

## **“Lead-catalyzed oxidation of DNA: oxidation of guanine by hydrogen peroxide”**

**Laura Banu, Voislav Blagojevic and Diethard K. Bohme (York University)**

The rates of the lead-catalyzed oxidation of guanine were measured with hydrogen peroxide in the absence and presence of  $\text{Pb}^{2+}$  using a variable temperature reactor coupled to a mass spectrometer by electrospray ionization. The signature ion of guanine,  $\text{GH}^+$ , observed in the mass spectra, was followed with time to provide kinetic data. The signature ion of the product,  $[\text{GH}^+ + \text{H}_2\text{O}_2]$ , was exposed to collisional activation to gain insight into its structure. The rate data provided clear evidence for the catalytic action of  $\text{Pb}^{2+}$ : the apparent rate constant of guanine oxidation at 333 K, for example, was found to be  $3.2 \times 10^{-3} \text{ s}^{-1}$  in the presence of  $\text{Pb}^{2+}$ , higher by 16 than that obtained in the absence of  $\text{Pb}^{2+}$ . The Arrhenius plot measured in the presence of  $\text{Pb}^{2+}$  from 308 to 333 K indicates an activation energy for oxidation of  $82 \pm 11 \text{ kJ mole}^{-1}$ . In the absence of  $\text{Pb}^{2+}$  the activation energy was found to be  $208 \pm 27 \text{ kJ mole}^{-1}$  from measurements in the range 328 to 335 K. Loss of 45, corresponding to the loss of aminoformaldehyde, was observed the main dissociation channel of the signature ion of the product in the presence and absence of  $\text{Pb}^{2+}$ . Deuteration experiments in  $\text{D}_2\text{O}$  as solvent revealed dissociation of the C4-N bond consistent with the formation of 5-carboxamido-5-formamido-2-iminohydantoin (2-Ih) by oxidation at carbon C5 of guanine as previously proposed in the literature.

Poster

## **“Locating metals in metal-peptide complexes using neutral losses”**

**Laura Banu, Voislav Blagojevic and Diethard K. Bohme (York University)**

The binding preference of  $\text{Pb}^{2+}$  and  $\text{Zn}^{2+}$  in doubly charged complexes with cysteine and histidine containing peptides (Pep),  $[\text{M}_n(\text{Pep}-2(n-1)\text{H})]^{2+}$ , has been investigated using tandem mass spectrometry. The N-terminus of these synthetically designed peptides were blocked with an acetyl group. Dodecapeptides possess four cysteine and/or histidine residues in positions 2, 5, 8 and 11, arranged in different motifs: CCHH, CHCH and CCCC. The  $\text{MS}^2$  spectra of  $\text{Pb}^{2+}$  and  $\text{Zn}^{2+}$  complexes show multiple losses of water and a single methane loss. The sequence of losses proved to be a sensitive probe for the location of the metal and indicative of its coordination. The elimination of a methane molecule indicated the position of the metal at  $\text{Cys}_2$  residue and the number of water losses preceding that of methane was sensitive to the number of metal dications in the complex. Lead is observed to preferentially bind to cysteine residues while zinc binds primarily to histidine residues and secondarily to cysteine residues. The predilection to lose PbS molecules is correlated with the number of thiol groups coordinated to lead, being high for one coordination, low for two and zero for three or more. The mechanism of water and methane loss has been investigated using comparative studies of G3 and G4 complexes with lead, and these have shown that the key to these dissociations is the ability of the metal to withdraw electrons from the proximal amidic nitrogen. This acidic nitrogen loses its hydrogen to an

amidic oxygen situated four atoms away leading to water elimination and the formation of a five-member ring.

**“Evaluation of the performance of molecularly imprinted polymers for trace analysis of 2,4-dichlorophenoxyacetic acid in the environment”**

***Adam G. Beaton, Geert Van Biesen, Christina S. Bottaro***

Molecularly imprinted polymers (MIPs) are cross-linked synthetic polymers that can selectively take up target analytes from solution. The Bottaro group has been working on MIPs in thin film format for direct analysis by desorption electrospray ionization – mass spectrometry (DESI-MS), and sensor applications. Encouraging results were obtained with an MIP for the herbicide 2,4-dichlorophenoxyacetic acid (2,4-D), and it was anticipated that the use of an internal standard would improve quantitation. There are two possible approaches to using internal standards with MIPs; internal standard can be added to the analyte solution with both analyte and internal standard taken up simultaneously by the MIP, or the internal standard can be pre-loaded onto the MIP then used later for uptake of the analyte. The latter procedure is more convenient for environmental analysis, since the MIP can be directly put into an aquatic environment, instead of spiking a collected sample with internal standard. Both procedures were used to prepare calibration curves using HPLC-MS of MIP extracts (DESI-MS analysis at Prosofia will be undertaken in a separate study), and yielded similar satisfactory results. Finally, to demonstrate the practical applications of these MIPs, they were used for uptake of 2,4-D from a complex matrix (spiked apple juice).

Poster

**“Differential Ion Mobility and Trimethylation in Quantitative Peptide Analysis”**

***Voislav Blagojevic, Gregory K. Koyanagi and Diethard K. Bohme (York University)***

In-vacuo trimethylation of peptides with methyl iodide isotopic tags has potential to substantially increase the dynamic range and accuracy of peptide quantitative measurements. The presence of quaternary amines created by the trimethylation, which have a permanent positive charge, offers exciting opportunities to exploit their unique properties to further increase detection limits and specificity of detection. Differential Mobility Spectroscopy (DMS), which increases separation by ion mobility by adding a chemical modifier, offers a suitable analytical tool. Use of high proton affinity modifiers allows suppression of non-trimethylated (protonated) peptides, while also increasing the ion mobility resolution of the DMS instrument. Furthermore, coupling with a Fused Droplet Electrospray Ionization (FD-ESI) source currently under construction, should allow the analysis of large samples without extensive cleanup or processing.

**“Structures of bare and hydrated [Pb(AminoAcid-H)]<sup>+</sup> complexes using infrared multi-photon dissociation spectroscopy: Deprotonation at amine”**

***Michael B. Burt, Sarah G. A. Decker, Chad G. Atkins, Mark Rowsell, and Travis D. Fridgen  
(Memorial University of Newfoundland)***

Infrared multiple-photon dissociation (IRMPD) spectroscopy was used to reveal the gas-phase structures of deprotonated Pb<sup>2+</sup>/amino acid (Aa) complexes with and without a solvent molecule. Five amino acid complexes with side chains containing only carbon and hydrogen (Ala, Val, Leu, Ile, and Pro) and three with more functionalized side chains (Phe, Lys, and Glu) were compared. These experiments demonstrated that the aliphatic [Pb(Aa-H)]<sup>+</sup> complexes are amine-deprotonated and have Pb<sup>2+</sup> covalently bound between the amine nitrogen and carbonyl oxygen. However, the more functionalized amino acids were deprotonated at the carboxylic acid and formed carboxylate salts instead. This structural difference is attributed to Pb<sup>2+</sup> being further stabilized by the functionalized side chains. IRMPD spectroscopy was also performed on the monohydrated analogues of the [Pb(Aa-H)]<sup>+</sup> complexes. The [Pb(Aa-H)H<sub>2</sub>O]<sup>+</sup> complexes, where Aa = Ala, Val, Leu, and Ile, exhibited two N-H stretches, as well as a carboxylic acid O-H and a PbO-H stretch. Hence, their structures are monohydrated versions of the amine-deprotonated [Pb(Aa-H)]<sup>+</sup> complexes where a proton transfer has occurred from the lead-bound water to the deprotonated amine. On the other hand, the absence of a carboxylic acid O-H stretch in the IRMPD spectra of [Pb(Pro-H)H<sub>2</sub>O]<sup>+</sup> and [Pb(Glu-H)H<sub>2</sub>O]<sup>+</sup> suggests that these complexes are hydrated carboxylate salts. The structure of [Pb(Lys-H)H<sub>2</sub>O]<sup>+</sup> is also carboxyl-deprotonated, but Pb<sup>2+</sup> is bound to the carbonyl oxygen and the amine nitrogen with one of the protons belonging to the water transferred to the basic side chain. This results in an intramolecular hydrogen bond that does not absorb in the region of the spectrum probed in these experiments. Structures were calculated, and the IRMPD spectra were compared to infrared spectra predicted by B3LYP/6-31+G(d,p) electronic structure calculations. 298 K enthalpies and Gibbs energies were calculated using the MP2(full)/6-311++G(2d,2p) method on the B3LYP geometries.

**“Optimization of protein digestion using microfluidic immobilized trypsin columns”**

***Carlos Canez, Jinal Patel and Jeffrey C. Smith (Carleton)***

An on-column digestion protocol was created and optimized using immobilized trypsin to digest large quantities of protein in an economical manner. The on-column digestion protocol was evaluated using a standard protein (bovine serum albumin) as well as a mixture of seven standard proteins ( $\alpha$ -casein,  $\beta$ -casein, albumin, concavalin A, hemoglobin, myoglobin and ovalbumin). The efficiency of the protocol was evaluated by RP-HPLC-ESI-MS/MS. Mascot software was used to interpret the results and evaluate their efficiency. Continuous flow digestion proved to be as effective as stationary digestion using micro-columns with inner diameters of 200 $\mu$ m. Our protocol decreased digestion times from several hours (typical of an in-solution digestion) to 30

seconds and showed stable activity for over three and a half hours. The continuous flow digestion provides a potential tool for an online, automated and economical microfluidic device.

**“Comparing the Energy/Structure Relationship of Peptides/Saccharides Complexes using Electrospray Mass Spectrometry, Tandem Mass Spectrometry, RRKM Theory, Ion Mobility Spectrometry and Molecular Dynamics”**

***Amanda Comeau, Justin Renaud, Paul Mayer (University of Ottawa)***

The noncovalent interaction of two short peptides (FLEEL and FLEEV) with six saccharides (glucose, lactose, D-panose, raffinose, stachyose and maltotetraose) was investigated using five methods; electrospray mass spectrometry, tandem mass spectrometry, ion mobility spectrometry, Rice-Ramsperger-Kassel-Marcus (RRKM) theory and molecular dynamics. A comparison of the results obtained from the different methods will give insight into the accuracy and credibility of each method. It has been observed that the location of the positive charge is integral to the structure of the interacting species.

Poster

**“Analysis of Interaction between microRNA and Protein p19 using Non-Equilibrium Capillary Electrophoresis of Equilibrium Mixtures”**

***Ana Gargaun, Nasrin Khan and Maxim V. Berezovski (University of Ottawa)***

MicroRNA (miRNA) expression profiles can be used as an analytical tool in the diagnosis of various types of cancer. A double-stranded RNA-binding protein, p19, was found to have size specific interactions with miRNA. The miRNA used in this research is miR-122 and is 22 nucleotides in length. The p19 binds strongly to miRNA that are between 21-23 nucleotides in length. This research focuses on studying the binding affinity between miRNA and p19 using Non-Equilibrium Capillary Electrophoresis of Equilibrium Mixtures (NECEEM). A complex between p19 and the miRNA was observed and separated from the dsRNA. Furthermore, the following reaction kinetic parameters were determined by using the developed method: rate constants of complex formation and dissociation and the equilibrium dissociation constant to illustrate complex stability.

Poster

**“Exploring the fragmentation pathways of phenanthrene and dihydrophenanthrene”**

***Mahta Ghodssi, Melanie Oulette, Brandi West and Paul M Mayer (University of Ottawa)***

Our lab is currently investigating the fragmentation of simple PAHs and compare their behaviour with the corresponding dihydro PAHs. Our study of the fragmentation (metastable and collision induced) of ionized phenanthrene and dihydrophenanthrene was performed on a VG-ZAB.

**“Gas phase activation of hydrocarbons by unsaturated bipyridinium ruthenium complex,  $\text{Ru}(\text{bipy})_2^{2+}$ : The effect of ligation of  $\text{Ru}(\text{bipy})_2^{2+}$  with CO on the dehydrogenation of i-butane, and propane”**

***Ameneh Gholami and Travis D. Fridgen (Memorial University of Newfoundland)***

Catalysts are required to selectively convert hydrocarbons into more useful petroleum compounds. Refining reactions of hydrocarbons consist of dehydrogenation, cracking, oxidation, or reduction processes. Dehydrogenation and cracking hydrocarbons lead to petroleum compounds of higher octane number. Metal-promoted catalytic activation of C-C and C-H bonds of hydrocarbons can also improve the octane rating of hydrocarbons [1,2]. Unsaturated transition metal complexes are known as active catalysis centers for activation of organic compounds. For example,  $\text{M}(\text{bipy})_2^{2+}$  (M=Cr, Ru, Os) have been used to activate neutral molecules such as  $\text{O}_2$  and hydrocarbons [3]. In this work, the reactions of  $\text{Ru}(\text{bipy})_2^{2+}$  with dioxygen, carbon monoxide, i-butane, propane, and propene have been investigated under the very low pressure conditions of the Fourier transform ion cyclotron resonance (FTICR) mass spectrometer. In these reactions, adduct ion-molecule products were observed. However, dehydrogenation of i-butane and propane were observed when  $\text{Ru}(\text{bipy})_2^{2+}$  was ligated with CO. At longer reaction times, between propane or i-butane with  $\text{Ru}(\text{bipy})_2(\text{CO})^{2+}$  C-C insertion reactions were evidenced by  $\text{Ru}(\text{bipy})_2(\text{CO})(\text{C}_2\text{H}_4)^{2+}$  and  $\text{Ru}(\text{bipy})_2(\text{CO})(\text{C}_3\text{H}_6)^{2+}$ , respectively. Density functional calculations were used to help elucidate the mechanism and energy requirement for the dehydrogenation reaction of i-butane mediated by  $\text{Ru}(\text{bipy})_2(\text{CO})^{2+}$ . The very interesting hydrocarbon dehydrogenation upon ligation with CO is attributed to a long-lived internally hot intermediate.

[1] Armentrout, P. B.; Chen, Y. M. J. Am Soc Mass Spectrom, 10, 1999, 821–839.

[2] Eller, K.; Schwarz, H. Chem. Rev. 91, 1991, 1121-1171.

[3]Nielsen, S. B.; Øiestad, A. L.; Bojesen, G.; Uggerud, E. Int. J. Mass Spectrom, 243, 2005, 231–239.

**“Optimized Buffer Conditions for Stabilizing Multiprotein Complex Structure in the Absence of Bulk Solvent: Development of a Gas-phase Hofmeister Series”**

***Linjie Han, Suk-Joon Hyung, and Brandon T. Ruotolo (University of Michigan)***

The application of ion mobility mass spectrometry (IM-MS) in structural biology requires that the structure of electrospray-generated ions of biomolecules to be conserved. However, the extent of ‘dehydration’ is often challenging to control leading to difficulties in downstream structural assignments. In recent experiments, we have screened a series of anions for their ability to stabilize protein complexes in the absence of bulk solvent. By using IM-MS, we observe both the dissociation and unfolding transitions for four tetrameric protein complexes bound to populations of twelve different anions using collisional activation. We quantify, for the first time, the influence of these anions on gas-phase protein structure, allowing us to rank and classify them as structure stabilizers. The rank order determined by our data is substantially different when compared to the known Hofmeister series in solution. Additionally, anion-resolved data acquired for smaller protein systems allows us to classify anions into three categories based on their ability to stabilize protein complex structure in the absence of bulk solvent. Recently, we have begun screening cations for their ability to stabilize gas-phase protein structure. Our ultimate goal is to project the optimal cation and anion additives to solutions for stabilizing the native-like structure of gas-phase proteins. —Withdrawn—

**“ Investigating the generation of metal anions from multiply charged metal cations using small organic molecules”**

***Dhiya Hassan, Julie Roy, Alex Mungham, Justin Renaud, Paul M Mayer (University of Ottawa)***

Fragmentation of some metal-cation/oxalate complexes results in the facile generation of unique metal anions. Previously limited to only singly charged metal cations; Ag<sup>+</sup>, Na<sup>+</sup>, K<sup>+</sup>...Could structure and properties of organic acids other than oxalic acid allow for generation of metal anions from multiply charged metals; such as Cu<sup>2+</sup>, Fe<sup>3+</sup>.

**“A quantitative mass spectrometry-based proteomic and phosphoproteomic analysis of VSV-infected K562 cells”**

***Matthew P. Huebsch and Jeffrey C. Smith (Carleton)***

The vesicular stomatitis virus (VSV) has been shown to selectively induce apoptosis in many cancer cell lines, however the mechanisms of its oncolytic properties remain poorly understood. In this work, a mass spectrometry-based approach was used to investigate the changes in protein phosphorylation and protein expression in a human leukemic cell line (K562) after a 30-minute

infection time with VSV. The analytical strategy incorporated tryptic digestion of cellular lysates, on-column stable-isotope dimethyl labeling, and proteomic and phosphoproteomic investigations by SAX/SCX-LC-MS/MS and IMAC-LC-MS/MS, respectively. A total of 53 proteins and 8 phosphorylation sites were found to be upregulated and 11 proteins and 9 phosphorylation sites were found to be downregulated in VSV-infected K562 cells versus control cells.

**“Enzyme Mechanisms and Dynamics Studied by Time-Resolved Electrospray Ionization:  
Pre-steady state kinetics of Chymotrypsin”**

***Araby Jeganathan and Derek Wilson (York)***

The pre steady state of an enzyme is that in which the intermediates of an enzymatic reaction are formed and populated. Studying the kinetics of the pre-steady state can provide insight into the enzyme's reaction mechanism as it allows for the detection of intermediates, and thereby the measurement of rate constants in individual reaction steps. The objective of this study was to determine whether enzymes undergo the same dynamics in the presence of substrate as they do in the absence of substrate, during the pre-steady state. Since the pre steady state kinetics occurs within a millisecond to second range, our experiments require specialized rapid mixing techniques. In this project, a continuous rapid flow mixing apparatus was coupled with mass spectrometry and electrospray ionization (ESI-MS), to achieve this effect. The device facilitates the rapid mixing of the enzyme (chymotrypsin) and substrate (p-NPA in D<sub>2</sub>O) immediately prior to online ESI. The incorporation of D<sub>2</sub>O allows us to detect the change in mass, after H/D exchange. By investigating the changes in mass over time, a dynamic mode associated with a specific intermediate step in the reaction mechanism steps was identified. This suggests, in contrast to the findings of previous studies, that enzyme dynamics are different in the presence and absence of substrate.

**“Quantitative lipidomics of K562 leukemic cells infected with Vesicular Stomatitis Virus”**

***Shira Joudan, Lennart Trouborst and Jeffrey C. Smith (Carleton)***

In addition to providing structural support, it has been demonstrated that membrane lipids play a role in cellular signalling. Cancer, diabetes and neurological diseases are known to alter lipids and their metabolic pathways, creating vast opportunities for lipidomic research. Recent studies have shown that Vesicular Stomatitis Virus (VSV) causes selective apoptosis in malignant tumours, yet the mechanism remains unknown. Lipids from an erythroleukemic cell line, K562, were extracted using the Bligh and Dyer method. A separate culture of K562 cells were infected with VSV and incubated at 37°C for 60 minutes. The phosphocholine content of each sample was independently separated using reversed phase HPLC and analyzed by ESI-MS/MS. Data were

analyzed to decipher relative differences in phosphocholine content of treated and untreated K562 cells.

(Poster)

**“Detection of microRNAs by CE-ESI-MS”**

***Nasrin Khan, Gleb Mironov and Maxim V. Berezovski (University of Ottawa)***

MicroRNAs have been identified in normal and malignant cells and are frequently deregulated in cancer cells. The deregulation of several, specific miRNAs produces a miRNA fingerprint that can distinguish between cancerous and non-cancerous cells. Here we introduce a capillary electrophoresis online coupled to a mass spectrometer with electrospray ionization (CE-ESI-MS) for detection of multiple unlabeled miRNAs. The limit of detection is ~1 nM. This method can be applied for quantitative detection of native miRNAs in blood serum and cell lysates.

**“Atmospheric Pressure Chemical Ionization Mass Spectrometry of Volatile Organic Compounds, Potential Disease Bio-markers in Breath”**

***Vitaliy Kapishon, Gregory K. Koyanagi and Diethard K. Bohme, York University***

Atmospheric pressure chemical ionization – mass spectrometry (APCI-MS) is a well-established analytical technique for the detection and measurement of volatile organic compounds (VOCs) and provides real time analysis of trace VOCs. This tool has recently become popular in the medical diagnostic field, since VOCs in exhaled breath can provide information about human health since they are biomarkers for numerous diseases. In this study we tested several VOCs (acetone, 1-propanol, pyridine, isoprene), known biomarkers, with a triple-quadrupole API2000 mass spectrometer, with the goal of understanding the chemistry of various VOCs in an APCI source and also to determine detection limits and quantitative capabilities. Since human breath is humid, and ideal real-time analysis would involve direct online breath sampling, a humidification system was designed in order to assess effect of humidity on instrument performance. It was found that most VOCs in this study could be detected in low-ppb levels but behaved non-linearly during calibration due to technical limitations and several other factors. For example, humidity by itself was found to have different effects on different VOCs, depending on the ionization chemistry of a particular VOC in the APCI. APCI-MS is a promising analytical tool in breath analysis with exceptional detection abilities, yet requires a highly controlled environment to allow online VOC quantification.

Poster

**“Techniques of Salt Suppression in Biological Mass Spectrometry”**

**Gregory K. Kovanaqi, Voislav Blagojevic and Diethard K. Bohme (York University)**

The quantitative analysis of biological samples, by mass spectrometry, is often hindered by the presence of high 'salt' concentration. Typically the salts are present to create representative liquid phase conditions for biological activity or as part of a medium for an orthogonal chromatographic method. Currently, we are investigating the use of Differential Mobility Spectrometry and Fused-Droplet Electrospray Ionization, individually and in tandem, to improve signal-to-noise ratios and to lower detection limits on small molecules and peptides/proteins eluted from a preceding Kinetic Capillary Electrophoresis experiment.

**“Exploring Amyloidogenic Surfaces on Acylphosphatase from *Sulfolobus solfataricus* (Sso Acp) in the Native and Native-like State”**

**Yanfang Liang, Tamanna Rob, Derek J. Wilson (York)**

Some proteins aggregate following partial unfolding, however it has recently been found that some other folded proteins can aggregate following pathways in which unfolding is not the first step. It is very important to study the mechanism of formation of potentially toxic oligomers which adopt initially a native-like folded conformation prior to aggregation. An ideal system with which to study the formation of pre-fibrillar aggregates under conditions is the acylphosphatase from *Sulfolobus solfataricus* (Sso AcP) since Sso AcP remains in a native-like conformation when placed in aggregating conditions and that such a native-like structure persists when the protein forms the initial aggregate. Here we study native and native-like conformation of Sso AcP by H/D exchange in millisecond scale using an integrated microfluidic device. Our experimental data reveals that the deuterium exchange level distribution is different between the native and native-like of Sso AcP in some regions.

**“Measuring Kinetic Isotope Effects Using Time-resolved Electrospray MAss Spectrometry”**

**Peter Liuni and Derek J. Wilson (York)**

Kinetic Isotope Effects (KIE's) give the unique ability to infer transition state structure and geometry from quantifiable changes in reaction rates by isotopic substitution. In order to measure KIE's that are directly related to the transition state of an enzyme, it is important to isolate the intrinsic bond-breaking step(s) involved in substrate to product conversion. Time-Resolved Electrospray Ionization Mass Spectrometry (TRESI-MS) provides the time resolution necessary to observe enzymatic reactions in the pre-steady state regime, which

eliminates contributions from other kinetically irrelevant steps. ESI-MS offers a simplified way to accurately detect subtle changes in substrate, enzyme, and intermediate populations by simultaneously monitoring variations in their mass to charge ratios. Measuring KIE's directly by online mass spectrometry for the reaction of chymotrypsin with  $^{12}\text{C}/^{13}\text{C}$  labelled substrate and the hydride transfer step in yeast alcohol dehydrogenase (YADH) with protiated/deuterated substrate is the focus of this work.

Poster

**"Threshold photoelectron spectroscopy of PAHs and Dihydro PAHs"**

***Paul Mayer, Christine Joblin and Valerie Blanchet (University of Ottawa)***

Imaging photoelectron photoion coincidence spectroscopy was used to derive threshold photoelectron spectra of naphthalene, anthracene, pyrene, 1,2-dihydronaphthalene and 9,10-dihydroanthracene. Agreement between experimental orbital IEs and Green's Function calculations are quite good. Vibrational detail is observed for the PAHs and 1,2-dihydronaphthalene. Accurate adiabatic IEs have been added for the two dihydro PAHs.

**"Kinetic Capillary Electrophoresis – Ion Mobility Mass Spectrometry (KCE-IM-MS) for Studying Conformational Changes of Nucleic Acid-Metal Complexes"**

***Gleb Mironov and Maxim V Berezovski (University of Ottawa)***

Kinetic Capillary Electrophoresis (KCE) coupled with Ion Mobility Mass Spectrometry (IM-MS) is used to study interactions between DNA/RNA with metal ions with formation of G-quadruplexes. KCE reveals affinity of the complexes and kinetics of the complex formation and decay in solution. IM-MS tracks conformational changes of the complexes in gas phase. In this study two DNAs with the same nucleotide proportion but different sequences are used: an anti-thrombin aptamer (ATA) and a scrambled DNA (SCR). It is shown that ATA forms a complex with potassium with half-life time  $\sim 0.5$  sec and  $K_d \sim 100 \mu\text{M}$ . Potassium-ATA complex has smaller collision cross section and Stokes radius than free ATA sequence.

Poster

**"Kinetic Capillary Electrophoresis - Mass Spectrometry (KCE-MS) reveals stoichiometry, rate and equilibrium constants of biomolecular noncovalent interactions"**

***Gleb Mironov, Jennifer Logie, Victor Okhonin, and Maxim V. Berezovski (University of Ottawa)***

Stoichiometry and kinetics of noncovalent interactions is of a great interest in bio-analytical chemistry and pharmacology. The purpose of this research was to measure binding parameters for noncovalent complexes between a small molecule drug (SM) 4,4'-(Propane-1,3-Diyl) Dibenzoic Acid (PDDA) and  $\beta$ -cyclodextrin (CD) using Equilibrium Capillary Electrophoresis of Equilibrium Mixtures (ECEEM) and mass spectrometry (MS). ECEEM is one of Kinetic Capillary Electrophoresis (KCE) methods studying noncovalent molecular interactions during electrophoretic separations. Equilibrium and rate constants can be determined by using time propagation pattern and peak shapes during separation with varying concentrations of cyclodextrin. In our work, we developed a math model to calculate the relative change in signal intensity of various ions in MS with changes in electrophoretic mobilities of the drug and its complexes in ECEEM. Both ECEEM and MS data were accounted to ensure accuracy and precision of math calculations. ECEEM experiments provide the weighted-mean or apparent equilibrium constant (Kd), while MS makes able to calculate exact Kds for different complexes.

**"IRMPD Spectroscopic Study of Solvated  $[\text{Ca}(\text{AlaGly-H})]^+$ ,  $[\text{Ca}(\text{GlyAla-H})]^+$ ,  $[\text{Na}(\text{AlaGly})]^+$ , and  $[\text{Na}(\text{GlyAla})]^+$  Complexes in the Gas Phase"**

***Maryam B. Moqaddam and Travis D. Fridgen (Memorial University of Newfoundland)***

Interaction of metal ions with amino acids and peptides occur in nature so the study of the their complexes is of great importance. Isomeric dipeptides with equal mass can not be identified by means of mass spectrometry technique. In one very recent study protonated AlaGly and GlyAla were separated and characterized by differential mobility spectrometry.<sup>1</sup> It is important, therefore, to try to determine if there are any major structural differences in isomeric peptides which can account for their being separated by differential mobility spectrometry. In this study, the structures of  $[\text{Ca}(\text{AlaGly-H})(\text{H}_2\text{O})_n]^+$ ,  $[\text{Ca}(\text{GlyAla-H})(\text{H}_2\text{O})_n]^+$  ( $n=1, 2, \text{ or } 3$ ), as well as  $[\text{Na}(\text{AlaGly})(\text{H}_2\text{O})]^+$  and  $[\text{Na}(\text{GlyAla})(\text{H}_2\text{O})]^+$  ion-molecule complexes were investigated with infrared multiple photon dissociation (IRMPD) spectroscopy. Also theoretical methods were used to determine the lowest energy structures of these complexes. The experimental spectra were compared with those predicted by the electronic structure calculations. Both experimental and theoretical studies showed that in the calcium clusters, both carboxylate oxygen atoms and the amide carbonyl oxygen interact with the calcium ion. Similarly, all water molecules bind to  $\text{Ca}^{2+}$  directly. In the case of  $\text{Na}^+$  clusters the nitrogen atom in terminal  $-\text{NH}_2$  and oxygen in  $-\text{C}=\text{O}$  group as well as the water molecule were bound to the sodium ion to form the lowest energy structure. In the case of  $\text{Ca}^{2+}$  clusters absence of O-H stretching of the carboxylic acid around  $3550 \text{ cm}^{-1}$  was evidence that the carboxylic acid is deprotonated and oxygen atoms participated

in cluster formation. As expected, the carboxylic acid O-H stretch absorption is observed around  $3550\text{ cm}^{-1}$ .<sup>2</sup>

1. V. Blagojevic, A. Chramow, B. B. Schneider, T. R. Covey, D. K. Bohme, *Anal. Chem.*, 2011, 83 (9), pp 3470–3476
2. C.G. Atkins, L. Banu, M. Rowsell, V. Blagojevic, D.K.Bohme and T.D. Fridgen, *J. Phys. Chem. B* 2009, 113, pp 14457–14464.

#### **“The Study of Electron Transfer and Bond Insertion Involving Metal Anions”**

**Alex Mungham, Julie Roy and Paul Mayer (University of Ottawa)**

A triple quadrupole mass spectrometer was used to investigate gas-phase collisions involving in-source electrospray generated metal anions with different volatile target gases. Insertion of the metal anions into acetonitrile and nitromethane along with the electron transfer from the metal anions to these collision gases were observed. The collision products were analyzed through the scanning of the final quadrupole. The comparison between the behaviours of the different generated metal anions with added translational energy colliding with the target gases was further analyzed and is thoroughly discussed.

#### **“Characterization and Quantification of free Fatty acids and triacyl glycerol during the development of Onopordum acanthium seeds”**

**Moufida Queslati and Paul Mayer (University of Ottawa)**

A comparative study was performed to determine the fatty acid (FA) and triacylglycerol (TAG) compositions during the development of onopordum seeds. The analysis of the two fractions, FA by GC/MS and TAG by electrospray-ionisation mass spectrometry (ESI-MS) showed the presence of fourteen free FA and twenty three TAG. By comparing both compositions, we identified the richness of the onopordum seeds in linoleic acid (C18:2) which coincided with the major TAG; Triacyl-linoleic-Glycerol (LLL).

(Poster)

#### **“Fragmentation pathway of 1-naphthalenol and 2-naphthalenol”**

**Melanie Ouillette, Mahta Ghosssi, Brandi West and Paul M Mayer (University of Ottawa)**

Preliminary data of two PAHs, 1-naphthalenol and 2-naphthalenol, collected using a modified VG-ZAB. Collision-induced dissociation using helium is performed on both PAHs. Possible

structures of the products based on preliminary data are shown.

**“A meticulous investigation of the effects of substrate structure and charge state upon the energetics and conformations of non-covalent complexes”**

***Justin Renaud and Paul M Mayer (University of Ottawa)***

A series of 51 non-covalent complexes between the synthetic polymer poly(methylmethacrylate) and different diaminoalkanes was examined using tandem mass spectrometry (MS/MS), ion-mobility spectrometry(IMS), and molecular dynamics(MD). MS/MS of each non-covalent complex was fit using RRKM unimolecular rate theory to better understand the energetics of these complexes, while MD and IMS were used to investigate the complex conformations. An interesting interdependent role of substrate structure and charge state was identified.

**“Structurally Resolved Snapshots of Protein Dynamics on Millisecond Time-scale using a Microfluidic Device incorporating TRESI, HDX and Rapid Proteolytic Digestion”**

***Tamanna Rob, Shaolong Zhu, Preet Kamal Gill and Derek Wilson (York University)***

Transient or weakly populated protein conformations play an important role in diseases such as Alzheimer’s, Parkinson’s and Type II diabetes. Recently, our group has integrated TRESI and rapid proteolytic digestion in a single microfluidic device for time-resolved, spatially resolved HDX measurements. This unique device enables us to collect a detailed time course (42 ms to 10 sec) HDX profile of DAHP synthase and Cytochrome C native state conformational dynamics. The relative D incorporations of peptides were correlated with the native state conformation of protein for spatially resolved profile of proteins. 14 peptides of Cytochrome C were identified with average of 8 residue spatial resolution. The observed dynamics are consistent with the NMR-based results reported by Englander in 1993[1]. We have identified 45 peptides from DAHP synthase yielding 56% sequence coverage. Our results indicate that in the absence of substrate, the secondary structures of DAHP synthase are well established at the tetramer interface, but weakly established around the active site.

Plenary

**“From diatomic molecules to eight thousand two hundred and three-atomic molecules:  
MS-based biomolecule analysis at the Trent Conference on Mass Spectrometry over the past  
decade: A tutorial and perspective by Jeffrey C. Smith”**

**Jeffrey C. Smith (Carleton)**

Over the past ten years, the Trent Conference on Mass Spectrometry (TCMS) has consistently proven to be an educational and stimulating forum for Canadian and international mass spectrometry scientists to discuss the latest trends in their field. Having a predominantly small molecule gas-phase ion chemistry focus early on, there have been an increasing number of presentations given over the past decade that have applied gas phase chemistry to investigate large biological molecules. In particular, the growing field of proteomics has introduced a host of new mass spectrometry and ancillary techniques, bridging the gap between chemistry, biochemistry and biology. This lecture will provide the audience with a historical perspective of proteomics at the TCMS and offer a tutorial on how mass spectrometry is used to study proteins and lipids.

**“Towards automating quantitative analyses of blood plasma lipids”**

**Lennart Trouborst, Shira Joudan and Jeffrey C. Smith (Carleton)**

Lipids have recently been found to play important roles in cellular functions beyond those of structure and energy storage, including cell signalling. Lipid molecules that vary in concentration between healthy and unhealthy cells represent potential candidates as disease biomarkers. Blood plasma lipids from several healthy patients have been analyzed using LC-MS/MS to establish a baseline lipidome; to this, blood plasma lipids from unhealthy patients have been compared in order to uncover potential disease biomarkers. Our analytical strategy generates large datasets which require many hours for manual analysis; therefore, methods to automate our data pipeline are concurrently being pursued.

**“Novel microfluidic methods to methylate peptides and permit quantitative analysis using  
tandem mass spectrometry”**

**Karl Wasslen and Jeffrey C. Smith (Carleton)**

Many of the current methods to quantitate peptides using mass spectrometry are expensive. The goal of this project is to optimize inexpensive on-column chemistry using diazomethane to permit peptide methylation and quantitation. This strategy will produce trimethylated peptide N-termini which fragment in MS<sup>2</sup> to preferentially form a<sub>2</sub> fragment ions permitting highly sensitive

analyses. Peptide methylation was tested in solution, on dry analyte and on a chromatographic resin; the latter technique provided the best results. MS<sup>2</sup> analysis confirmed that both tri- and tetramethylated hexaglycine fragmented to form the a<sub>2</sub> ion, demonstrating that peptide N-termini are methylated prior to carboxylic acid groups in our experimental setup. Recent experimental optimization has led to 100% conversion of peptides to a<sub>2</sub> ion-forming products.

### **"Energetics of dissociation of PAHs and Dihydro-PAHs"**

***Brandi West, Paul Mayer, Christine Joblin, Valerie Blanchet, Andras Bodi and Balint Sztaray  
(University of Ottawa)***

Imaging photoelectron photoion coincidence spectroscopy was used to construct breakdown curves for naphthalene, anthracene, 1,2-dihydronaphthalene and 9-10,dihydroanthracene. These breakdown diagrams were then fit using RRKM calculations in order to extract energetics data. There is good agreement between experiment and calculations and initial trends have been observed.

### **"Quantification of low abundance plasma proteins by immunoaffinity purification, reverse phase nanoflow chromatography and selected reaction monitoring"**

***Declan Williams, Leroi V. DeSouza, and K. W. Michael Siu (York University)***

Techniques for the quantification of plasma proteins have applications in the development of medical diagnostics and therapies. The mass spectrometry of plasma proteins is impeded by the diversity of matrix components and their wide range of concentrations. Combining immunoaffinity-based sample preparation with targeted mass spectrometry of tryptic peptides provides sensitivity and specificity for the quantification of plasma proteins considered to be of low abundance with throughput suitable for large scale studies. Here an assay for chaperonin 10 (cpn10), an intracellular and circulating protein with diagnostic and therapeutic utility, based on batch immunoaffinity chromatography with analyte detection by selective reaction monitoring (SRM) is described. The method was applied to normal human serum spiked with recombinant cpn10 at physiologically significant plasma concentrations as well as lysates of the cell line Hec1a, a source of endogenous cpn10. Samples were analyzed on two high performance liquid chromatography-mass spectrometry platforms, a cHiPLC-QTRAP 5500 and a TempoLC-QTRAP 4000 (MDS Sciex). The lower limit of detection of the QTRAP 4000 system for tryptic cpn10 peptides in aqueous solution and in the serum preparations was 50 fmol (55 ng) and 1 ng/ml respectively whereas a concentration of 109 ng /ml was required for analyte detection in serum depleted of 20 high abundance proteins. Sensitivity was dependent on the immunoprecipitation substrate, antibody concentration, washing conditions, reversed phase chromatography parameters, SRM transitions and the analytical platform used. Sample

contamination was evaluated in parallel with analyte detection by targeting tryptic peptides of the abundant plasma proteins clusterin and albumin with unique SRM transitions.

**“Characterizing the Resolution and Accuracy of a Second-Generation Traveling-Wave Ion Mobility Separator for Biomolecular Ions”**

***Yueyang Zhong, Suk-Joon Hyung, and Brandon T. Ruotolo (University of Michigan)***

High-accuracy, high-resolution ion mobility measurements enable a vast array of important contemporary applications in biological chemistry. With the recent advent of both new, widely available commercial instrumentation and also new calibration datasets tailored for the aforementioned commercial instrumentation, the possibilities for extending such high performance measurements to a diverse set of applications has never been greater. Here, we assess the performance characteristics of a second-generation travelling-wave ion mobility separator, focusing on those figures of merit that lead to making measurements of collision cross-section having both high precision and high accuracy. Through performing a comprehensive survey of instrument parameters and settings, we find instrument conditions for optimized drift time resolution, cross-section resolution, and cross-section accuracy for a range of peptide, protein and multi-protein complex ions. Moreover, the conditions for high accuracy IM results are significantly different from those optimized for separation resolution, indicating that a balance between these two metrics must be attained for traveling wave IM separations of biomolecules. We also assess the effect of ion heating during IM separation on instrument performance.

—Withdrawn—

**“Analysis of protein dynamics by H/D exchange using FORTRAN language”**

***Shaolong Zhu and Derek J. Wilson (York)***

H/D exchange (HDX) is a chemical reaction whereby N-H, O-H and S-H bonds in a protein can be exchanged with deuterium atoms. HDX used to study the dynamics of the protein folding pathways as well as their conformational changes. However two kinds of exchanges could occur, fast and slow exchange. Fast exchange occurs in the regions of the protein that are exposed to the deuterium solvent such as the side chains or end terminals whereas the slow exchange occurs in the protected regions of the protein which are usually the backbones of the peptides. This project was to create a program using FORTRAN language to calculate the theoretical percentage of deuterium exchange on “backbones” of peptides/proteins from experimental spectra. Slow exchangeable sites are studied because they rarely go through back exchange (exchange back to hydrogen) unlike the fast exchangeables. The program is made based on the convolution of the natural isotopic distribution with the binomial distribution of the slow exchangeable hydrogens.