébastien Gagné, Jacques Lesage , Lekha Sleno

Nour Mashmoushi, University of Waterloo

Employing Differential Mobility Spectrometry to Characterize Cannabinoid Derivatives

Cannabinoid derivatives exhibit unique psychoactive and pharmaceutical potencies, dependent on their interactions with human receptors. Thus, new cannabinoids are synthesized at a greater rate every year. Our research employs differential mobility spectrometry to investigate the physicochemical properties and binding effects of cannabinoids. Density functional theory computations and molecular modelling are employed to estimate the analytes' interaction potentials and clustering behaviour. The correlation between our experimental/calculated data and the physicochemical/biological properties of cannabinoids will be used to train machine learning algorithms to predict the properties of newly-designed cannabinoids.

Contributing Authors Mashmoushi, Nour Campbell, J. Larry Hopkins, W. Scott Hopkins

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Benilde Mizero, University of Manitoba

Peptide retention time prediction for TMT-labeled peptides in RP-HPLC for proteomic applications

TMT isobaric labelling has been a leading method for relative quantification; mainly due to its multiplexing capability and it is not amino acid specific. In parallel, there is an urging need to use Retention Time (RT) prediction to improve the identification confidence in RPLC-MS bottom up proteomic workflows. Such studies need to consider any modification that can alter the RT. In our attempt to determine the RT shift upon labelling with TMT to update our Sequence Specific Calculator model (SSRCalc) to include TMT modification, we measured retention values for 144000 unique non-labelled (NL)/TMT labelled (L) peptide pairs. Our prior knowledge on peptide behavior in RP chromatography helped to develop a sequence specific prediction algorithm that yield a solid correlation (R2~0.989) between RT of NL and L peptides. Such update of our SSRCalc should permit a transition from NL to L peptide workflow and vice-versa.

Contributing Authors B Mizero, C Villacres, V Spicer, R Viner, J Saba, B Patel, S Snovida, P Jensen, A Huhmer, O Krokhin

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Vivaldy Prinville, UQAM

Quantitative analysis of bile acids to study effect of acetaminophen in rat

Bile acids play a crucial role in metabolism and liver health. It is known that bile acids are important for liver function, and therefore should be affected by drug-induced hepatoxicity. Acetaminophen is a very commonly used drug to relieve pain and fever, however it is also the main cause of acute liver failure in North America. A targeted liquid chromatography-tandem mass spectrometry assay was developed for the quantitative analysis of 47 bile acids, with 14 isotopically-labeled internal standards. With this optimized method, bile acid profiles in plasma were studied at four different doses of acetaminophen in a rat model.

Contributing Authors Vivaldy Prinville, Leanne Ohlund, Lekha Sleno

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Gabriel Robert, Université de Sherbrooke

Fate of 5-methyl radicals of thymine within DNA in the presence of oxygen: analysis and mechanistic studies in the formation of tandem lesions

The integrity of the genetic material is susceptible to oxidative damage originating mainly from exposure to reactive oxygen species (ROS), which are generated endogenously as by-products of aerobic

respiration, or exogenously from toxic xenobiotics. One of the main reactions of hydroxyl radical (°OH) with DNA is H-atom abstraction from the methyl group of thymine. Because the methyl group protrudes into the major groove, it is highly susceptible to attack by °OH and accounts for approximately 50% of the total damage to pyrimidines in DNA. This reaction generates the 5-(uracilyl)-methyl radical (T°), which can propagate damage by reacting further, with another nucleobase or notably under aerated conditions.

The reaction with O2 leads to methylperoxyl radicals (TOO°) that can react with neighboring purine bases leading to so-called double or tandem lesions. Tandem lesions are refractory to traditional repair systems and constitute a greater mutagenic potential compared to isolated single lesions. Here, we investigate the mechanism of their formation by using a photolabile precursor that generates T° and in turn TOO° at a specific position in DNA. Upon photolysis in oxygenated solutions, the resulting products were characterised by LC/MS-MS and HRMS. Thereby, we show that TOO° can react with an adjacent guanine moiety to produce double lesions involving the oxidation of guanine to either fapyG or 8-oxoG together with the oxidation of thymine to either 5-hmU or 5-fU.

Contributing Authors Gabriel Robert, J. Richard Wagner

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