14th Toronto Post-ASMS Symposium

Free-to-attend Mass Spectrometry Users’ Meeting

Sep. 18, 2014, Toronto (Plaza Airport Hotel)

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PROGRAM AGENDA

04:00pm-04:20pm – Welcome Coffee/Tea and Refreshments

Plenary Oral Session - Chair: Dr. Lars Konermann

04:20pm-04:40pm – "Ex vivo and in vivo solid phase microextraction in metabolomic profiling of apple (Malus x domestica Borkh.)" - Presented by Dr. Sanja Risticic - (University of Waterloo/Apotex Pharmachem, Brantford, ON)

04:40pm-05:00pm – "Nanodiscs and CaR-ESI-MS: A novel method for the discovery of protein-glycosphingolipid interactions" - Presented by Prof. John Klassen (University of Alberta, Edmonton, AB)

05:00pm-05:20pm – "Integrating mass spectrometry with structural biology in the Mass Spec Studio" - Presented by Prof. David Schriemer (University of Calgary, Calgary, AB)

05:20pm-06:20pm – Hot Buffet Dinner

Parallel Oral Session 1 - Chair: Dr. Lekha Sleno

06:20pm-06:40pm – "Optimization of Solid Phase Extraction Procedure for Leuprolide in Human Plasma" - Presented by Dr. Nicki Hughes (Bioanalytical Laboratory Services, a Division of LifeLabs LP, Toronto, ON)

06:40pm-07:00pm – "Absolute quantitation of NAPQI-modified serum albumin from rat plasma samples by LC-MS/MS: monitoring acetaminophen toxicity" - Presented by Prof. Lekha Sleno (UQAM, Montreal, QC)

07:00pm-07:20pm – "Mass Spectrometry as a tool for the detection of Emerald Ash Borer attack" - Presented by Dr. Naomi Stock (Trent University, Peterborough, ON)
07:20pm-07:40pm – "Investigation of the chemical interactions of the triad: sample, solvent and surface and their effects in DESI-MS imaging analysis" - Presented by Prof. Demian Ifa (York University, Toronto, ON)

07:40pm-08:00pm – Parallel Poster Presentation Session 1 Chaired by Prof. Rebecca Jockusch (McMaster University, Hamilton, ON)

Parallel Oral Session 2 - Chair: Prof. Christoph Borchers

06:20pm-06:40pm – "Development of a Novel 2D LC/MRM-MS Approach for Deeper and Broader Quantitation of Putative Protein Biomarkers in Human Plasma" - Presented by Prof. Christoph Borchers (University of Victoria-Genome BC Proteomics Centre, Victoria, BC)

06:40pm-07:00pm – "Recent Developments in HDX/MS for Exploring Protein Folding, Structure, Dynamics, and Interactions" - Presented by Prof. Lars Konermann (University of Western Ontario, London, ON)

07:00pm-07:20pm – "Epitope Mapping and Interrogation of Allostery in Protein-ligand Interactions Using an Integrated, Electrospray-coupled Microfluidic Device" - Presented by Prof. Derek Wilson (York University, Toronto, ON)

07:20pm-07:40pm – "Fluorescence resonance energy transfer measurements for the structural characterization of gaseous proteins generated by electrospray ionization" - Presented by Prof. Rebecca Jockusch (University of Toronto, Toronto, ON)

07:40pm-08:00pm – Parallel Poster Presentation Session 2 Chaired by Prof. Rebecca Jockusch (University of Toronto, Toronto, ON)

08:00pm-08:30pm – Dessert and Coffee/Tea
08:30pm-09:10pm – "Challenges in Environmental Analysis" - Presented by Prof. Bert van Bavel (Örebro University, Örebro, Sweden)

09:10pm-09:30pm – "Quantification of Growth Hormone Receptor Antagonist Pegvisomant by LC-MS/MS in Rat Plasma: Method Development Considerations for PEGylated Proteins" - Presented by Dr. Fabio Garofolo (Algorithme Pharma, Laval, QC)

List of Poster Presentations

Parallel Poster Session 1 Chaired by Prof. Philip Britz-McKibbin

1. "Simultaneous Quantitation of Nebivolol and Valsartan in Human Plasma" - Presented by Mei Li Mei Li; HongZhi Liu; Anita Dalko; Helen Deng; Surya Kandukuri; Nicola Hughes Bioanalytical Laboratory Services (a division of LifeLabs LP), Toronto, Canada

2. "Trimethylation and Chemical Modifiers in IMS/MS Peptide Analysis: Performance Enhancement Through Solution- And Gas-Phase Chemistry" - Presented by Voislav Blagojevic Voislav Blagojevic; Amanda De Filippis; Diethard K. Bohme York University, Toronto, CANADA

3. "Strategies for direct coupling of SPME to mass spectrometry" - Presented by German Augusto Gomez-Rios German Augusto Gomez-Rios; Nathaly Reyes-Garces; Barbara Bojko; Janusz Pawliszyn University of Waterloo, Waterloo, CANADA

4. "Rapid Confirmatory Testing of Thyroxine Status by Multi-Segment Injection Capillary Electrophoresis-Mass Spectrometry with Chemical Derivatization" - Presented by Meera Shanmuganathan Meera Shanmuganathan1, Osama Aldirbashi2 and Philip Britz-McKibbin1 1Department of Chemistry and Chemical Biology, McMaster University, 1280 Main St. W., Hamilton, Ontario, Canada, L8S 4M1; 2Newborn Screening Ontario, Children’s Hospital of Eastern Ontario
Parallel Poster Session 2 Chaired by Prof. Rebecca Jockusch

   Modupeola Sowole; Lars Konermann Univ. of Western Ontario, London, ON

2. "Unambiguous Characterization of Analytical Markers in Complex, Seized Opium Using an Enhanced Ion Mobility Spectrometry-Mass Spectrometry Method" - Presented by Peter Liuni
   Peter Liuni1, Vladimir Romanov2, Marie-Josée Binette3, Hafid Zaknoun3, Maggie Tam3, Pierre Pilon3, Jan Hendrikse2 and Derek J. Wilson1 1Department of Chemistry, York University, Toronto, ON, Canada; 2Smiths Detection, Inc., Mississauga, ON, Canada; 3Canada Border Services Agency, Ottawa, ON, Canada

3. "High-throughput solid phase microextraction (SPME)-LC-MS as a convenient method for the simultaneous determination of multiple prohibited substances in urine and plasma" - Presented by Nathaly Reyes-Garces
   Nathaly Reyes-Garces; Ezel Boyaci; Krzysztof Gorynski; Angel Rodriguez-Lafuente; Barbara Bojko; Janusz Pawliszyn University of Waterloo, Waterloo, CANADA

5. "Hydrogen-Deuterium Exchange Combined with Electron Capture Dissociation to Probe the Conformation of Gaseous Peptide Ions" - Presented by Rita Straus
   Rita Straus; Rebecca A. Jockusch University of Toronto, Toronto, ON

Oral Sessions Abstracts & Speaker Biographies

Oral 01: “Ex vivo and in vivo solid phase microextraction in metabolomic profiling of apple (Malus x domestica Borkh.)”

Speaker: Dr. Sanja Risticvic (University of Waterloo/Apotex Pharmachem, Toronto, ON)

Introduction: Long-term storage of fruits may deteriorate fruit quality and result in production of disorders. The development of postharvest disorders during fruit storage also depends on a range of preharvest factors, including climate conditions and harvest maturity. Considering that food quality attributes can be fully defined in terms of metabolome composition, food metabolomics has been utilized for comprehensive characterization of food metabolome and assurance of food quality and safety. Metabolomic analyses consist of a sequence of steps including collection of representative sample, sample preparation, metabolism quenching, metabolite extraction, metabolite separation, detection, and data treatment. Considering that the objective of metabolomics analysis is unbiased metabolome coverage, nonselective methods are required for extraction of as many as possible compounds in the food samples.
Method: Sample preparation: SPME-LC-MS method used for analysis of different groups of apple samples ('Honeycrisp' apples from Ontario). 96-blade SPME coatings were developed and used for apple metabolomics studies to characterize metabolic changes associated with storage of apple samples. In vivo SPME was employed in combination with GCxGC-ToFMS for on-site sampling of apple samples.

Chromatography: UPLC PFP column (kinetex 2.6 PFP (100x2.1mm, 100Å) was used to develop LC-MS method for separation of extracted metabolites. For GCxGC-ToFMS analysis, Rxi-5SilMS (30 m x 0.25 mm ID x 0.25 μm) and Stabilwax (1 m x 0.25 mm ID x 0.25 μm) columns (Restek (Bellefonte, PA, USA)) were employed.

Mass spectrometry: MS detection was carried out using Exactive benchtop Orbitrap system (Thermo, San Jose, CA) operated in both positive and negative modes using high resolution, full MS (m/z 100-1000). For GCxGC analyses, LECO Pegasus 4D GCxGC-ToFMS system (LECO, St. Joseph, MI, USA) was used.

Data processing: XCMS R-package was used for data processing to provide ion annotation on the list of features. For GC platform, data acquisition and processing were performed with ChromaTOF (version 4.24) software. SIMCA-P+ software (Umetrics, NJ, USA) was used for statistical analyses.

Preliminary data: Different coating chemistries for 96-blade SPME (PS-DVB-WAX, HLB, Si-IL, Si-RP-WAX, C18, Strata-X, PBA, DioI, Tris amide (HILIC), PS-DVB:HLB 80:20 (w/w), PS-DVB:Si-IL 80:20 (w/w), PS-DVB:HLB 50:50 (w/w)) were tested and applied for extraction of targeted metabolites. Results showed that HLB 96-blade extraction phase provides the best extraction recovery with the least carryover.

96-blade SPME method was developed using HLB coating for nontargeted apple metabolomics. Optimized condition includes:

- preconditioning: methanol:water: 50:50 (v/v) (60 min)
- extraction: apple samples (120 min)
- wash: water + 0.1% formic acid (20 seconds)
- desorption: methanol:acetonitrile:water 40:40:20 (v/v) (120 min)

About 10000 features were detected using XCMS R-package. Metlin database was used to match obtained m/z values with a mass tolerance window of 5 ppm resulting in provision of a database composed of about 1000 metabolites, including sugars, fatty acids, amino acids, peptides, sugar alcohols, organic acids, prenol lipids, glycerophospholipids, flavonoids, sphingolipids, ethers, hydrolyzable tannins, steroids and steroid derivatives, thiamines, cinnamic acid derivatives, phenols and derivatives, fatty amides, cyclic alcohols and derivatives, alkyl glycosides, isoindoles and derivatives, pyrrolidines, fatty aldehydes, furans, pyrans, indoles, pyridines and derivatives, and acetals.

The other part of study is using PCA along with PLS-DA analyses to differentiate groups of samples and identify potential biomarkers of fruit storage and fruit development. Results showed that 96-blade SPME using HLB coating is capable of detecting metabolic responses of apple samples in response to cold storage. The accumulation of carbohydrates such as glucose, fructose, and raffinose was demonstrated using S-PLOT. Also, changes in aminoacid, sugar and sugar alcohol levels resulted in response to the storage conditions. Based on the statistical interpretation of data and detection of biomarkers of fruit maturation, in vivo SPME has demonstrated great potential in quantitative metabolomics and metabolome coverage of GC amenable analytes.

Novel aspects: 96-blade SPME prior to LC-MS analysis was applied to high-throughput analysis of complex food matrices without any need for sample pretreatment. Application of in vivo SPME in quantitative plant metabolomics was highlighted.
Area: Food "omics": MS Characterization of Food and Nutritional Supplements, Food Safety

Speaker Biography

BSc, Honours Chemistry, University of Waterloo, Canada, 2005
MSc, Analytical Chemistry, University of Waterloo, Canada, 2008
PhD, Analytical Chemistry, University of Waterloo, Canada, 2012

Graduate work: Solid phase microextraction coupled to gas chromatography – time-of-flight mass spectrometry and comprehensive two-dimensional gas chromatography – time-of-flight mass spectrometry for determination of food quality and metabolomic profiling of foods

Work experience:

Scientist in Exova, R & D Health Sciences Department, Mississauga, Ontario (September 2012-November 2013)

Development, optimization and validation of GC, GC-MS and HPLC analytical methods for quantitative determination of residual solvents, extractables and leachables, contaminants and impurities in pharmaceutical products

Chemist in Apotex Pharmachem Inc., Analytical R&D Laboratory, Brantford, Ontario (November 2013-present)

Supporting process R&D department for the analysis of raw materials, intermediates and finished active pharmaceutical ingredients using a variety of analytical methods and techniques. Method development, optimization and validation activities for assay determination and analysis of related compounds and residual solvents in starting materials, process intermediates and finished active pharmaceutical ingredients.

Oral 02: “Nanodiscs and CaR-ESI-MS: A novel method for the discovery of protein-glycosphingolipid interactions”

Speaker: Prof. John Klassen (University of Alberta, Edmonton, AB)

Introduction

Catch and release electrospray ionization mass spectrometry (CaR-ESI-MS) has tremendous potential for rapidly screening carbohydrate libraries against target proteins to identify and quantify new protein-carbohydrate interactions. However, the method is limited to the analysis of carbohydrates that are relatively soluble in water. Here, we describe a new method, employing nanodiscs and CaR-ESI-MS, for the discovery of protein interactions with insoluble glycosphingolipids. Glycosphingolipids are readily incorporated into nanodiscs, which are discoidal phospholipid bilayers surrounded by a membrane scaffold protein, allowing them to mimic their natural cellular environment while being soluble in aqueous solution. The results of control experiments reveal that the assay allows for simultaneous detection of both low and high affinity glycosphingolipid ligands and has the ability to rank their relative affinities.

Methods

Nanodiscs were prepared by dissolving lipid films made up of a mixture of DMPC and glycosphingolipid with membrane scaffold protein (MSP1E1) and sodium cholate (25 mM) to give a final molar ratio of 100:1 lipid:MSP1E1. Nanodiscs were then formed by the slow removal of sodium cholate at 25 °C by the addition of biobeads. Finally, nanodiscs were purified using size exclusion chromatography and concentrated into 200 mM ammonium acetate pH 6.8 prior to ESI-MS analysis. A Synapt G2S HDMS mass spectrometer (Waters) was used to detect protein-glycosphingolipid complexes present in solution. Collision-induced dissociation was performed on
the protein-glycosphingolipid complexes in the Trap region to release the ligands. Accurate mass measurement allowed for ligand identification.

Preliminary Results/Abstract

Two proteins were selected as model glycosphingolipid-binding proteins for this study: cholera toxin B subunit homopentamer (CTB$_5$) and the heat labile toxin B subunit homopentamer (HLTB$_5$), which both bind preferentially to the ganglioside GM1. Application of the CaR-ESI-MS assay to CTB$_5$ and a library of nanodiscs that each contains one of three gangliosides (GM1, GM2 or GM3) or nanodiscs that contain a mixture of all three glycosphingolipids demonstrated that the assay allows for the simultaneous detection of both high and low affinity glycosphingolipid ligands. To determine whether the specificity of binding can also be determined using this method, the CaR-ESI-MS assay was applied to CTB$_5$ and a nanodisc containing seven gangliosides (GM1, GM2, GM3, GD1a, GD1b, GD2 and GT1b). Using collision-induced dissociation to release bound ligands, it was found that CTB$_5$ binds to GM1, GM2, GM3 and GD1b in aqueous solution at pH 7. Moreover, the relative abundances of the ligands released from the CTB$_5$-glycosphingolipid complexes were found to qualitatively reflect the affinities of the oligosaccharide ligands for CTB$_5$ in solution. Finally, to demonstrate the utility of the CaR-ESI-MS assay for discovering new glycosphingolipid ligands, it was applied to solutions of CTB$_5$ or HLTB$_5$ with nanodiscs containing glycolipids extracted from porcine brain. Notably, these measurements led to the discovery of a neolacto glycosphingolipid as a new cholera toxin ligand.

**Novel Aspect** Novel method based on nanodiscs and electrospray ionization mass spectrometry for screening glycosphingolipid libraries for protein interactions is described.

**Speaker Biography:**

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

**Oral 03: “Integrating mass spectrometry with structural biology in the Mass Spec Studio”**

**Speaker:** Prof. David Schriemer (University of Calgary, Calgary, AB)

Combining biophysical data from multiple sources is critical for developing accurate structural and functional models of dynamic multi-protein systems. Mass spectrometry can be used to measure the insertion location for a wide range of chemical probes, and such insertion data provide a rich but disparate set of topographical modeling restraints. We have developed routines that integrate mass spec data with protein modeling activities. These
routines are built on a novel architecture for app development (the Mass Spec Studio). The presentation will briefly highlight the concepts and technologies behind this new approach to software development in mass spectrometry, and then describe an app package useful for MS-driven integrative structural biology.

The structure biology app package mines any labeling data from any mass spectrometer in a proteomics-grade manner, and converts label data into molecular docking restraints. These restraints are implemented within the HADDOCK framework to drive complex protein modeling problems. Specifically, support is provided for covalent labeling chemistries and hydrogen/deuterium exchange (HX). It includes novel acquisition strategies such as targeted HX-MS² and data-independent HX-MS² (CID and ETD-based). The MS² modes extend measurements to highly complex protein systems, which we demonstrate through the analysis and modeling of microtubule-ligand interactions.

Speaker Biography:

Dr. David Schriemer studied chemistry and mathematics at the University of Manitoba and the University of Winnipeg and obtained his Ph.D. in chemistry at the University of Alberta (1997). As a postdoctoral fellow and in research leadership for two biotechnology companies, he developed MS-based technologies for drug discovery and protein interaction analysis. He returned to academia in 2001, where he is an Associate Professor in the Department of Biochemistry & Molecular Biology, and an adjunct in the Department of Chemistry. He is also the Director of the SAMS Centre for Proteomics at the University of Calgary.

His research group fuses a fundamental interest in measurement science with a fascination for the inner workings of cell division. One goal is to understand the role of microtubules in orchestrating the separation of replicated chromosomes, and to turn this enhanced understanding into novel therapies. The group has been driving mass spectrometry as an approach for modeling complex protein systems – in both structural and conformational terms. It has allowed them to generate new knowledge about tubulin self-assembly and most recently, it has supported a program in rational drug design centred on a novel strategy for antimitotic drug therapy. Structural mass spectrometry is data-intensive, requiring a strong focus on computational methods and informatics. His group has produced software adopted by researchers and instrument vendors alike.

Dr. Schriemer holds a Canada Research Chair in Chemical Biology and is an AIHS Heritage Senior Scholar.

Oral 04: “Optimization of Solid Phase Extraction Procedure for Leuprolide in Human Plasma”

Speaker: Dr. Nicki Hughes (Bioanalytical Laboratory Services, a Division of LifeLabs LP, Toronto, ON)

Introduction Leuprolide, a synthetic nonapeptide analog of naturally occurring gonadotropin releasing hormone (GnRH or LH-RH), is used in the treatment of prostate cancer or breast cancer. In order to develop a sensitive and rapid quantitation method for Leuprolide using a low volume of plasma sample, the solid phase extraction (SPE) procedure was systematically optimized. Leuprolide (0.0200 ng/mL to 20.00 ng/mL) was extracted from 300 µL of human plasma sample by SPE without evaporation and quantified using AB Sciex API 4000 triple quadrupole mass spectrometer using TurboIonSpray ionization in positive ion mode.

Methods Leuprolide in 300 µL of human plasma, fortified with deuterated internal standard (IS) and diluted with water, was extracted using a Waters HLB 96-well plate. After washing with acetonitrile/water solution, the analytes were eluted with 2 x 150 µL of acidified methanol/water solution. The SPE method was partially automated using a
Tomtec. A 15 µL aliquot of the eluent was injected onto LC-MS/MS system. The chromatographic separation was achieved on a C8 column using gradient mobile phase of methanol and acidified ammonium formate solution. The detection was performed on AB Sciex API 4000 using TurbolonSpray in positive ion mode. The MRM transitions (doubly charged precursor ion) were 605.5/249.2+221.0 and 608.2/249.2+221.0 for Leuprolide and IS. Total run time was 4.0 minute.

**Preliminary Results/Abstract** During method development, adsorption test was performed to determine the optimal container type for the storage of Leuprolide solutions. It was observed that Leuprolide solutions in methanol, methanol/water, acetonitrile/water adsorbed avidly to glass containers in various magnitude and an almost complete response loss was observed in the most severe case. The methanolic Leuprolide solution in a polypropylene container showed most consistent response after going through adsorption test and storage. SPE procedure was systematically optimized for sample preparation, washing and elution, using Waters Oasis HLB and WCX cartridge or 96-well plate, and waters Oasis HLB 96-well plate was finally chosen. It was noticed that the analyte response was similar with high and low percentage organic solvent wash. Washing with organic solvent/water of ~50/50 resulted in the best analyte response. After the optimization, a sensitive and simple SPE procedure without evaporation was established using 300 µL of plasma sample. The method was developed for the calibration curve range of 0.02000 ng/mL to 20.00 ng/mL for Leuprolide. A signal to noise ratio of ~20 was obtained for LLOQ. The assay performance was confirmed with a few accuracy and precision batches. The extraction recovery as approximately 70%. Hemolysis and lipemic matrix showed no impact on the quantitation.

**Novel Aspect** A sensitive SPE procedure without evaporation/reconstitution was established for Leuprolide in human plasma after systematic method optimization.

**Speaker Biography:**
Nicki obtained a BSc in biochemistry (toxicology) from the University of Surrey (UK) and PhD from the University of London (UK) in the field of Chemical Carcinogenesis in 1991. In 1992, she moved “across the pond” to take on a research role in the clinical Pharmacology department at the Hospital For Sick children in Toronto, where her research focus was in determining the relationship between pharmacogenetics and aromatic amine exposure as risk factors for bladder cancer susceptibility. In 1997 she joined Biovail Contract Research, where she led the R&D team and subsequently the whole lab in the development, validation and application of highly sensitive LC-MS/MS bioanalytical assays to support clinical trials. In 2010, she joined Cetero Research (which later became PRACS) and led an operational reorganization and in doing so developed an exceptional bioanalytical team. In 2012, Nicki joined CML HealthCare (now LifeLabs) initially leading a number of operational changes and then took on a leadership role in expanding the specialized clinical trials laboratory services. This included a strategic partnership with the clinical group Inflamax and the acquisition and operationalization of the laboratory assets of the former PRACS site in Toronto, which is now the Bioanalytical Laboratory Services (BLS) division of LifeLabs. Nicki is the division lead at BLS and works with a highly experienced team, who has worked together in the CRO industry for greater than 15 years.

**Oral 05:** “Absolute quantitation of NAPQI-modified serum albumin from rat plasma samples by LC-MS/MS: monitoring acetaminophen toxicity”

**Speaker:** Prof. Lekha Sleno (UQAM, Montreal, QC)

**Introduction** Acetaminophen (APAP) toxicity is the most common cause of acute liver failure in the United States. The hepatotoxicity of acetaminophen is caused by an electrophilic reactive metabolite of acetaminophen, N-acetyl
p-benzoquinone imine (NAPQI), that forms covalent adducts with nucleophilic thiol groups in liver proteins, causing hepatic necrosis. Serum albumin (SA) is known to be covalently modified by NAPQI and is present at high concentrations in the bloodstream. SA is therefore a potential biomarker to assess the levels of protein modification by NAPQI. To date, no quantitative method exists for NAPQI modified serum albumin. Here, we present a highly sensitive method for the absolute quantitation of modified serum albumin from in vivo rat plasma samples by LC-MS/MS.

**Methods** Plasma samples were digested using pepsin and the albumin active site peptide containing Cys34, modified by NAPQI, was targeted for quantitation. For accurate absolute quantitation, a standard was designed using an iodo-analog of acetaminophen to quantitatively modify RSA cysteines. This protein standard yields a target peptide that is a positional isomer to the one modified by NAPQI. A deuterated version was used to produce an internal standard added prior to digestion to all standards and unknown samples, correcting for all steps during sample preparation. The digested plasma samples undergo solid phase extraction, and ion exchange fractionation prior to injection. LC-MS/MS analysis was performed on an AB Sciex 5500 QTRAP in positive MRM mode.

**Preliminary Results/Abstract** An LC-MS/MS method was developed to quantify NAPQI-modified serum albumin from rat plasma with a limit of quantitation of 31.25 ng modified RSA present in 100 µl plasma, representing approximately 0.0006 % of the total RSA. The linear dynamic range spans two and a half orders of magnitude. The precision and accuracy of the method were assessed using a 10 point standard curve prepared in triplicate with excellent accuracies and reproducibility. The method was applied to quantify modified RSA in plasma samples from rats (n=4) dosed at low (non-toxic) levels of acetaminophen (75 mg/kg). While no peaks of interest were detected in blank plasma samples, we measured concentrations between 380 and 480 ng/100 µl plasma in dosed samples, illustrating the potential of this approach to directly monitor acetaminophen covalent binding from in vivo samples. The developed quantitation method relies on a novel protein standard design based on iodoacetamide chemistry for modifying cysteine residues in standard albumin. This modified RSA, spiked into blank rat plasma, is used for an external calibration curve while a deuterated (d4) version of the analog is used as internal standard. Several clean-up steps were shown to be necessary in order to remove complex background from digested plasma. Mixed-mode anion exchange solid phase extraction as well as strong cation exchange HPLC fractionation is crucial for achieving the high sensitivity of the final method. After digestion, the resulting peptides are positional isomers of NAPQI modified RSA peptides. Using synthesized standards of our targeted peptic peptide (LQKC*PYEE) containing both the NAPQI and the iodo-APAP modifications, we show that the standard and target peptides’ chromatographic, ionization and fragmentation behaviors are extremely similar. We also show that this standard is essential to the accuracy of the method by comparing to other methods commonly used for protein quantitation.

**Novel Aspect** Absolute quantitation of NAPQI-modified albumin for monitoring acetaminophen toxicity able to monitor non-toxic levels of adducts in plasma

**Speaker Biography:**

Lekha Sleno received her PhD in chemistry from Dalhousie University in 2006, where her work involved small molecule mass spectrometry. She then completed two postdoctoral fellowships at the University of Geneva in Switzerland (in pharmaceutical analytical chemistry) and the University of Toronto (at the Donnelly CCBR in proteomics research). She is currently associate professor in the chemistry/biochemistry department at UQAM (Université du Québec à Montréal). Her research interests include bioanalytical mass spectrometry applied to metabolomics and covalent binding of reactive drug metabolites.
Oral 06: “Mass Spectrometry as a tool for the detection of Emerald Ash Borer attack”

Speaker: Dr. Naomi Stock (Trent University, Peterborough, ON)

Abstract:

Introduction The ash trees of North America have been under attack by the Emerald Ash Borer (EAB), an invasive species, since 2002. Some 80 million ash trees are at risk of EAB attack that is specific to the ash and fatal. Physical symptoms produced by EAB attack are loss of foliage, D-shaped holes in trunk, epicormic growth, and bark splitting. Detection of EAB outbreaks can be difficult in the early stages because often the tree does not display any outward visible symptoms until death is inevitable. It has been reported that marked changes in volatile metabolite production are caused by EAB stress. In this study, mass spectrometry is employed to investigate metabolic characteristics and develop a method to detect EAB attack.

Methods Leaves were collected from approximately one hundred ash trees which were assigned to one of three groups: healthy trees, trees under attack by EAB, or trees stressed from factors other than EAB attack, such as drought, trunk damage, other insect attack etc. Two grams of leaves from each tree were analyzed. Leaves were cut into small pieces, extracted with methanol and analyzed using liquid chromatography-mass spectrometry (LC-MS). The ion chromatograms obtained were subjected to principal components analysis (PCA) in order to identify changes in the distribution of normal metabolites and the observation of novel metabolites induced by EAB attack. Key metabolites were identified and analyzed using high resolution mass spectrometry.

Preliminary Results/Abstract

Initial research by our group reported (1) that electrospray ionization mass scans of methanolic extracts from an ash tree within an EAB-infected area, and an ash tree well beyond the EAB-infected area, are strikingly different. Subsequently, a relatively large scale study to analyze the foliage of approximately one hundred trees was carried out.

LC-MS analysis of leaf extracts combined with principle components analysis, clearly show differentiation between the three groups of trees: healthy trees, trees under attack by EAB, or trees stressed from factors other than EAB attack. Key peaks corresponding to metabolites of interest were also identified. Specific metabolites were observed in trees attacked by EAB, but not in healthy trees. Other metabolites were observed in healthy trees, but not in trees that attacked by EAB. Metabolites common to both healthy and non-EAB stressed trees, but not EAB-attacked trees were also observed.

Some of these key metabolites of interest have been examined by high resolution mass spectrometry and the corresponding elemental compositions have been obtained. Efforts are underway to combine elemental compositions and product ion mass spectra to obtain plausible ion structures of such peaks. All samples were subjected to LC-MS in negative ion mode although a small sub-set of samples were examined in positive ion mode to assist in the identification of metabolites.

As expected, not all of the trees sampled from EAB-infested areas indicated EAB attack; however, among those trees attacked by the EAB, there is indication of the time sequence in which trees were attacked.

Preliminary results indicate mass spectrometry may be an effective tool for the detection of EAB attack and could play an important role in treatment and protection of ash trees.

Novel Aspect: Rapidity of response and identification of biological marker compounds characteristic of ash tree health.

Speaker Biography

Naomi Stock is a Senior Research Scientist at the Water Quality Centre at Trent University and has been working there since 2008. She is the principle operator of the Centre’s organic mass spectrometers. When all the instruments are working well and students are analyzing their own samples, she is able to spend some time on research. Her current research focuses on environmental mass spectrometry – primarily development of analytical methods for determination and identification of organic contaminants in environmental samples, and naturally occurring compounds of environmental interest.

Naomi is also an Adjunct Professor in the Environmental and Life Science graduate program at Trent University. She completed her PhD in Environmental Chemistry at the University of Toronto.

Oral 07: “Investigation of the chemical interactions of the triad: sample, solvent and surface and their effects in DESI-MS imaging analysis”

Speaker: Prof. Demian Ifa (York University, Toronto, ON)

Introduction: The performance of DESI analysis is dependent on the choice of the solvent, surface, and the system geometry, as well as, the solubility of the analytes. Keeping the system geometry unchanged, the interactions among the chemical properties of the analytes, solvent and surface may determine the success of the DESI droplet pickup mechanism. These interactions have significant effect in DESI-MSI analysis of imprinted samples and determine the overall quality of the ion images. To understand the chemical interactions of the triad: sample, solvent and surface; this study present the evaluation of the behavior of four β-blockers (Atenolol, Nadolol, Acebutolol and Propranolol) against different hydrophobic and hydrophilic membrane surfaces, as well as, their interactions with different solvent mixtures.

Methods: A standard solution of β-blockers (5 mg/mL) was prepared in methanol/water/black ink (5:3:2) resulting in final solution of 3.3 mg/mL. The final solution was transferred into an empty deskjet printer cartridge. Six membrane filters with hydrophobic and hydrophilic surfaces (M1 to M6) were cut and placed side-by-side on white office paper using a double side tape. A symmetric figure with four squares was printed on the membrane surfaces. The same set of squares was printed four times in each membrane. The imprinted membranes were analyzed using a Thermo Fisher LTQ mass spectrometer with a lab-built moving stage DESI source with four solvent mixtures with different polarity (S1 to S4).

Preliminary Results/Abstract: According to ion images created, the higher intensities for all β-blockers were reached using the membrane M1 (hydrophobic). In terms of the solvent selected, S3 (methanol/water 1:9) showed the higher intensity for all analytes. All membranes analyzed with S2 (methanol/water 1:1) showed good intensity (lower than S3) for all evaluate ions, and images with better definition, S2 promotes the solubilisation of the compounds and also good spray solvent volatility. This combination of effects leads to efficient ionization and minimize effects of wash out or splashing. Hydrophilic membranes (M2, M4 and M6) presented lower intensities compared to hydrophobic membranes because of the affinity of the membrane surfaces for the polar compounds.

Novel Aspect: The evaluation of the interactions among the triad: sample, solvent and surface was performed by DESI-MSI analysis.

Speaker Biography
Demian Ifa received his BSc in Pharmacy from the State University of São Paulo (UNESP), Brazil; MSc in Organic Chemistry from the University of Rio de Janeiro (UFRJ); and PhD in Pharmacology from the University of São Paulo (USP). He worked as postdoctoral fellow and associate research scientist at the Aston Labs for Mass Spectrometry at Purdue University, USA. He joined the Chemistry Department at York University as an Assistant Professor in 2011. His major interests are ambient ionization techniques, imaging mass spectrometry, structural biology and clinical mass spectrometry.

Oral 08: “Development of a Novel 2D LC/MRM-MS Approach for Deeper and Broader Quantitation of Putative Protein Biomarkers in Human Plasma”

Speaker: Prof. Christoph Borchers (University of Victoria-Genome BC Proteomics Centre, Victoria, BC)

Introduction Rapid, sensitive, and reproducible protein quantitation methods are required to verify and validate the growing list of disease biomarker candidates in human biofluids such as blood plasma. Although immunoassays are ideal for use in clinical validation and employment, a bottom-up proteomic approach utilizing stable isotope-labeled standards (SIS) and LC/MRM-MS has emerged as a powerful and preferred technique for biomarker verification. The multiplexing ability and detection sensitivity of this method, however, remains rather limited, especially at levels below 5 ng/mL. We have therefore developed a novel 2D RPLC/MRM-MS method, utilizing high and low pH separations with internal SIS peptides for the highly multiplexed and sensitive quantitation of >250 high-to-low abundance disease biomarker candidates in undepleted human plasma.

Methods Tryptic digestion of a pooled plasma control sample was conducted following a standard, sample pretreatment workflow. A complex mixture of 1035 synthetic SIS peptides (corresponding to 423 proteins) was spiked in post-digestion, for normalization. Samples were then extracted and fractionated by rapid 2D RPLC, configured in an off-line alkaline (ammonium hydroxide, pH 10) separation, followed by an on-line acidic (formic acid, pH 3) separation. The on-line LC step was interfaced to a triple quadrupole mass spectrometer for dynamic MRM measurements via positive ESI on an Agilent 6490 triple quadrupole mass spectrometer. Development involved optimization and interference testing, with quantitation conducted on 13 LC fractions. Application of this method to patient samples is currently being explored.

Preliminary Results/Abstract We have previously demonstrated the ability to quantitate the top 6 order-of-magnitude concentration range of plasma proteins with only a simple sample pre-treatment and without the need for antibody depletion or enrichment, or additional chromatographic or electrophoretic fractionation (Percy et al. BBA, 2013). There, standard-flow RPLC/MRM-MS enabled the quantitation of proteins with concentrations as low as 44 ng/mL (myeloblastin). To enhance the depth and breadth of quantitation beyond that level, a novel 2D-LC fractionation method was developed. This method utilized alkaline and acid separations, with the former using an ammonium hydroxide-based mobile phase, in contrast to the ammonium formate or ammonium acetate mobile phases conventionally used in proteomics. Preliminary optimization involved tuning the transition-specific parameters, adjusting the high-pH LC gradient, and evaluating several fraction-pooling strategies. The final method involved the analysis of 13 LC fractions (consisting of ca. 80 peptides/fraction) which were obtained by selectively pooling some of the 47 fractions which were obtained over a 31 min run. Subsequent analysis of technical replicates (n = 3) showed a high level of consistency in peptide elution and signal, and good quantitative performance. Concentrations of 256 disease-related plasma proteins spanning an 8 order-of-magnitude range (from 15 mg/mL to 450 pg/mL) could be determined. This represented a 2 order of magnitude enhancement in the quantitative depth over that attainable without fractionation, as revealed by parallel 1D LC/MRM-MS experiments.
with matched proteomic samples. From this comparison, 83 proteins were quantified only by 2D, with 18 proteins quantified below 5 ng/mL and 55 presenting concentrations below the 44 ng/mL limit found in our 1D results. The improvements provide a springboard for application to our complete panel (634 interference-free peptides corresponding to 256 disease-linked proteins), or a subset thereof. We are currently exploring method applications to patient samples for protein biomarker verification.

**Novel Aspect** Simple and fast LC fractionation toward more comprehensive and multiplexed MRM quantitation of high-to-low abundance proteins in undepleted human plasma.

**Speaker Biography:**

Christoph H. Borchers, Ph.D., Full Professor, University of Victoria

Dr. Borchers received his B.S., M.S. and Ph.D. from the University of Konstanz, Germany. After his post-doctoral training and employment as a staff scientist at NIEHS/NIH/RTP, in North Carolina, he became the director of the UNC-Duke Proteomics Facility and held a faculty position at the UNC Medical School in Chapel Hill, NC (2001-2006). Since then, Dr. Borchers has been employed at the University of Victoria (UVic), Canada and holds the current positions of Professor in the Department of Biochemistry and Microbiology and the Don and Eleanor Rix BC Leadership Chair in Biomedical and Environmental Proteomics. He is also the Director of the UVic – Genome BC Proteomics Centre, which is one out of five Genome Canada funded Science & Technology Innovation Centres and the only one devoted to proteomics. Dr. Borchers is also appointed as Professor at McGill University in the Department of Oncology, Montreal, QC.

His research is centred around the improvement, development and application of proteomics technologies with a major focus on techniques for quantitative targeted proteomics for clinical diagnostics. Multiplexed LC-MRM-MS approaches and the immuno-MALDI (iMALDI) technique are of particular interest. Another focus of his research is on technology development and application of the combined approach of protein chemistry and mass spectrometry for structural proteomics. Dr. Borchers has published over 190 peer-reviewed papers in scientific journals and is the founder and CSO of two companies, Creative Molecules. Inc. and MRM Proteomics Inc. He is also involved in promoting proteomic research and education through his function as HUPO International Council Member, Scientific Director of the BC Proteomics Network and Vice-President, External of the Canadian National Proteomics Network.

**Oral 09: “Recent Developments in HDX/MS for Exploring Protein Folding, Structure, Dynamics, and Interactions”**

**Speaker:** Prof. Lars Konermann (University of Western Ontario, London, ON)

**Introduction** This talk will provide an overview of the latest developments in the field, partially based on a recent review article [Konermann et al. Anal. Chem. 86, 213 (2014)]. HDX/MS provides a highly sensitive tool for probing protein-protein and protein-ligand interactions. Recent examples of such investigations include studies on protein-DNA binding, interactions of antibodies with HIV proteins, phospholipase inhibition, as well as lipid-induced protein conformational changes. It is tempting to use HDX/MS for mapping binding sites, implicitly postulating that the largest reduction in structural dynamics will occur where protein and ligand interact. Unfortunately, such a simplistic data interpretation can be misleading because binding often involves allosteric effects, where major effects occur far away from the site of interaction.
Methods In intrinsically disordered proteins (IDPs) defy the classical structure-function paradigm, according to which a well-defined structure is required for biological function. HDX/MS is increasingly being used for exploring the properties of IDPs, as well as conformational switching events that are induced by ligand binding. Disordered regions undergo deuteration with rate constants that approach kch, i.e., the value expected for unprotected amides. Accurate measurements of these rapid kinetics are challenging when using conventional HDX/MS technology. The application of customized rapid mixing devices is an elegant solution to this problem. Another approach is to exploit the pH dependence of kch for slowing down the deuteration kinetics.

Preliminary Results/Abstract Regardless of the experimental technique used, studies on integral membrane proteins (IMPs) remain difficult. In recent years a number of research groups have begun to tackle this issue by HDX/MS. IMP studies are most commonly conducted on detergent-solubilized species. A number of investigations were successful in characterizing the deuteration behavior of IMPs in a membrane environment. The results obtained in this way can be markedly different from those seen for solubilized proteins. Pulsed HDX/MS is an essential tool for exploring protein conformational changes in time-resolved experiments. The most common application is the characterization of short-lived folding intermediates. Owing to the rapid time scale of typical folding reactions, many of these experiments require rapid mixing devices. The level of structural information that can be extracted from pulsed HDX/MS studies is impressive, as recently demonstrated in experiments on ribonuclease H1. The characterization of cytotoxic protein aggregates is not an easy task, largely because of their considerable heterogeneity. Abeta aggregates represent the most thoroughly studied systems in this context. Pulsed HDX data suggest that the formation of these oligomers proceeds in a self-catalyzed fashion, where the center regions of the polypeptide chains interact first, followed by the formation of contacts in the C and N termini. HDX/MS was also applied to study the conversion of the prion protein PrPC to its cytotoxic PrPSc form. The data obtained are consistent with a large-scale conformational switch from the monomeric alpha-helix-rich PrPC conformation to an aggregated PrPSc structure that is dominated by beta-strands. In addition to the topics outlined above, we will also briefly discuss the application of HDX/MS to protein therapeutics, as well as recent work towards experiments with single residue resolution.

Novel Aspect Using examples from various research groups, this presentation will provide a brief overview of the field.

Speaker Biography

Lars Konermann holds a Canada Research Chair in the Chemistry Department at The University of Western Ontario. He is cross-appointed to the Department of Biochemistry. Konermann received his PhD in 1996 for studies on plant photosynthesis at the Max-Planck-Institute in Mulheim/Germany. After a post-doctoral fellowship at UBC in Vancouver he moved to Western where he rose through the ranks to full professor. The work in Konermann’s laboratory focuses on protein biophysical chemistry. Many of his projects involve the development and application of new mass spectrometry techniques. Konermann is recipient of the UWO Florence Bucke Science Prize, the Fred Beamish Award of the Canadian Society for Chemistry, the ETP Ken Standing Award, the Fred Lossing Award of the Canadian Society for Mass Spectrometry, as well as several undergraduate teaching awards.


Speaker: **Prof. Derek Wilson** (York University, Toronto, ON)
Introduction  Mapping ligand-binding epitopes in proteins in their native conformation is largely limited to X-ray crystallography and NMR studies. Conformational epitopes, that often depend on an 'induced-fit' mode of interaction with ligands are particularly inaccessible to conventional analytical approaches. We previously introduced a microfluidic device integrating the full 'bottom-up' workup for site-specific, sub-second HDX analysis by ESI-MS and applications to studying dynamics of weakly-structured regions. Here, we expand its applications as a tool for epitope mapping and evaluation of allosteric changes in dynamics following ligand binding. Sub-second labeling pulses appear to enhance binding site predictions and capture of the evolution of protein dynamics. The microfluidic format reduces total workup cycle to less than 10 seconds, and has potential high-throughput applications.

Methods  Time-resolved HDX was performed with a capillary-based rapid mixer, integrated onto a microfluidic device. Target protein, incubated with ligand, was continuously infused into the inner capillary of the mixer, where it would mix with outer capillary-supplied D$_2$O. The analyte was then quenched with acetic acid, continuously supplied via a third device-integrated channel, and entered a digestion chamber filled with pepsin immobilized on agarose beads. The worked-up sample was sprayed directly into the QStar Elite ESI-MS. At least 7 timepoints were analyzed for relative deuterium incorporation ranging from 180ms to 7.5s to construct the kinetic curves for each peptide. The data is an average of three independent runs. Minimum primary sequence coverage was 70%, and average spatial resolution was 6 residues.

Preliminary Results/Abstract  Two model systems were utilized to examine the application of the device to epitope mapping and interrogation of allosteric changes following ligand binding: Glutathione-S-Transferase (GST) interacting with its ligand, GSH; Signal Transducer and Activator of Transcription 3 (STAT3), a transcription factor dysregulated in cancer, binding to a novel class of small molecule dimerization inhibitors, BP-1-102 and SF-1-066. The GST study revealed that GST-GSH complexation results in a significant relative decrease in deuterium uptake in peptides mapping to the active site residues in the G-binding pocket, while the rest of the protein exhibits non-significant changes. The binding of the ligand to GST was accompanied by the formation of new backbone hydrogen bonds at the active site, observed as a decrease in deuterium uptake in the peptides spanning residues 53-58/53-61 and 62-69. These peptides formed hydrogen bonds to the cysteinyl carbonyl and ?-glutamyl carboxylate of GSH, respectively. These findings were consistent with predictions from crystallographic studies of the GST-GSH complex, which implicate residues Leu55 and Ser68, mapping to the observed peptides.

The STAT3 study enabled mapping of binding site epitopes of two different SH2 domain-binding STAT3 dimerization inhibitors, BP-1-102 and SF-1-066, observed as hotspots of decreased deuterium uptake. The data provided an experimental validation of binding site predictions based on in silico docking and biological activity assays. Significant allosteric changes were observed by this technique, propagating from the inhibitor binding site towards STAT3 domains involved in DNA binding and regulation of nuclear trafficking. These dynamic changes presented as increases in deuterium uptake, consistent with possible destabilization or increased ‘breathing’ motion in those regions. The two models interrogated by our technique represent two contrasting protein-ligand binding modes. While GST-GSH binding exemplifies a binding mechanism with a large 'lock-and-key' character, the STAT3 inhibitor binding model reflects an 'induced-fit' mechanism with a significant allosteric effect.

Novel Aspect  This comprehensive investigation of conformational dynamics of protein-ligand interactions describes novel applications of TRESI-MS/HDX for epitope mapping and allostery evaluation.

Speaker Biography
Derek J. Wilson was born face-up in Richmond Hill on the cool spring night of April the 25th, 1978. As a child, he showed reasonable aptitude for everything, but particular aptitude for nothing, setting the stage for his trademark 'jack-of-all-trades-master-of-none' approach to research. His secondary training was similarly 'diverse', with a high school career focused on acting and vocal performance in the 'Arts York' program, leading naturally to a BSc in biochemistry at Trent which culminated in an undergraduate research project under Prof. Steve Rafferty that received an 88% from Prof. Mark Parnis, who is a very tough marker. This set the stage for a PhD in bioanalytical chemistry under the gentle tutelage of a very tall german (Prof. Lars Konermann) at Western. These efforts were successful enough to attract an NSERC post-doctoral fellowship, which was taken up in the laboratory of the largely absent, but still somehow brilliant Dr. Chris Dobson at Cambridge. At the same time, Dr. Wilson interviewed and was ultimately hired at York University, where he now runs a research group focused (if that's the right word) on understanding the molecular basis of protein pathogenesis using bioanalytical mass spectrometry, microfluidics and biophysical nuclear magnetic resonance.

Oral 11: “Fluorescence resonance energy transfer measurements for the structural characterization of gaseous proteins generated by electrospray ionization”

Speaker: Prof. Rebecca Jockusch (University of Toronto, Toronto, ON)

Introduction Various mass spectrometry-based methods, including ion mobility, ion-molecule reactions, and tandem mass spectrometry techniques, can give structural footprints of gaseous, ionized proteins. Despite developments in these areas, structural information for gaseous proteins lacks completeness in detail. Here, fluorescence resonance energy transfer (FRET), which relies on the distance-dependent non-radiative transfer of electronic excitation energy between a donor and acceptor dye, is applied to infer inter-residue separation distance information in mass-selected proteins in the gas phase. The efficiency of energy transfer, which encodes structural information, is explored as a function of charge state and other experimental parameters in the immunoglobin G-binding domain of protein G (GB1), and the proteins frataxin and prothymosin. Comparisons are made with solution-phase single-molecule FRET measurements.

Methods Protein mutants containing two cysteine point-mutations were expressed to enable site-specific labeling with thiol-reactive forms of a donor (ATTO 532) and acceptor (ATTO 647N) FRET dye pair. Using nano-electrospray ionization, fluorescent protein conjugates were delivered to a quadrupole ion trap mass spectrometer modified for laser-induced fluorescence experiments of trapped ions. Mass-selection allowed fluorescence measurements to be done in a charge-state-resolved manner. A titanium-sapphire laser was frequency-doubled to provide 485 nm excitation light for selective donor excitation. Fluorescence light emitted by ions was collected, filtered, and sent to one of two optical detectors. Emission spectra were measured using a spectrograph interfaced with an electron-multiplying charge-coupled device. Time-resolved donor fluorescence was measured using a single-photon avalanche diode by time-correlated single-photon counting methods.

Preliminary Results/Abstract Fluorescence emission spectra and time-resolved fluorescence from the donor in various charge states of fluorescent GB1 conjugates show dependence of the FRET efficiency on the degree of charging on the protein. In solution, GB1 adopts a conformation similar to ubiquitin over a wide range of charge states, consisting of a four-stranded ?-sheet spanned by an ?-helix. In the gas phase, for the two lowest charge states of GB1 examined (4+ and 5+), a high FRET efficiency is observed, as evidenced by sensitized emission from the acceptor and quenching of donor fluorescence. Since the dyes are located near the termini of the protein, these data suggest that the 4+ and 5+ charge states correspond to GB1 conformational ensembles which place the dye pair in close proximity, and are therefore somewhat compact. Upon addition of more charge to the protein.
(i.e., in the 6+, 7+, and 8+ states of GB1), a progressive recovery of donor fluorescence, and concomitant decrease in emission from the acceptor is observed, which indicates a gradual loss in FRET with increasing charge. This suggests that the higher charge states of GB1 have more elongated conformations, and supports the view of a Coulombically-driven extension of its structure in the gas phase. Estimates of dye-dye separation distances derived from the measured FRET efficiencies suggest that, even in the most compact GB1 structures probed, the protein is significantly unfolded in the gas phase relative to its solution structure. Preliminary measurements on a destabilized variant of GB1, which show a similar dependence of the FRET efficiency on charge state, support this view. Efforts are currently underway to: (i) assess the validity of the estimated value of the critical Forster distance for this FRET pair using a labeled polyproline peptide, and (ii) extend this technique to other proteins.

**Novel Aspect** These results represent the first gas-phase FRET measurements of mass-selected proteins.

**Speaker Biography**

Rebecca Jockusch an Associate Professor in the Chemistry Department at the University of Toronto, where she holds a Canada Research Chair in Biophysical Analytical Chemistry. She is the current Secretary, and member of the Board, of the American Society for Mass Spectrometry (ASMS). Rebecca majored in physics at Carleton College (Northfield, MN). After a two year stint teaching English in Japan as part of the JET program, she returned to science and started graduate studies in Chemistry at the University of California, Berkeley. Her doctoral studies were conducted under the guidance of Prof. Evan R. Williams and focused on the development of tools and techniques to further biological mass spectrometry. She was awarded a PhD in Chemistry in 2001. She then held a Royal Society USA Research Fellowship at Oxford University, pursuing post-doctoral research using laser spectroscopy to analyze carbohydrate conformation and hydration effects under the guidance of Prof. John P. Simons. She is the recipient of Early Researcher Awards from the Province of Ontario and from ASMS.

Dr. Jockusch’s research program employs techniques from chemistry, biology and physics to investigate the properties of biological molecules, both in isolation and in complexes. Factors affecting protein conformation and dynamics, including the role of water in biological systems, are of particular interest. The unique instrumentation developed for these studies in her laboratory combine trapping mass spectrometers with recent advances in technology for optical spectroscopic experiments. Research highlights include the implementation of sensitive fluorescence detection in order to monitor the conformation of biomolecular ions inside a mass spectrometer via fluorescence resonance energy transfer (FRET) techniques. Her group has also used fluorescence to visualize images of the cloud of trapped ions under different mass spectrometry conditions.

**Oral 12: “Challenges in Environmental Analysis”**

**Speaker:** **Prof. Bert van Bavel** (Örebro University, Örebro, Sweden)

Within the UNEP program ‘Assessment of Existing Capacity and Capacity Building Needs to Analyze POPs in Developing Countries’ several activities were undertaken the last 5 years. The program is focused on the analytical capacity for the POPs under the Stockholm Convention including several pesticides (DDT, chlordane, and toxaphene) and industrial (by)-products (dioxins, PCBs). Recently brominated flame retardants (BDE) and an organic fluor compound (PFOS) were added. One of the conclusions of the program was that it is quite a challenge to analyze all POPs in the sample types proposed for the global monitoring program (GMP).
Traditionally low resolution GC/MS is used for the analysis of the chlorinated POPs, mostly operating in the EI mode or NCI mode for specific compounds including toxaphene and the brominated BDEs. For dioxins high resolution GC/MS systems are often required to avoid inferences and to achieve the low LODs needed.

One of the difficulties to develop a universal method for the Stockholm convention POPs is that different ionization techniques are needed for different POPs. With the advances in LC/MS ionization using electro spray with or without corona needle (APCI) more reliable equipment for atmospheric pressure ionization is now becoming available. The use of atmospheric pressure ionization for GC might be a possibility to efficiently ionize the majority of the POPs on the Stockholm convention.

Not only detection technology is moving into a more general technology for Stockholm Convention POPs, also in the field of chromatography similar developments are seen and for example supercritical fluid are making a comeback. In addition, untargeted screening technologies, combining metabolomics or screening for small molecules, with POP analysis is a new trend in POP analysis.

Speaker Biography

Bert van Bavel is a professor in analytical chemistry at the MTM Research Center at the Örebro University in Sweden. After receiving his master degree at the University of Amsterdam, he received a PhD from the Umeå University, he spent time working in the US as a senior scientist at Test America and two periods as a guest professor at the Kyoto University and Shimadzu Techno Research in Japan.

He is currently head of MTMs analytical laboratory including the dioxin lab. He is the co-author of more than 200 publications in environmental chemistry and organizes each year one of the largest QA/QC programs on dioxin analysis. His research interest covers all from the analysis of dioxins and traditional chlorinated POPs, brominated flame retardants to perfluorinated compounds. Professor Bert van Bavel is one of the experts for UNEP leading laboratory inspections to assess existing capacity and capacity building needs to analyze POPs in developing countries. In 2004 he won the prestigious Cancer and Allergy Foundations Environmental Medicine Award for his research on the relation between POPs and cancer.

Oral 13: “Quantification of Growth Hormone Receptor Antagonist Pegvisomant by LC-MS/MS in Rat Plasma: Method Development Considerations for PEGylated Proteins”

Speaker: Dr. Fabio Garofolo (Algorithme Pharma, Laval, QC)

Introduction PEGylation is a widely used approach to improve the pharmacokinetic properties of therapeutic protein drugs. However, quantification of PEGylated proteins in plasma by LC-MS/MS encompasses challenges that may not be encountered in non-PEGylated proteins and therefore requires careful consideration. Depending on the method of conjugation, PEGylation at the targeted amino acids may not be stoichiometric or consistent, therefore requiring a surrogate peptide to be void of potential PEGylation sites and thus reducing the pool of candidate signature peptides amenable for quantification. Herein, we present an LC-MS/MS approach for the quantification of Pegvisomant, a recombinant human growth hormone (hGH) structural analog PEGylated on several lysine residues and used as a therapeutic hGH receptor antagonist indicated for the treatment of acromegaly.

Methods Rat plasma was spiked with a Somavert formulation containing 5 mg/mL of Pegvisomant protein. As internal standard, the hGH was also spiked in the plasma samples. Samples were diluted 5-fold with NH₄HCO₃ to allow protein reduction with DTT (5 mM) at 60°C for 20 minutes, followed by alkylation with iodoacetamide (10 mM) for 15 minutes at 20°C. Reduced and alkylated plasma proteins were then subjected to tryptic digest at 60°C for
60 minutes (400 mg/mL final trypsin concentration). A 0.1% HCOOH and acetonitrile gradient was applied to a Waters Acquity CSH column (50 x 2.1 mm, 1.7µm) using a UHPLC pump (Waters) and the Pegvisomant peptide was monitored in a QTRAP triple-quadrupole instrument (AB Sciex) operated in ESI(+) MRM mode.

**Preliminary Results/Abstract** Pegvisomant is a PEGylated version of the hGH with several amino acid substitutions that allow the molecule to bind the growth hormone receptor while preventing its dimerization and thus, activation. The protein portion of Pegvisomant has a molecular weight of 22 kDa, to which 4 to 6 molecules of polyethylene glycol (PEG) are covalently attached to lysine residues, resulting in a heterogeneous population of molecules with molecular weights of 42, 47 and 52 kDa. To avoid any possible inconsistencies and in order to ensure specificity, the arginine-flanked, Pegvisomant-specific tryptic peptide (R)VSTFLR (corresponding to the tryptic peptide (K)VETFLR in the hGH) was chosen as the signature peptide for quantification. During the early phase of tryptic digestion optimization, it was found that urea-mediated sample denaturing was not only unnecessary but also resulted in lower detection levels. However, reduction with DTT and subsequent alkylation with iodoacetamide was essential to the detection of the signature peptide VSTFLR, most likely due to its proximity to an adjacent cysteine involved in a disulfide bond. Direct plasma tryptic digest was performed without enrichment or purification steps, thus rendering the method attractive for high throughput purposes. The major product ions generated from fragmentation of the VSTFLR doubly charged parent ion (m/z 361.7) corresponded to the y4 (m/z 536.3) and y5 (m/z 623.4) fragments which confirmed the identity of the selected signature peptide. For quantification purposes, the y5 fragment was used due to lower associated interfering ions. Under these conditions, the assay was linear and quantitative between 50 ng/mL - 50 mg/mL. This research highlights how the judicious choice of a surrogate peptide, optimization of sample preparation steps, as well as optimal tuning of UHPLC and MS parameters can circumvent the challenges of PEGylated protein quantification and result in the development of industry-tailored, cost-efficient bioanalytical methods.

**Novel Aspect** Quantification by UHPLC-MS/MS of the PEGylated protein Pegvisomant in the ng/mL range without prior protein purification or enrichment.

**Speaker Biography**

Dr. Fabio Garofolo has been working in the pharmaceutical bioanalytical and LC-MS analytical fields for more than 20 years. He has also been heavily involved and committed to working as a volunteer for pharmaceutical and scientific non-profit organizations with the mission to promote the interactions among industrial, academic and regulatory bodies to provide education and forums for discussion in the pharmaceutical practices.

Dr. Garofolo has progressed throughout in his career in both the pharma/biotech and CRO industries and benefited from successfully overcoming the prevalent challenges in drug development. Since 2005 he has held the position of Vice-President Bioanalytical Services at Algorithm Pharma. Dr. Garofolo has over 130 publications & presentations in international conferences. He developed around 300 innovative analytical methods. He designed and invented 3 innovative bioanalytical approaches and is the recipient of the following Lilly awards: Achievement (2001); Global (2002); Emmerson (2003). His current interests include analysis of large molecules by LCMS, HRMS, DBS, and Emerging Technologies.