Colleagues,

On behalf of the National High Magnetic Field Laboratory and Florida State University, we welcome you to the 10th North American FT MS Conference! We hope that your stay in Key West will be personally and professionally rewarding. Topics span a broad range of techniques and applications. Posters will remain up throughout the meeting, to encourage discussions.

The primary effort for organizing this conference has been provided by our administrative staff, Karol Bickett and Krista Jemmott. They have done an excellent job with the many required logistical and personal arrangements.

Your registration fee covers only part of the expenses of this conference. The generous contributions of our sponsors have kept the meeting costs affordable for participants, and made it possible for us to assist with the expenses of the invited speakers and the numerous graduate student poster presenters. Please take an opportunity to thank our participating sponsors at their display tables.

Thank you for joining us, and we look forward to a splendid conference!

Sincerely,

Alan G. Marshall
Robert O. Lawton Professor of Chemistry & Biochemistry, FSU
Chief Scientist, Ion Cyclotron Resonance Program, NHMFL
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• Name tags must be worn at all times during the conference, including the oral sessions, the poster sessions, all mixers, all breaks and all meals.

• Paid accompany guests are welcome to the opening mixer, the dinner Monday night and the banquet dinner Wednesday night. Name tags must be worn at these events.

• Oral and poster sessions will be held in the Flagler Ballroom.

• Please see conference program agenda for location of meals and breaks.

• This is a non-smoking facility. Smoking is not permitted inside the buildings.

• Photography is not permitted at the oral or poster sessions.

• All cell phones must be turned off or set to vibrate during all oral sessions. Courtesy is expected.

• A message board is located by the conference registration table.

• Posters may be set up beginning on Sunday, April 12th between 5:00 p.m. and 10:30 p.m. All posters should be in place by Monday, April 13th at 5:00 p.m. Posters must be removed no later than Wednesday, April 15th at 1:00 p.m. Please see the conference program for the appropriate poster number. Any posters remaining after 3:30 p.m. on Wednesday, April 15th will be discarded.

• Speakers may review talks prior to sessions. Please see Greg Blakney as early as possible but, no later than, one session break prior to your scheduled talk. If you are the first talk in the morning, please see Greg the evening prior.

• There is wireless available in the hotel. No password is required. Additionally, there is hard wired internet in the lodging rooms.

• Those leaving early from the conference, but after hotel check out, may store their luggage with hotel bell staff.

• Downtown shuttle from the hotel begins at 8:00 a.m. and every hour on the hour and drops off at Margaritaville. Return from Margaritaville to the hotel at 20 past every hour. Last shuttle return from Margaritaville is at 11:20 p.m.
Jean H. Futrell

Jean Futrell is internationally recognized for his pioneering research in mass spectrometry fundamentals. A principal focus is reaction kinetics and reaction dynamics of ions; his research initially emphasized ion-molecule reactions and now emphasizes high energy ion-neutral and ion-surface collisions. Dr. Futrell has a particular interest in development or significant modification of mass spectrometry instrumentation for specialized research purposes. These have included high-pressure chemical-ionization mass spectrometers, tandem, triple-quadruple instruments, tandem ion-cyclotron resonance mass spectrometers, very high resolution FTICR, and molecular beam instruments. Collision-induced dissociation of polyatomic ions and electron-transfer reaction dynamics are topics of special interest. Recent research extends these concepts to ion-surface collisions of complex ions, their recoil excitation and soft-landing.

Jean obtained his B.S. in Chemical Engineering at Louisiana Polytechnic Institute and, earned his M.S. and Ph.D. degrees in Physical Chemistry at the University of California (Berkeley). He joined PNNL as the first Director of DOE’s Environmental Molecular Sciences Laboratory (1998-2002) and in 2002 was appointed Battelle Fellow. In 2013, he was appointed Battelle Fellow Emeritus. He also served as chairman and Willis F. Harrington Professor of Chemistry, Biochemistry and Chemical Engineering at the University of Delaware, Professor of Chemistry at the University of Utah and, Air Force officer and civilian scientist at Wright-Patterson Air Force Base.

Jean was awarded the FH Field and JL Franklin Award for Outstanding Achievement in Mass Spectrometry by the American Chemical Society in Mass Spectrometry, the German Mass Spectrometry Society’s Wolfgang Paul Distinguished Lecturer, the PNNL Director’s Award for Lifetime Achievement in Science and Technology, the Erwin Schroedinger Gold Medal by the International Symposium on Atomic and Surface Physics, was was in the honor issue of International Journal of Mass Spectrometry and Ion Physics and, was the first American honored by special issue of the European Journal of Mass Spectrometry and Honor Symposium in Konstanz, Germany.
10th North American FT MS Conference

KEY WEST MARRIOTT BEACHSIDE HOTEL
Key West, Florida
April 12 – 16, 2015

Conference Program

Sunday April 12

5:00 p.m.-10:00 p.m. Registration – Flagler Foyer

Session I. Flagler Ballroom

7:00 p.m. Welcome: Alan Marshall
National High Magnetic Field Laboratory/Florida State University

7:05 p.m. Joe Loo
University of California-Los Angeles
“Linking Tandem MS of Native Proteins and Complexes to Structural Biology”

7:35 p.m. Carol Nilsson
University of Texas Medical Branch at Galveston
“High Resolution Mass Spectrometry and Bioinformatics Elucidate the Dark Matter of the Human Proteome”

(There will be a mixer following the last speaker in the Flagler Terrace/Foyer)

Monday April 13

8:00 a.m. - 8:55 a.m. Breakfast – Flagler Terrace/Ballroom
Registration Open – Flagler Foyer

Session II. Flagler Ballroom

9:00 a.m. Liz Kujawinski
Woods Hole Oceanographic Institute
“Mass Spectrometry-Based Metabolomics Reveals New Metabolic Pathways for Carbon and Petroleum Remineralization”

9:30 a.m. Michael Gross
Washington University
“Top Down, FTICR MS, and Ion Mobility for MS-Based Biophysics”

10:00 a.m. Chris Hendrickson
National High Magnetic Field Laboratory

10:30-11a.m. Break – Flagler Foyer/Terrace
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<td>11:00 a.m.</td>
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|              | Evan Williams  
*University of California, Berkeley*  
“Charge Detection Mass Spectrometry for Single Ions” |
| 11:30 a.m.   | Jon Amster  
*University of Georgia*  
“FTMS and Its Advantages for GlycoseAminoGlycan Characterization” |
| 12:00-1:00 p.m. | Lunch – Flagler Terrace/Ballroom                                                              |
| 1:00 p.m.    | **Session IV. Flagler Ballroom**                                                              |
|              | Mike Westphall  
*University of Wisconsin*  
“High-Resolution Measurements of the Metabolome and Proteome” |
| 1:30 p.m.    | Jarrod Marto  
*Dana Farber Cancer Institute, Harvard University Medical School*  
“Systematic Interrogation of Phosphatase Biology Using Quantitative Proteomics” |
| 2:00 p.m.    | Thomas Oldenburg  
*University of Calgary*  
| 6:00-7:15 p.m. | Dinner- Flagler Ballroom                                                                     |
|              | **Sponsor Presentations. Flagler Ballroom**                                                 |
| 7:15-7:30 p.m. | Announcements                                                                               |
| 7:30-7:45 p.m. | ThermoFisher                                                                                 |
| 7:45-11:00 p.m. | Poster Presentations                                                                         |

**Tuesday April 14**  
8:00 a.m. - 8:55 a.m.  
Breakfast – Flagler Terrace/Ballroom  
Registration Open – Flagler Foyer

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|              | Livia Eberlin  
*Stanford University*  
“Visualizing Cancer Tissue Metabolism by High Resolution Mass Spectrometry Imaging” |
9:30 a.m.  Forest White  
M.I.T.  
“High Resolution Analysis of Receptor Tyrosine Kinase Signaling Networks”

10:00 a.m.  Jim Bruce  
University of Washington  
“Development of an Ion Cyclotron Resonance Mass Spectrometer Array”

10:30-11 a.m.  Break – Flagler Foyer/Terrace

Session VI.

11:00 a.m.  Lissa Anderson  
National High Magnetic Field Laboratory  
“Analyses of Intact Histones Using a Front-End Electron Transfer Dissociation Enabled Orbitrap Velos Pro”

11:30 a.m.  Yury Tsybin  
Ecole Polytechnique Federale de Lausanne  
“Toward Next Generation FTMS: High Resolution, Accurate, and Fast”

12:00 p.m.  Conference Photo on the beach

Open afternoon to explore Key West

Wednesday April 15  
8:30 a.m.-9:25 a.m.  Breakfast – Flagler Terrace/Ballroom  
Registration Open – Flagler Foyer

Session VII.  Flagler Ballroom

9:30 a.m.  Roman Zubarev  
Karolinska Institutet  
“Monoisotopic Proteomics”

10:00 a.m.  Ljiljana Pasa-Tolic  
Pacific Northwest National Laboratory  
“FTMS for Functional Characterization of Microbial Proteins”

10:30 a.m.  Michael Freitas  
The Ohio State University Medical Center  
“Peering into the Functional Role of Linker Histone Modifications Through the High Resolution “Lens” of Fourier Transform Mass Spectrometry”

11-11:30 a.m.  Break – Flagler Foyer/Terrace
11:30 a.m. student (selected from poster presentations)

11:50 a.m. student (selected from poster presentations)

12:10 a.m. student (selected from poster presentations)

12:30-2:00 p.m. Lunch – Flagler Terrace/Ballroom
ICR Facility Advisory Panel meeting – Private Dining Room

Session VIII. Flagler Ballroom

2:00 p.m. Philip Compton
*Northwestern University*
“Proteomics at the Next Hierarchical Level in Protein Organization”

2:30 p.m. Helen Cooper
*University of Birmingham, UK*
“What’s at the Surface? Latest Developments in LESA Mass Spectrometry of Biological Substrates”

3:00 p.m. Eugene Nikolaev
*Institute for Energy Problems of Chemical Physics, Moscow*
“Two Topics: The New Non-FT Method of ICR Mass Spectrometry, Based on Measuring of “Antenna” Ions Cyclotron Frequency Time Dependence and, Is Dynamically Harmonized Cell Harmonized Statically”

3:30-5:15 p.m. Off

Session IX. Flagler Ballroom

5:15-5:30 p.m. Final Recap – Alan Marshall

5:30-6:30 p.m. Jean Futrell
*Pacific Northwest National Laboratory*
“Five Decades of Invention in Fifty Minutes”

6:30 p.m. Depart to Hemingway House

7:00-11:00 p.m. Conference Banquet and Final Mixer
Hemingway House

Thursday April 16

Checkout and Depart by 11:00 a.m.
SPEAKERS
FIVE DECADES OF INVENTION IN FIFTY MINUTES

Jean Futrell

Battelle Fellow Emeritus, Pacific Northwest National Laboratory, Battelle Boulevard, Richland, WA

The second half century of mass spectrometry and its central role in advancing our understanding of the molecular sciences reflects the close-coupling of advances in instrumentation and novel application of new capabilities enabled by these advances. This conference has presented important new examples of novel instruments and new applications, including early results from 21-Tesla FTICR. In marked contrast to these advances, this retrospective describes some early work of the author and his collaborators in attempting to couple initially incompatible ion sources and mass analyzers.
Advanced mass spectrometry (MS) has capabilities to offer structural biologists layers of insight into the details of protein complexes. Mass measurements deliver information on stoichiometry of binding partners directly, even for multi-ligand hetero-complexes and molecular machines with masses well beyond 1 MDa. With electrospray ionization (ESI), MS can measure proteins and complexes from aqueous solution at near neutral pH, i.e., “native” MS. ESI’s gift for transforming solution-phase macromolecules into gas-phase ionized counterparts without disrupting covalent bonds and weak noncovalent interactions is key for applying MS to study protein complexes.

Top-down mass spectrometry can be an effective tool for protein sequencing. We use top-down high resolution Fourier transform ion cyclotron resonance (FT-ICR) MS to probe ligand-binding sites and to generate topological information for large proteins and complexes. We are using electron capture dissociation (ECD)/FT-ICR MS to investigate the molecular action of compounds that prevent amyloid fibril formation in neurodegenerative diseases such as Alzheimer’s and Parkinson’s disease. Other means for activation/dissociation, including IRMPD, UVPD, and EID generate complementary structural information. Native top-down MS generates information on the surface topology, ligand binding sites, and post-translational modifications of protein complexes and membrane proteins. We aim to relate the 3D architecture of the gas phase protein to the solution phase state as a means to further develop MS for structural biology. Native top-down MS should be one of several approaches to provide important data for an integrated approach to structural biology.

Support from the US National Institutes of Health (R01GM103479, S10RR023045, S10RR028893), the US Department of Energy (DE-FC-02-02ER63421), the American Society for Mass Spectrometry Postdoctoral Award Program (to HL), and the Development and Promotion of Science and Technology Talents Project (DPST), Royal Thai Government (to PW) are acknowledged.

HIGH RESOLUTION MASS SPECTROMETRY AND BIOINFORMATICS ELUCIDATE THE DARK MATTER OF THE HUMAN PROTEOME

Carol L. Nilsson¹

¹The University of Texas Medical Branch, Department of Pharmacology and Toxicology, Galveston, TX

High sensitivity, high mass accuracy mass spectrometry in combination with improved bioinformatics have transformed the way we examine biological and biomedical questions. With modern analytical tools, proteome-wide studies of protein post-translational modifications and single amino acid variants (SAVs) are enabled¹. Expression levels can be accurately quantified by single reaction monitoring (SRM). Furthermore, with the recent integration of the ENCODE data into our proteomic workflow, we are positioned to identify the unknown human proteins at an accelerated pace². By use of databases that contain predicted sequences of known and novel proteoforms, we identified dozens of SAVs in proteins derived from glioma stem cells that may be associated with genomic instability and invasivity, and novel alternative spliceforms derived from fusions between known and previously unknown exons¹,³. The identification of an overexpressed metabolic protein variant, BCAT2 T186R, has opened up studies of a target never associated with cancer pathology. The integration of RNA-Seq data and proteomics has allowed us to study the somatic-proteomic landscape of GSCs, thereby determining the roles of SAVs and novel fusion proteins in GSC pathobiology. Bioinformatic searches of ENCODE data translated into a searchable database for proteomics (proteoENCODE db) yielded eighty-one chimeric peptides in GSCs, the result of fusion of known and unknown exons. The identification of novel proteins and proteoforms in GSCs will allow further studies of their role in pathogenesis, tumor recurrence, and resistance to chemotherapy and radiation. The next step is to validate and quantify the expression of 225 SAVs detected in our samples by SRM across our biobanked cell lines, at baseline conditions and following standard-of-care treatments. We expect that among the newly identified proteins, new therapeutic targets (“driver” SAVs) and biomarkers will be established. Our results are enabled by high resolution mass spectrometric methods and the development of databases that encompass the complexities of protein features and serve to demonstrate how improved analytical methods have translated into increased ability to produce breakthrough discoveries in cancer research.

MASS SPECTROMETRY-BASED METABOLOMICS REVEALS NEW METABOLIC PATHWAYS FOR CARBON AND PETROLEUM REMINERALIZATION

Elizabeth B. Kujawinski, Krista Longnecker, Melissa C. Kido Soule, Yina Liu

Department of Marine Chemistry & Geochemistry; Woods Hole Oceanographic Institution, Woods Hole, MA

Microbial metabolism plays a primary role in shaping the global carbon cycle and thus fundamentally affects the response of the oceans to environmental perturbations such as oil spills, eutrophication, and changing climate. Prediction of these responses requires quantitative estimates of the salient metabolic rates and an understanding of the factors that govern the relative balance among different metabolic strategies. Gene-based surveys (e.g. [meta-]genomics and transcriptomics) have provided a window into the metabolic potential within marine microbes. Although incredibly important, these data are not easily transformed into the quantitative analysis of metabolic function needed for development of biogeochemical models of the carbon cycle. Metabolomics, or the study of small molecules produced by a cell, can establish the link between genome-encoded potential and realized metabolic function through the identification, and quantification, of intermediates within specific biochemical pathways. These techniques are just now emerging in marine and environmental chemistry but their application shows tremendous potential to elucidate novel pathways and to provide quantitative estimates of biogeochemical reactions. We have recently applied a coupled targeted and untargeted metabolomics approach to the analysis of marine microbial metabolism in axenic laboratory cultures, in mesocosm experiments and in field settings. Together, these data suggest that marine microbes are expressing biochemical pathways in unique ways to effect carbon and petroleum remineralization in the surface and deep oceans. When combined with complementary gene-based data and environmental chemical parameters, metabolic profiling of microbes in laboratory culture and in field settings will lead to transformative insights into our understanding of the molecular reactions at the foundation of the global carbon cycle.
TOP-DOWN, FTICR MS, AND ION MOBILITY FOR MS-BASED BIOPHYSICS

Michael L. Gross, Hao Zhang, Weidong Cui, Ben Niu, Ying Zhang, Yue Lu

Department of Chemistry, Washington University in St. Louis, Box 1134, One Brookings Drive, St. Louis, MO

We are exploring applications of native spray MS with top-down sequencing on a 12-T FTICR and with Q-ToF and ion-mobility technologies. We showed earlier [1,2] that native spray and top-down sequencing can identify the proteins in an assembly and reveal flexible regions in those assemblies, providing complementary information to that of collisional activation and Q-ToF technology. We are expanding these efforts to test application to homogeneous and heterogeneous assemblies, to aggregating proteins, and to antibody/antigen interactions. In a second application, we characterize membrane-associated proteins, which constitute 20-30% of all expressed proteins. Their structure and function are not well understood, and there are limited numbers of high-resolution X-ray crystal structures. We are developing an approach that uses both top-down sequencing and lipid nanodiscs [3] as a “carrier” for membrane proteins to probe membrane-bound protein complexes. One example is an ongoing Nanodisc-based MS study of Vitamin K epoxide reductase (VKOR), a small protein. VKOR is a membrane-embedded enzyme and the target of warfarin, the most widely prescribed oral anticoagulant drug in North America. We assembled a VKOR bacterial homolog into a Nanodisc, and the intact VKOR-nanodisc and directly introduced it by native MS and analyzed it by top-down strategies, mainly collision-induced dissociation and electron-capture dissociation. We seek conformational information of VKOR in the Nanodisc and ultimately in the native membrane in vivo is ongoing by using protein footprinting using NEM labeling of Cys, GEE labeling of Asp and Glu, and Fast Photochemical Oxidation of Protein (FPOP).

21 TESLA FT-ICR MASS SPECTROMETER: A NATIONAL RESOURCE FOR ULTRAHIGH RESOLUTION MASS SPECTROMETRY

Christopher L. Hendrickson¹², John P. Quinn¹, Nathan K. Kaiser¹, Donald F. Smith¹, Greg T. Blakney¹, Tong Chen², Alan G. Marshall¹²

¹National High Magnetic Field Laboratory, 1800 East Paul Dirac Drive, Tallahassee, FL; ²Florida State University, Department of Chemistry, Tallahassee, FL

Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry offers the highest achievable broadband mass resolving power and mass accuracy of any mass analyzer. Resolving power and scan rate improve linearly, and mass accuracy and dynamic range improve quadratically with magnetic field strength, such that resolving power greater than 1 million and mass accuracy better than 1 ppm become routine at sufficiently high magnetic field strength. We report here the design, construction, and characterization of the first 21 tesla FT-ICR mass spectrometer. The instrument is a part of the National High Field FT-ICR User Facility at the National High Magnetic Field Laboratory, and is available to all qualified users.

The 21 tesla magnet (Bruker) features a 123 mm warm temperature bore diameter, less than 5 ppm spatial inhomogeneity over an 60 mm diameter by 100 mm long cylinder, ~3 ppb/hour drift rate, and no cryogen consumption. The magnet cryostat was designed to use no liquid nitrogen and consume no liquid helium. We have observed no helium loss throughout the first nine months of operation. The mass spectrometer combines a Velos Pro (modified for front electron transfer dissociation (FETD)) with an NHMFL-designed external linear quadrupole ion trap, rf quadrupole ion transfer optics, and a novel dynamically harmonized ICR ion trap (DHC).

At 21 tesla, ions of m/z 200-2000 oscillate at 1.6 MHz-160 kHz, which allows high resolving power at high scan rate. For example, the isotopic distribution of bovine serum albumin (BSA, 66 kDa) is resolved by use of a 0.38 second detection interval, which facilitates top-down proteomic analysis of up to 100 kDa proteins by online LC/MS. Longer detection interval generates higher resolving power, with >1 million RP routinely achievable for an isolated charge state of transferrin (78 kDa) or other large proteins.

High magnetic field limits the magnitude of ion cyclotron frequency shifts that occur due to ion-ion and ion-image charge forces, and imperfect magnetic and trapping electric fields. Consequently, mass errors due to variation in trapped ion number scale inversely with the square of magnetic field strength. At 21 tesla, variation in measured masses of a mixture of ten peptides is limited to <150 ppb rms over thousands of measurements (taken over the course of ~1 hour). Single ion monitoring reduces the error to <50 ppb.

Efficient dissociation methods and high magnetic field combine to facilitate rapid, high resolution tandem mass spectra of large proteins. For example, electron transfer dissociation and collisional dissociation combine to produce hundreds of fragment ions of carbonic anhydrase. Overlapping isotopic multiplets are easily resolved, even at high (>1 Hz) scan rate. A barium fluoride window on the ICR cell flange allows future coupling of photodissociation induced by UV (193 or 266 nm), vis (532 nm), or IR (10.6 micron) lasers.

Work supported by the National Science Foundation through DMR-1157490, CHE-1016942, CHE-1019193, and the State of Florida.
CHARGE DETECTION MASS SPECTROMETRY FOR SINGLE IONS

Andrew Elliott, Satrajit Chakrabarty, Evan R. Williams

Department of Chemistry, University of California – Berkeley, Berkeley, CA

Obtaining information about structures of macromolecular complexes can be challenging when the complexes are large and/or heterogeneous. Heterogeneity can be a result of many different factors, including multiple different stoichiometries of the complex, heterogeneity of the molecular constituents within a complex, or the propensity of the complex to adduct salts and/or other molecules in solution. Although the complexes can often be readily ionized, the heterogeneity can lead to incomplete or no separation between the different charge states making assignment of charge states, and hence masses of the ions difficult or impossible. Strategies involving tandem MS have been used to obtain some information when charge state distributions are unresolved. Interferences between ions with overlapping $m/z$ values can be eliminated by weighing individual ions. Individual ions of large synthetic polymers and biopolymers have been measured using both charge detection mass spectrometers and also FT-ICR mass spectrometry [1,2]. Here, results from a new charge detection mass spectrometer that uses multiple pick-up electrodes between cone electrodes to trap and measure individual ions multiple times will be presented. Progress on the development of this instrument and its use to measure the molecular masses of individual ions up to 100s of MDa will be presented.

FTMS AND ITS ADVANTAGES FOR GLYCOSAMINOGLYCAN CHARACTERIZATION

I. Jonathan Amster, Isaac Agyekum, Yuejie Zhao, Morgan Stickney, Jiana Duan

Department of Chemistry, University of Georgia, Athens, GA

Glycosaminoglycans (GAGs) are highly anionic, linear carbohydrates that play an important role in a number of biological processes, generally through specific interactions with proteins, particularly cell surface proteins. The pattern of sulfation is known to play a role in the specificity for a few well-studied cases, but the challenging nature of GAG sequencing is an impediment in advancing our understanding of GAG-protein interactions. FTMS offers many advantages for sequencing GAGs, both because of its high resolution, accurate mass capability, and the wide variety of ion activation methods that can be implemented. These advantages for GAG sequencing will be presented.
HIGH-RESOLUTION MEASUREMENTS OF THE METABOLOME AND PROTEOME

Nicholas W. Kwiecien¹, Anna E. Merrill¹, Matthew J. P. Rush¹, Arne Ulbrich¹, Alexander S. Hebert¹, Michael S. Westphall¹, Joshua J. Coon¹,²,³

¹Genome Center of Wisconsin, University of Wisconsin, Madison, WI; ²Department of Chemistry, University of Wisconsin, Madison, WI; ³Biomolecular Chemistry, University of Wisconsin, Madison, WI

The fundamentals of using Neutron-Encoded Mass Signatures (NeuCode) for multiplexed quantitative proteomics will be described. NeuCode capitalizes on the phenomenon of mass defect wherein differential nuclear binding energies of isotopically heavy atoms incorporated into essential amino acids are used to create subtle mass differences between peptides labeled with these residues. Descriptions and evaluations of NeuCode quantitation using the in-house software package NeuQuant for biological samples prepared at varying ratios analyzed using a number of FTMS instruments will be presented. Also to be described are experiments designed to characterize quantitative accuracy using the NeuCode method. The following points are highlighted: 1) how is NeuCode data collected, 2) how is NeuCode data quantified, 3) what is the quality of this data, 4) what are the limits of NeuCode quantitation, and 5) how does NeuCode perform across multiple systems?

The benefits of high mass resolution to metabolomics will also be highlighted through the examination of data collected using a gas chromatography/Orbitrap mass spectrometer. Gas chromatography/mass spectrometry (GC/MS) is a premiere analytical tool for qualitative and quantitative analysis of volatile organic compounds and is key technology for the emergent field of metabolomics. Harnessing this potential, however, has been challenging for numerous reasons: pseudo-molecular ion information is often not present, co-elution of multiple compounds confounds identification, and the largest spectral libraries exclusively contain low resolution spectra. All of these factors have limited the impact of GC/MS for metabolomic application. A novel combinatorial informatics approach, High-Resolution Filtering (HRF), which exploits the accurate mass capabilities of newly introduced GC/MS discovery platforms will be presented. Our HRF method augments traditional database searching by in silico fragment matching for greatly increased confidence in metabolite identifications.
SYSTEMATIC INTERROGATION OF PHOSPHATASE BIOLOGY USING QUANTITATIVE PROTEOMICS

Jarrod A. Marto

Department of Biological Chemistry and Molecular Pharmacology, Harvard Medical School, Department of Cancer Biology and Blais Proteomics Center, Dana-Farber Cancer Institute, Boston, MA

Phosphorylation signaling networks have primarily been studied from an activation perspective, with phosphatases viewed as simple counter-balances that functioned passively in the wake of kinase activity. However data from recent studies, including quantitative phosphoproteomic approaches, suggest the possibility of an initiating role for dephosphorylation in key cellular processes. The relatively modest number of phosphatase subunits as compared to kinases suggests that phosphatases may rely on a wider repertoire of molecular mechanisms for coordinate regulation. Hence a major challenge is to experimentally capture the diverse molecular architecture that mediates phosphatase activity. Our goal in this work is to utilize genetic, proteomic, and biochemical approaches to systematically identify substrates and binding partners of phosphatases as a starting point to interrogate and define their functional roles in normal cell physiology and human disease.
FTICR-MS – MORE THAN JUST MASS SPECTROMETRY? TOWARDS REACTION SYSTEMS MODELS IN PETROLEUM GEOCHEMISTRY

Thomas B.P. Oldenburg¹², Renzo C. Silva¹, Ryan W. Snowdon¹², Gabriela P. Gonzalez-Arismendi¹, Melisa Brown¹, Steve Larter¹²

¹Petroleum Reservoir Group, Department of Geoscience, University of Calgary, 2500 University Drive NW, Calgary, AB, Canada; ²Aphorist Inc., Calgary, AB, Canada

Over the last five decades, Petroleum Geochemists have developed a great selection of tools and molecular markers, or proxies, to better understand the processes involved in the generation, migration and alteration of petroleum. Unfortunately, several of these proxies are altered during in situ biodegradation in oil reservoirs and are thus, not available for petroleum system studies in many biodegraded oil provinces. Historically, most of the petroleum system proxies used by geochemists are predominantly hydrocarbon in nature, but recent advances in analytical technology, especially Fourier Transform Ion Cyclotron Resonance – Mass Spectrometer (FTICR-MS), now permit routine analysis of non-hydrocarbon species which are commonly not accessible using standard gas chromatography-mass spectrometry (GC-MS) technology. These non-hydrocarbon species, in addition to providing new and more robust petroleum system proxies (Oldenburg et al., 2014), are also the most critical components, to assess in terms of understanding fluid rock interactions and key phenomena such as solid phase wettability, emulsion formation, water washing effects, and petroleum migration to name but a few topical areas. These fluid-solid and fluid-fluid interactions mediated by petroleum non-hydrocarbons can be termed interfacial phenomena and are a badly neglected area for petroleum geochemists.

About a decade ago, a new technology named ultrahigh resolution Fourier Transform Ion Cyclotron Resonance -Mass Spectrometry (FTICR-MS) found its way into petroleum geochemistry. This technology allows the comprehensive analysis of tens of thousands of molecules containing polar functional groups as they occur in crude oil. FTICR-MS is still in its early days and needs further developments especially to improve its rudimentary quantitation capabilities but it is already starting to produce interesting and usable results with practical implications for organic and petroleum geochemists (Oldenburg et al, 2014).

In this presentation, we would like to give an overview of recent advances in improving the quantitation capabilities of FTICR-MS and how to convert the tremendous amount of produced data now available into useful information for applications in the areas of petroleum geochemistry. Recent software developments, a better understanding of the competition of oil constituents for ionization resulting in different response factors and the use of statistical methods such as chemometrics allows us to develop reaction systems models for several new petroleum system proxies for the assessment of oil maturity, source facies and alteration processes such as biodegradation.

VISUALIZING CANCER TISSUE METABOLISM BY HIGH RESOLUTION MASS SPECTROMETRY IMAGING

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Desorption electrospray ionization mass spectrometry imaging (DESI-MSI) has been shown to be particularly powerful for identifying lipids and metabolites directly from tissue sections and providing a chemical map of their distribution. We used high mass resolution DESI-MSI and molecular biology approaches to investigate changes in lipid profiles that occur in animal and human lymphomas associated with the overexpression of the MYC oncogene. Using statistical analysis, we identified 86 lipids that have an altered abundance in MYC-induced transgenic mouse models of lymphoma when compared to normal tissue. Most of the increased lipids were glycerophosphoglycerols and cardiolipins with a higher content of monounsaturated fatty acids when compared to control tissue. We then used DESI-MSI to investigate human cancer tissue metabolism. Interestingly, the lipid profiles of human lymphomas with overexpression of MYC resemble closely those observed in MYC-induced transgenic mouse models. These results suggest that there is a correlation between lipid abundance and MYC oncogene expression in human lymphomas. The lipid species might provide new biological insights into how MYC regulates cellular metabolism in cancer.
HIGH RESOLUTION ANALYSIS OF RECEPTOR TYROSINE KINASE SIGNALING NETWORKS

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The epidermal growth factor receptor (EGFR) is highly expressed or mutated in a wide array of human cancers and plays a critical role in normal human physiology. Signaling network dynamics following EGFR stimulation have been previously reported, through either quantitative tyrosine phosphorylation analysis or through global phosphorylation analysis. Most previous studies have focused on quantification relative to a basal state, but absolute levels of phosphorylation, recorded at high temporal resolution under multiple different stimulation conditions, should enable the identification of kinetic parameters governing network activation. To this end, we have developed mass spectrometry based methods enabling the absolute quantification of dozens of tyrosine phosphorylation sites in the EGFR network at high temporal resolution following EGFR stimulation with different EGFR ligands. Quantitative phosphorylation data generated in this analysis provides insight into the relative site occupancy of multiple tyrosine phosphorylation sites on the receptor, highlighting mechanisms of differential regulation in response to different ligands.
DEVELOPMENT OF AN ION CYCLOTRON RESONANCE MASS SPECTROMETER ARRAY

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A critical challenge for mass spectrometry analysis of highly complex biological samples relates to the need for both higher spectral acquisition rates and high mass accuracy measurements. Current hybrid-type mass spectrometers offer a compromise of these two constraints by combining rapid, low mass accuracy MS/MS analyzers in parallel with slower, high mass accuracy analyzers for precursor mass determination. However, recent studies suggest that high mass accuracy measurements in the MS/MS stage may also help resolve co-isolation of multiple precursors that can adversely affect peptide identification. Data-independent strategies have been developed to forgo precursor selection whereby all ions within a range are simultaneously fragmented and the fragment ion population is analyzed with high mass accuracy to allow simultaneous identification of multiple peptides in each fragmentation spectrum. While narrow precursor mass ranges increase effective dynamic range that can be achieved, this also comes as a compromise in terms of how many such ranges can repeatedly be sampled during chromatographic separations. A mass spectrometer array could significantly benefit both data-dependent and data-independent operation by simultaneously providing accurate mass analysis on fragment ion populations from more than a single precursor or single mass range of precursor ions. Our lab is currently developing a mass spectrometer array system that combines a set of Fourier transform ion cyclotron resonance detectors. Each element detector is independently used to analyze fragments from a distinct precursor ion or range of precursor ions. After each element ICR cell is populated with ions, a single cyclotron excitation and detection event is performed on all array elements to allow multiplexed detection of fragment ions trapped in each cell. This presentation will describe this MS array concept, illustrate initial design features and show initial ICR array results.
ANALYSES OF INTACT HISTONES USING A FRONT-END ELECTRON TRANSFER DISSOCIATION ENABLED ORBITRAP VELOS PRO

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Comprehensive characterization of histones by mass spectrometry (MS) has proven challenging for several reasons. While most methods of sequence determination and post-translational modification (PTM) site localization involving enzymatic digestion preserve the combinatorial PTM profiles of histone tails, it is not always possible to obtain a complete combinatorial PTM profile or to confidently identify the protein isoforms from which these peptides originate. Histones are ideal candidates for intact analyses due to their size, the high, electron transfer dissociation (ETD)-compatible charge states they exhibit, and the potential to gain valuable information concerning combinatorial PTMs and sequence variants.

Development of a front-end ETD (FETD) source for the Orbitrap Velos Pro™ was reported in 2013 [1]. This source facilitates multiple fills of the C-trap with product ions from ETD of intact proteins prior to high resolution mass analysis. The result is a dramatic enhancement of the observed fragment ion current and significant improvement in signal-to-noise ratios in fragment ion spectra. This eliminates the need for the acquisition of several time consuming transients and allows for direct coupling to chromatography. The FETD source can also be used to ionize reagents for ion-ion proton transfer (IIPT) reactions. These reactions are used to simplify the resulting spectra and to disperse ETD fragment ions over the available mass range in a controlled manner [2]. These methods were used to obtain high-quality MS/MS spectra of histones derived from human cell lines. The spectra were used to unambiguously identify over 35 unique intact proteoforms derived from butyrate-treated HeLa cells with up to 80% sequence coverage. Several of these contained multiple PTMs including methylation, acetylation, phosphorylation and ubiquitination, as well as single amino acid substitutions. Additionally, well over 100 forms of truncated H2A and H2B were detected and characterized. All experiments were performed on-line with picomole quantities of sample and spectra were acquired using data-dependent instrument methods.

TOWARD NEXT GENERATION FTMS: HIGH RESOLUTION, ACCURATE, AND FAST

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In addition to the leading position in analytical performance among all mass spectrometers, FTMS possesses yet unexplored reserves of further improvement of its characteristics and capabilities. One of the vectors for FTMS further development is in advanced acquisition and processing of time-domain signals (transients) – enabled by recent and on-going rapid developments in electronics, computers, and mathematical methods of signal processing. Another vector is from the improved understanding of ion motion in ion traps – enabled by increasingly powerful simulations and advances in analytical theory development [1]. When joined, these vectors give rise to what can become a next generation FTMS, based on novel ion traps generating transient signals with higher-frequency sinusoidal or non-sinusoidal components which can be accurately acquired with high-performance data acquisition systems and processed with advanced signal processing algorithms. The performance of the next generation FTMS should demonstrate a substantial, 2-10 fold, increase of resolution per unit of ion detection time while keeping or further improving mass and ion abundance accuracies. These advances should translate into improved FTMS applications, especially those with time-constraints, e.g., LC-based proteomics and metabolomics.

To start the journey toward the next generation FTMS, we have developed and implemented the following tools:

- narrow aperture detection electrodes (NADEL) ICR cells for FT-ICR MS [2]
- least-squares fitting of transients for FTMS [3]
- filter-diagonalization method with errors estimation for FTMS [4]
- extended basis FT (xFT) for FTMS with high harmonics content;
- pyFTMS framework for batch file transient processing with multicore workstations & clouds
- high-performance DAQ system with high sampling frequency and data transfer rates

With the developed tools in hands we have managed to achieve the following first preliminary results:

- speed up resolution gain via software: improved multiplexed protein and lipid quantitation
- speed up resolution gain via hardware: frequency multiples & harmonics with xFT analysis
- improved mass accuracy in routine FTMS: FT-ICR MS at unperturbed cyclotron frequency
- improved ion abundance accuracy: via advanced FT and non-FT signal processing

In this presentation we will overview the outlined vision and describe our most recent developments in this direction.

MONOISOTOPIC PROTEOMICS

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In shotgun proteomics, the proteins are digested by trypsin prior to LC-MS/MS analysis. At conventional terrestrial isotopic abundances and the dynamic range of 1:100, an average peptide gives in a mass spectrum 3-4 isotopic peaks separated by a 1 Da interval. The number of isotopic peaks increases by 2-3 for every decade of the dynamic range added. As a result, in a proteomics LC-MS/MS run where hundreds of peptides elute simultaneously, and a typical survey mass spectrum has a 1:1000 dynamic range, isotopic distributions of many peptides overlap. This overlap interferes with the precursor selection for MS/MS and complicates data processing. In top-down proteomics, where intact proteins are analyzed, the situation is even worse, as the proteins and their fragments have broad isotopic distributions, sometimes stretching for tens of daltons.

A general solution to the problem of wide isotopic distributions has been known since at least 1998, when A. Marshall et al. have used doubly depleted (depleted with $^{13}$C and $^{15}$N) growth media to recombinantly express a protein of interest. The resultant protein had its monoisotopic mass as the most abundant member of the isotopic cluster. Later, other research groups have explored similar approaches. However, the method has gradually faded away before the modern proteomics approaches have become firmly established. Here, we reinvent this approach, branding it “monoisotopic proteomics”, and explore its benefits in typical tasks solved the contemporary proteomics. In general, monoisotopic proteomics gives greatly simplified mass spectra, especially in top-down analysis, possesses 2-3 times higher sensitivity, facilitates de novo sequencing, PTM assignment and revolutionizes hydrogen-deuterium exchange (the latter has first been shown by Marshall et al. in early 2000s). These benefits, as well as some pitfalls, of monoisotopic proteomics, will be discussed.
FTMS FOR FUNCTIONAL CHARACTERIZATION OF MICROBIAL PROTEINS

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Combinatorial post-translational modifications (PTMs), signal peptide cleavages, proteolytic processing and site mutations are all important biological processes that largely go undetected in bottom-up proteomics but could be gleaned from top-down studies. Recent advances in MS instrumentation, separation, and bioinformatics significantly increased the throughput of top-down proteomics, allowing the identification of hundreds of intact proteoforms. However, most of these efforts involve additional sample pre-fractionation steps, which are often labor intensive, require large sample sizes, and are inadequate in terms of quantitation. Herein, we describe practical one-dimensional LC-FTMS workflows developed to tackle these challenges and enable high-throughput, comprehensive and sensitive top-down proteomics. Selected applications will be highlighted, including characterization of histone PTMs involved in control of gene expression in fungi and complex and heterogeneous proteoforms present in fungal secretomes to provide important information for their biotechnological applications. A brief update on the status of the EMSL’s 21T FTICR spectrometer, as it relates to top-down proteomics, will also be presented.
Histone H1 has been reported as a potential biomarker in breast and other cancers due to its maximal phosphorylation during mitosis. We applied orthogonal tandem MS techniques to further our understanding of variant specific H1 phosphorylation. Top-down MS/MS was used to quantify proteoforms in breast cells as a function of cell cycle. FT-ICR MS analysis revealed that histone variants H1.2 and H1.4 were the major species in MDA-MB-231 metastatic breast cells. In addition to these variants, histone H1.3 was identified in non-neoplastic MCF-10A cells. Progressive phosphorylation of histone H1.4 was observed in both cell lines in M phase-enriched cell populations. Phosphorylation occurred on N-terminally acetylated H1.4, first at S172 followed successively by S187, T18, T146, and T154. Phosphorylation of S2 was also observed. Phosphorylation at S173 of histone H1.2 and S172, S187, T18, T146, and T153 of H1.4 increases during mitosis, suggesting that these events are cell cycle-dependent and may serve as markers of proliferation during breast or other proliferative cancers. Notably, T146 and T154 are the only phosphorylated sites observed at higher rates in metastatic versus non-neoplastic cells. We also report the observation of the H1.2 SNP variant A18V in MCF-10A cells. CE-MS/MS lends itself as a complementary approach to efficiently separate hydrophilic H1 peptides. Improved peak capacity increases sensitivity relative to RPLC and permits identification of low abundance modifications that do not produce enough ions to be detected by FT-ICR analysis. CE-MS/MS reinforces the presence of phosphorylation sites on H1.2 (S173) and H1.4 (S2, T18, T146, T154, S172, S187) as well as unique sites on H1.2 (S36, T146, T154) and H1.4 (S36, S150). CE-MS/MS additionally identified phosphorylation sites on H1.0 and H1.X and drastically increased sequence coverage. Phosphorylation at observed residues on H1.0 and H1.4 has not previously been reported in the literature. Future directions include mapping these phosphorylation changes at more precise cell cycle time points by releasing cells from a thymidine block and monitoring the abundance of H1 phosphosites as the cells proceed through the cell cycle toward mitosis.
Herein, we describe advances in separations, mass spectrometry and bioinformatics that form the foundation of a platform for the untargeted analysis of protein complexes. Native, gel-based separations have been developed that produce samples amenable to downstream mass spectrometric analysis. Separating a fungal secretome, mouse heart mitochondrial extract, and HeLa cell extract demonstrated the broad applicability of the method. Subsequent application of novel multistage activation strategies within the mass spectrometer enabled the interrogation of the proteins and protein complexes within these samples down to subunit backbone fragment ions. These analyses revealed isoform specific composition as well as novel posttranslational modification. A computational approach to identify specific isoforms within multi-protein assemblies will also be described. We combined the CORUM and UniProt databases to create candidates for an error-tolerant search engine designed for top-down analyses of protein complexes by native mass spectrometry. Taken together, these efforts have begun to yield a never-before-seen view of protein complexes from endogenous systems.
WHAT'S AT THE SURFACE? LATEST DEVELOPMENTS IN LESA MASS SPECTROMETRY OF BIOLOGICAL SUBSTRATES

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Liquid extraction surface analysis (LESA) is an ambient surface mass spectrometry approach which looks particularly promising for protein analysis. The benefits of LESA are speed of analysis, reduced sample preparation requirements and sample loss, potential for multiple sampling of the same location and, in the case of tissue sections, the potential to image multiple analytes. We have previously demonstrated LESA of dried blood spots and thin tissue sections for top-down and bottom-up proteomics.

High field asymmetric waveform ion mobility spectrometry (FAIMS) is emerging as a powerful technique in biomolecular analysis. FAIMS relies on differences in ion mobility in high and low electric fields to achieve gas phase separation of ions at atmospheric pressure, offering advantages including reduced interference from ions of similar mass-to-charge (m/z), and separation of isomers and positional variants.

Here, we demonstrate the coupling of LESA with FAIMS for the analysis of proteins and lipids from thin tissue sections of mouse liver and brain, and bacterial colonies growing on agar. Advantages and challenges of the combined approach will be discussed.
TWO TOPICS: THE NEW NON-FT METHOD OF ICR MASS SPECTROMETRY, BASED ON MEASURING OF “ANTENNA” IONS CYCLOTRON FREQUENCY TIME DEPENDENCE AND, IS DYNAMICALLY HARMONIZED CELL HARMONIZED STATICALLY?

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Implementation of dynamically harmonized measuring cell [1] caused an almost order of magnitude jump in resolving power of FT ICR mass spectrometry. Resolving power of around 10 million is now routinely achievable on the next generation of FT ICR mass spectrometers with moderate magnetic field. There are still some problems to solve one of which is to increase the acquisition rate of spectra which is getting inappropriate at this level of resolution approaching to some minutes per spectrum which is not acceptable for LC and other separation methods. FDM and other parameter estimation methods offered to decrease necessary detection time do not work for complex spectra and low S/N [2]. The other problem is a proper averaging of the field for off axes ions via dynamic harmonization. It is not clear yet to what extend dynamically harmonized FT ICR cell is harmonized statically at given number of shaped segments.

Regular 7 T Bruker Apex Q FT ICR mass spectrometer with dynamically harmonized cell is used for the experimental research. Different types of antenna ions are tested. Among them are ions of highest intensity in isotopic fine structure clusters. Supercomputer simulation of antenna-analite ions interactions were performed using in house developed Particle in Cell algorithm. Filter diagonalization method was used for “antenna” ion signal processing. Different approaches to electric field simulations were used to analyze the extend of static harmonisity of dynamically harmonized cell with different number of shaped segments.

In the new method of mass spectra measurements on FT ICR instruments mass spectra are measured using so called antenna ions. These ions of particular m/z are permanently introduced into the measuring cell together with ions from analite. They are excited cyclotronically together with analite ions but ICR signal from only these antenna ions is detected in resonant narrow band mode of detection. Cyclotron frequency of these antenna ions are modulated by Column interaction with ions from analite and are changing with time. Spikes in frequency dependence on time are corresponding to periodic approaching in the course of cyclotron motion of analite ions of particular m/z to antenna ion cloud and the periods of such spikes are inversely proportional to cyclotron frequency difference of antenna and analite ions. Because of high and permanent intensity of antenna ions there is no problems with their frequency-time domains analyses by other than FT methods such as parameter estimation methods like FDM and linear prediction. Our simulations of the electric field distribution in dynamically harmonized cell with different numbers of shaped electrodes show that the volume of the ICR cell with statically harmonic field is increasing with increase of the number of shaped cell segments. It means that by choosing a proper technology it is possible to make the field statically harmonic in practically the whole volume of the cell and magnetron motion causing off axis rotation of ions will not ruin resolving power of such cell.

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A UNIVERSAL METHOD FOR PEPTIDE IDENTIFICATION ON ORBITRAP FUSION MS

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Achieving the maximum identifications from different peptide samples requires optimization of MS methods. Optimizations are time/sample intensive to determine the best balance of scan rate and number of ions per spectrum. This is particularly true when accurate sample concentration, complexity, and dynamic range are unknown, often the case following fractionation/enrichment. Unfortunately, optimizations are often not performed due to sample/time restraints. Here we present a universal method which adjusts parameters “on-the-fly” according to spectral complexity/intensity, eliminating the requirement for optimization.

We analyzed various samples including HeLa digests and immunoprecipitations. Analysis was performed on an Orbitrap Fusion MS. The resulting LC-MS/MS data were searched using Proteome Discoverer, matches were filtered to 1% FDR. Each sample was analyzed with varying ion targets and maximum injection times to determine optimal parameters.

Novel instrument control software, now implemented on Orbitrap Fusion MS, was used to develop a Universal Method which makes “on-the-fly” decisions about length of injection time per precursor based on the ion flux, complexity of full scan and available cycle time without user input.

Maximum identifications are obtained by reaching a balance between scan rate and quality of spectra. With 1 microgram HeLa digest, Orbitrap Fusion achieves maximal identifications (~25,000 unique peptide identifications (UPI)) using 35 ms maximum injection time and 1e4 ion target. At 1 ng, however, maximal identifications are achieved (~700 UPI) using 500 ms maximum injection time and 1e4 ion target. Depending on the sample load, complexity, and dynamic range, optimal values change dramatically. A single Universal Method achieved maximal identifications in all sample types.

Our results show that it is possible to achieve maximal peptide identifications from samples with unknown concentrations without method optimization and lengthy reanalysis, thereby, increasing the throughput of the instrument while simultaneously improving the quality of the data acquired.
STUDENT AWARDS
DETERMINATION OF HEXURONIC ACID STEREOCHEMISTRY IN HEPARIN AND HEPARAN SULFATES GLYCOSAMINOGLYCANS USING ELECTRON DETACHMENT DISSOCIATION

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The structural elucidation of Heparin (Hp) and heparin sulfate (HS) glycosaminoglycans continue to attract a lot research efforts due to their biological and clinical significance. However, this effort has been hampered by their high heterogeneity arising from their non-template biosynthesis making them very difficult to analyze. Recent advances in tandem mass spectrometry activation methods, especially electron detachment dissociation (EDD), has been employed to confidently assign sites of modifications and to differentiate hexuronic acid epimers of HS tetrasaccharides. Whereas preceding studies focused on non-sulfated uronic acid epimers, this current work extends the assignment of the hexuronic acid stereochemistry to 2-O sulfated uronic acid epimeric tetrasaccharides. The presence of 2-O sulfation on the central uronic acid was found to greatly influence the formation of B₃ and Y₁ ions in glucuronic acid, whiles Y₃ and Y₂ were diagnostic for iduronic acid. Using diagnostic ratios \( DR = (B_3 + Y_1)/(Y_3 + Y_2) \), we are able to confidently assign the uronic acid stereochemistry in a single MS/MS spectra.

TARGETED HIGH RESOLUTION ION MOBILITY SEPARATION COUPLED TO ULTRA HIGH RESOLUTION MASS SPECTROMETRY OF ENDOCRINE DISRUPTORS IN COMPLEX MIXTURES

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Detection of endocrine disrupting compounds (EDCs) from environmental matrices is traditionally performed using lengthy pre-fractionation steps or high resolution mass analyzers due to the large number of chemical components and their large structural diversity (highly isomeric). In the present work a variant of trapped ion mobility spectrometry (TIMS) is coupled to Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) for direct separation and characterization of targeted EDCs from a complex environmental matrix in a single analysis. In particular, targeted identification based on accurate mobility (R~70-120) and exact mass measurements (R>400,000) of seven commonly targeted EDC and their isobars (e.g., bisphenol A, (Z) and (E) diethylstilbestrol, hexestrol, estrone, α-estradiol, and 17-ethynylestradiol) is shown from a complex mixture of water soluble organic matter (e.g., Suwannee River Fulvic Acid standard II) complemented with reference standard measurements and theoretical calculations (<3% error).
CHARACTERIZATION OF INTERFACIAL MATERIAL ISOLATED FROM PHOTOCHEMICALLY WEATHERED CRUDE OIL BY FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

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Wind and ocean currents provide sufficient mixing energy to form stable emulsions once crude oil enters the environment. Emulsions increase the viscosity of the crude oil, which reduces the effectiveness of recovery strategies (i.e. skim boats used to remove crude oil from the environment). Knowledge of compounds that exist at the oil/water interface and how their composition changes as a function of environmental weathering can help in the development of more effective remediation techniques. Here, interfacial material (IM) isolated from the parent Deepwater Horizon (DWH) crude oil, photochemically weathered DWH crude oil, and an environmentally weathered sample collected ~30 days after the DWH blowout are characterized by Fourier-transform ion cyclotron resonance mass spectrometry to provide insight into degradation pathways (biotic or abiotic) of crude oil in the environment.

Initial FT-ICR MS analyses reveal that photochemical weathering results in an increase in the relative abundance of higher-order O\(_x\) and O\(_x\)S\(_y\) (\(x \geq 4\)) species in the IM fraction. A similar trend is also observed in IM isolated from the environmentally weathered sample. These results suggest that abiotic degradation plays an important role in the chemical transformation of DWH IM in the environment.

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TOP-DOWN ANALYSIS OF HISTONES H2A/H2B AND THEIR CHANGES DURING HIV LATENCY

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Although histones H2A and H2B are key components of the nucleosome in eukaryotic cells, their biological functions are less studied due to the analytical challenge. Unlike histones H3 and H4, which exhibit numerous post-translational modifications (PTMs), histones H2A and H2B carry fewer modifications but are rich in sequence variants, rendering antibody-based methods and bottom-up proteomics incapable of identifying their proteoforms. However, the ultrahigh mass accuracy and resolving power of Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometry enable us to fully characterize histones H2A and H2B by top-down MS/MS. By comparing the changes in histones H2A and H2B during HIV latent infection, we provide a first glimpse of the biological function of these histones.

Mass spectra were acquired with our custom-built 9.4 T FT-ICR mass spectrometer with a positive nanoelectrospray ionization (ESI) source. Precursor ions were isolated by an external quadrupole mass filter and internal stored waveform inverse Fourier transform (SWIFT) excitation to within a nominal mass-to-charge ratio (m/z) range of ~1. Fragmentation was achieved by electron capture dissociation (ECD). Data was analyzed by our in-house software and verified manually. Proteoforms of histone H2A and H2B have been assigned and semi-quantified.

Work supported by NSF Division of Materials Research through DMR-1157490, NIH through R01 DA033775, and the State of Florida.

SIMULATION OF OFF-AXIS ION MOTION AND ITS EFFECT ON PERFORMANCE OF A DYNAMICALLY HARMONIZED FT-ICR ANALYZER CELL

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Dynamically harmonized analyzer cells represent one method of harmonizing trapping potentials to increase ion cloud coherence and thus, mass resolution. This works by using specially shaped electrodes that cause an ion undergoing cyclotron motion around the center of the cell to ‘see’ an average radial electric field equivalent to that of an ideal penning trap. In practice however, ions generally do not rotate perfectly around the center of the cell; they are offset. This is caused by forces generated by variables such as improper tuning of the excite pulse or using sidekick electrodes. This work uses 3D particle-in-cell simulations to study the causes and results of such offsets on the performance of dynamically harmonized cells.

This work is focused on understanding how off-center cyclotron motion affects performance of dynamically harmonized cells. By simulating monoisotopic clusters of Cs⁺ ions under a variety of excitation conditions, we are able to observe how this affects the magnetron offset and, in turn, how it changes the free inductive decay. Additionally, starting the ions off axis allows simulation of the effects of forces like those generated by sidekick ion accumulation. By studying what magnitude of offset this begins to have an adverse effect on performance compared to a static ideal electric field, we can suggest potential ways to alter excitation and experimental parameters to obtain better performance from these dynamically harmonized analyzer cells.
AUTOMATED ANALYSIS OF GLYCOSAMINOGLYCAN FTICR-MS/MS

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Automated analysis and assignment of glycoasminoglycan (GAG) MS/MS data is challenging due to their non-template synthesis. Structure assignment requires de novo analysis of tandem mass spectra. The number of combinations of GAG sequences that match a specific composition increases exponentially with respect to the number of sugar residues and modifications. Assigning the positions of modifications, specifically O- or N-sulfation and N-acetylation, has been accomplished previously by manual interpretation of a spectrum, a process that is slow, tedious, and requires a great deal of expertise.1 We report here a method that successfully annotates and identifies heparin and heparan sulfate MS/MS spectra with minimal user input.

Our approach utilizes the genetic algorithm, a search heuristic designed to mimic the evolutionary process. The program begins by randomly generating theoretical structures that matches a GAG composition, derived from the accurately measured molecular weight using FTICR-MS. These structures undergo two forms of genetic operations – crossover and mutation – and then are tested against the user given mass spectra for their fitness quality. The most-fit structures are then passed onto the next iteration of the software. This process is fundamental to all genetic algorithms and is repeated until the fitness of the structures plateaus at a maximum, at which point the best structure is found. This approach has the advantage of an exhaustive search of all possible structures, but is much more efficient, arriving at the correct match by examining only a small percent of all possibilities. Our fitness function is designed to look at specific combinations of glycosidic and cross-ring cleavages common in GAG MS/MS. The weight of each cleavage and combination is statistically determined using intensity dependent functions. We avoid any tedious pre-programming of databases by generating structures on demand, allowing the software to work with GAGs of any size and/or number of modifications.

CHANGES IN ORGANIC MATTER COMPOSITION WITH PERMAFROST THAW REVEALED BY FT-ICR MS COMPOUND CLASS CHARACTERIZATION

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Since permafrost-zone peatlands contain over 1/3 of the amount of carbon in the atmosphere, the fate of this carbon as permafrost thaws represents a major question for the development of earth system models. The susceptibility of carbon to decomposition and subsequent release into the atmosphere depends largely on its reactivity, which is in turn determined by molecular structure. In this study, we used FT-ICR MS to analyze the molecular composition of dissolved organic matter (DOM) from different peatland types along a permafrost thaw succession in Stordalen Mire, a thawing subarctic peatland in northern Sweden. In approximate order of succession, our site types include: (a) a recently-thawed thermokarst sinkhole, (b) partially-thawed Sphagnum-dominated bogs, (c) a mostly-thawed poor fen with a combination of Sphagnum and Eriophorum angustifolium, and (d) fully-thawed Eriophorum-dominated fens. Our samples (27 total) were run on four different dates several months apart, during which the instrument received several upgrades, which unfortunately precluded detailed analysis of changes in individual molecular formulas over the entire sample set. Despite this variability in instrumental performance, we could still discern changes in bulk molecular composition based on the total relative abundances of different compound classes defined by their positions in the van Krevelen diagram [1,2]. Principal component analysis revealed that our samples fall into two broad categories according to habitat type, with markedly different molecular composition in the fully-thawed Eriophorum-dominated fens compared to the earlier thaw stages. The fully-thawed fen sites contained more protein-like, amino sugar-like, and carbohydrate-like compounds, and slightly more lignin-like compounds and unsaturated hydrocarbons, whereas the partially-thawed sites contained more tannin-like compounds and slightly more condensed aromatics. These trends in compound class abundances are supported by trends in overall elemental ratios, with DOM from fully-thawed fen sites having higher H/C and lower O/C ratios than DOM from non-fen sites. Combined with previous data that show increased CH₄ and CO₂ production along the thaw progression [2], these results indicate that permafrost thaw drives shifts in DOM composition from more recalcitrant unsaturated high-oxygen compounds in the partially-thawed sites, to more labile longer-chain reduced compounds in the fully-thawed sites. This shift to more labile and reduced DOM along the thaw progression may help to drive higher release of greenhouse gasses, particularly CH₄, as permafrost thaws.

GAS-PHASE STRUCTURAL CHARACTERIZATION OF ANIONIC RNA OLIGONUCLEOTIDES

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Ions produced by electrospray ionization (ESI) can retain a memory of their solution-phase structures for subsequent mass spectrometric interrogation in the gas phase. For example, protein complexes have been shown to retain their stoichiometry and architecture upon desolvation. However, much less is known about the gas-phase structures of other biomolecules such as RNA. Even less is known about the structure of RNA anions, a state strongly favored through the acidic phosphate backbone structure of this molecular class. Here, the gas-phase structure of a 16S hairpin RNA oligonucleotide is examined via ion mobility as function of solvent composition. We also seek to apply negative ion electron-capture dissociation (niECD) to probe the binding of aminoglycoside antibiotics to this RNA.

From a native-like solvent (10 mM ammonium acetate) a bi-modal ion abundance profile was observed for the generated RNA charge states, with the two sub-distributions spanning from the -7 to -13 and the -5 to -7 charge states, respectively. The same charge state profile was observed with increasing methanol concentration (10-40%), suggesting that methanol (in this range) has a small effect on the RNA structure. The measured IM drift time distributions for the -4 to -13 charge states as function of methanol content varied by ≤1.5%. At higher charge states, above -7, IM arrival time distributions remained constant across the varied methanol concentrations.
Nanoelectrospray ionization (nanoESI) has gained popularity due to increased sensitivity, compatibility with capillary separations, and the ability to maintain "native" structures into the gas phase. NanoESI is typically utilized in positive-ion mode in which acidic molecules may not ionize well. Negative ion nanoESI presents challenges not observed in positive-ion mode, e.g., dielectric breakdown of air at the needle tip. Corona discharge results in greatly suppressed signal and rapid degradation of nanoESI emitters. Solutions include addition of low surface tension solvents and utilization of SF$_6$ as a dielectric barrier. These approaches often involve dilution of analyte and modification of instrumental interfaces. With the addition of small amounts of trifluoroethanol (TFE), aqueous solutions exhibit improved spray stability and suppression of corona discharge. Mass spectrometric experiments were performed using a Waters Micromass LCT Premier with in-house pulled borosilicate needles. A series of proteins, peptides, and small molecules were analyzed in solutions of varying composition. The modifiers examined were TFE, methanol (MeOH), and ethanol (EtOH). Water solutions were prepared with 0.2% NH$_4$OH, and 0.2, 0.5, 1.0, 2.0, and 5.0% total composition of modifier. An α-S1-casein, β-casein, and bovine serum albumin (BSA) tryptic digest was analyzed with a Proxeon EASY-nLC coupled to a Bruker 7T quadrupole-Fourier transform ion-cyclotron resonance mass spectrometer operated in negative-ion mode.

A mixture of five peptides (sulfated cholecystokinin, Arg$^8$-vassopressin, angiotensin I, TSTEPQpYQPGEN, and YFYLIPLEYLK) at 5 µM each was studied in negative-ion mode for 30 min at a nanoESI voltage of -1,350 V. Absolute abundance values for water only solutions were observed to initially be 1.00±0.09×10$^6$ counts. After 30 minutes, corona discharge was clearly visible and counts were reduced to 0.55±0.09×10$^6$. When the analyte mixture was examined with 0.2% EtOH, the absolute intensity was 1.50±0.02×10$^6$ for 30 min. With 0.2% TFE, the absolute intensity observed was 1.55±0.004×10$^6$. NanoESI spray current stability is improved 22-fold compared to water only solutions and 5-fold compared to 0.2% EtOH solutions. NanoLC analysis of the tryptically digested proteins was performed without TFE and with 0.2% TFE in each mobile phase. When TFE is excluded, the first observed peptides occur at approximately 23 min and ion current drops sporadically until 38 min. Peptides are observed eluting as soon as 14 min and ion current is stabilized when TFE modified mobile phases are implemented. By including TFE in negative-ion mode nanoESI analysis, corona discharge is suppressed while nanoESI stability and sensitivity is improved in separation and direct infusion experiments.
HEPARAN SULFATE LIBRARIES FOR ROBO1 AFFINITY PULLDOWNS

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The secreted protein Slit binds to its transmembrane protein Robo (Roundabout) to form a complex that is a signaling pathway with many diverse functions, including neural development and angiogenesis. The complex is modulated by heparan sulfate (HS), a sulfated polysaccharide chain that is abundant on the cell surface and in the extracellular matrix. HS binds to both Slit2 and Robo1 thus facilitating ligand-receptor interaction. To better understand how this complex is activated we aim to determine the details of the heparan sulfate sequence that leads to specific binding with Robo1.

Separate libraries containing HS hexasaccharides, decasaccharides, and dodecasaccharides are provided by a collaborator after digestion with Heparanase1. The structural compositions of the isolated oligosaccharides are determined by high resolution mass spectrometry (HRMS). 0.1mg/ml sample solutions in 50/50 methanol:water were infused at a rate of 120uL/hr and ionized by electrospray. Diethylamine (DEA) is added to the samples to remove sodium for better identification. An exhaustive database containing the calculated mass of every possible conformation of HS polysaccharides up to a chain length of twelve is employed to facilitate identification. Collision Induced Dissociation (CID) is used to verify the identification. A small amount of sodium (Na) is added to samples to induce Na/H+ exchange to prevent sulfate loss during CID.

The use of DEA in the spray solvent significantly suppresses salt adduction in the samples, and has allowed assignment of HS compositions in a clean spectrum. Abundant components in the mixture can have their structures assigned by tandem mass spectrometry. Na/H+ exchange facilitates the production of useful fragmentation processes during tandem mass spectrometry by reducing loss of SO3. In this way the structures of HS present in each library have been determined. There are one or two prominent HS compositions present in each library, each with a varying degree of sulfation. The DP6 library is found to be predominantly [1,2,3,1,x] 1 delta uronic acid, 2 uronic acids, and 3 glucosamine [1 of which is n-acetylated], with varying degrees of sulfation. The DP10 library is predominantly [1,4,5,2,x] 1 delta uronic acid, 4 uronic acids, and 5 glucosamine [2 of which are n-acetylated], and varying degrees of sulfation.

Tandem mass spectrometry provides composition and structure of heparan sulfate polysaccharides responsible for modulating the Slit-Robo signaling pathway.
PROBING LYSYL-TRNA SYNTHETASE (KRS)-INDUCED CONFORMATIONAL CHANGES TO THE 37-KDA LAMININ RECEPTOR PRECURSOR (37LRP)-NANODISC COMPLEX BY H/D EXCHANGE FT-ICR MS

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Laminin is a major component of the extracellular matrix (ECM). It induces a signal cascade that leads to proteolytic degradation of ECM, and promotes cell migration.¹ The 67-kDa laminin receptor (67LR) is a metastasis marker and expressed at elevated level various carcinomas. Expression level is correlated with cancer progression and malignancy.

KRS produces Lysyl-tRNA for translating mRNA at the ribosome. Emerging evidence indicates that KRS has novel functions associated with cell migration and metastasis in cancer.² When cytosolic KRS translocates to the cell membrane, it stabilizes 67LR binding to the cell membrane, a function implicated in cancer metastasis.

Previous experiments revealed that the N-terminal domain 1-207 of KRS is responsible for interacting with 67LR. We compared the deuterium uptake pattern of 1-207 KRS (KRS for short), 37LRP, nanodisc, alone and in the complex they form, to reveal the contacting surfaces of the KRS, 37LRP, and nanodisc complex.

With our hybrid linear ion trap 14.5 T FTICR instrument, we achieved 100% sequence coverage for each of the proteins, and >90% sequence coverage from common peptides. Contacting surfaces of KRS and 37LRP are revealed. Our results support the previous finding that KRS enhances the LR binding to the membrane, and identify a possible binding surface of the complex. This finding paves the way for developing an anti-metastatic drug that targets the KRS and LR-nanodisc complex.

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OPTIMIZED EXCITATION ELECTRIC FIELD FOR FREQUENCY-MULTIPLE DETECTION

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Fourier Transform ion cyclotron resonance mass spectrometry offers the highest broadband mass resolving power and mass accuracy. Resolving power improves with increasing magnetic field strength, but at great expense and difficulty. It has long been recognized that multiplying the detected image current frequency by detection with multiple pairs of electrodes can multiply the mass resolving power accordingly at a given magnetic field strength [1-5]. However, this method requires an excitation electric field that can excite ions to a sufficiently large orbital radius to yield a reasonable signal magnitude. Therefore, the quality of the excitation electric field is essential to frequency-multiple detection.

In this work, we compare different excitation electric fields, namely 60° excitation electric field, 90° excitation electric field, 120° excitation electric field, compensated excitation electric field and circular polarized excitation electric field. The comparison is based on theoretical analysis and simulation. For a conventional cylindrical ICR cell of four azimuthal sections, excitation electrodes of 120° angular extent offers the best excitation electric field. Additional azimuthal sections due to frequency-multiple detection can be used as compensated electrodes to further optimize the excitation electric field. Furthermore, the compensated excitation electric field can be adapted for circular polarized excitation electric field which requires only a half of the excitation voltage magnitude to excite ions to a given ion cyclotron orbital radius. The present compensated excitation electric field and circular polarized excitation electric field are based on the ICR cell geometry of six azimuthal sections for triple-frequency detection. ICR cell for higher order frequency-multiple detection has more azimuthal sections as compensated electrodes which can further improve the excitation electric field. The optimized excitation electric field facilitates exciting ions to large cyclotron orbits which benefit not only the frequency-multiple detection but also the fundamental frequency detection.

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DEVELOPMENT OF NOVEL INTERROGATION TOOLS FOR SURFACE ANALYSIS USING ULTRA-HIGH RESOLUTION MASS SPECTROMETRY

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Mass Spectrometry Imaging is emerging as a powerful tool to map the distribution of molecules and macromolecules in a cell or tissue. Correlating the distributions then indicates whether any two identified molecules colocalize and thus potentially interact, and this could then create an unbiased map of the 3-dimensional interactome in and on cells. This information would be enormously valuable for understanding the fundamental interactions in a cell and identifying disease-associated defects in the interactome. In the present work, we describe the instrument development of a high-resolution imaging mass spectrometer for cell analysis that will permit the analysis of native (or untreated) surfaces with a 10-100x fold sensitivity increase compared to available technology in molecular ion detection at high spatial resolution. We have previously shown that gold nanoparticles (Au₄₀₀⁺, AuNP) provide higher secondary ion yields (100 fold) in comparison to other cluster based projectiles (e.g., Bi₃⁺ or C₆₀⁺) [1] and that the complexity of biological samples requires ultrahigh resolution mass measurements. [2] Theoretical simulations and preliminary experiments characterizing the AuNP-FT-ICR SIMS instrument performance will be presented.

POSTERS
CALCULATION OF TOTAL SULFUR CONCENTRATION BY (+) APPI FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETRY

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The current paradigm shift to unconventional crude oils has led to the production of oils rich in heteroatom-containing species that are problematic during refining. Bulk properties are often used to classify crude oils based on density, viscosity, and heteroatom concentration; however, these measurements reveal little or no information about chemical composition. The percentage of sulfur in crude is an important bulk characteristic due to the contribution of sulfur compounds to environmental pollution, corrosion, and fouling of conversion catalysts. Here, we describe a method to calculate the total sulfur concentration in petroleum samples based on the chemometric modeling of data obtained by positive atmospheric pressure photoionization Fourier transform ion cyclotron resonance mass spectra (APPI FT-ICR MS). Analysis by FT-ICR MS provides both a measurement of total sulfur concentration and characterization of chemical composition and complexity. Thirty crude oil samples ranging from 0.2 to 4.6 wt.% sulfur, determined by XRF and CHNOS elemental analyzer, were selected to train the model. Data processing, data calibration, molecular formula assignments, and chemometric analysis were conducted with PetroOrg N8.6©. A visual observation of three selected mass spectra shows that the ratios of signal magnitude for sulfur and hydrocarbon peaks increase with increasing sulfur concentration. To investigate this trend for a large number of samples and multiple heteroatom classes, principle component analysis (PCA) was performed. The relative abundances (RA) for sulfur heteroatom classes (S$_1$, S$_2$, and S$_3$) were normalized with the relative abundance of hydrocarbons observed for each sample. The ratio of the %RA between the sulfur classes (S$_x$) the hydrocarbons classes (HC) were then used as variables for PCA analysis. The PCA results show a clear linear trend along the principal component 1 (PC1) which accounts for 92.2% of the explained variance, whereas PC2 accounts for only 6.8% of the explained variance. Analysis of the loadings plot reveals that the S$_1$/HC ratio governs the trend in PC1. Values for PC2 are governed by S$_1$/HC, S$_2$/HC, and S$_3$/HC and provide the ability to distinguish between oils with higher numbers of sulfur heteroatoms. These results suggest that sulfur concentration for crude oils can be modeled by a linear combination of variables when utilizing S$_x$/HC ratios. A model was successfully developed and sulfur concentrations were predicted for 11 test samples, where the S% for each sample was predicted with an average error of 0.3% deviation from sulfur concentrations obtained from bulk elemental analysis.

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UNDERSTANDING SOLAR INDUCED TRANSFORMATION OF CRUDE OIL USING ION MOBILITY SPECTROMETRY COUPLED TO FT-ICR MS

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The exposure of crude oils in water to UV light causes the degradation and generation of different chemical compounds and their analysis requires ultrahigh resolution, complementary post-ionization separation techniques. [1] We have recently shown the advantages of trapped ion mobility spectrometry coupled to mass spectrometry (TIMS-MS) for the separation and characterization of polyaromatic hydrocarbon mixtures. [2] In the present work, a variant of TIMS-MS, Selective Accumulation TIMS coupled Fourier Transform- Ion Cyclotron Resonance Mass Spectrometry (SA-TIMS-FT-ICR MS, [3]) is used for the analysis of the water absorbed fraction before and after photo-induced transformation of a crude oil. Preliminary results shows that molecular components can be identified based on their mass accuracy (i.e., chemical formula and double bond equivalents) and their mobility values ($R_{\text{IMS}} = 75-120$). Inspection of the SA-TIMS-FT-ICR MS data shows that the most abundant heteroatom hydrocarbons can be easily identified and that over 1000 new compounds are observed after photo transformation. This is the first application of high resolution mobility measurements and ultra-high mass resolution to the study of photolysis of oils.

EPITOPE MAPPING OF 7S CASHEW ANTIGEN IN COMPLEX WITH ANTIBODY BY SOLUTION-PHASE H/D EXCHANGE MONITORED BY FT-ICR MASS SPECTROMETRY

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Tree nut allergies are one of the most common, severe, and lifelong persistent food allergies among children and adults.1,2 Food allergies appear early in life and the incidence of severe food allergies has increased over the past few decades.3-6 Currently, the related costs associated with food allergies in the United States are estimated to be $25 billion annually.7 Reactions to peanut and tree nuts can trigger anaphylaxis in allergic patients. The basis for understanding how these molecules are recognized by the immune system is epitope mapping.

Here we demonstrate that the cashew 7S globulin allergen, Ana o 1 2G4 epitope is susceptible to denaturation, and we apply HDX-MS to identify likely regions of mAb contact. HDX analysis was accomplished by comparing backbone H/D exchange of free recombinant (r) Ana o 1 to rAna o 1 bound to the mAb 2G4 (rAna o 1:2G4). We show that the 2G4 epitope is conformational, and as revealed by HDX, five discontinuous regions likely contribute to the 2G4 epitope. Two of the regions overlap previously reported linear epitope-contributing regions and three are newly identified.

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Absorption mode is a different way of viewing your FT-MS data – giving better resolution, signal-to-noise ratio and accuracy, and you don’t have to adapt your instrument, just process the data in a different way. Methods for producing absorption mode spectra have been around for nearly 10 years, but have a couple of weaknesses – the techniques have relied on the phase correction function being quadratic in nature and the spectra produced often exhibit a severe baseline deviation which must be corrected before the MS data can be interpreted (this correction is both computationally difficult and time-consuming). Here we show how these problems can be solved by the application of some well-chosen curves that improve resolving power, remove the necessity for baseline correction,[1] and provide absorption mode specific isotopic models.[2] We also show how some instruments can require a non-standard, higher order (>2) phase correction function in order to produce absorption mode spectra.[3] As a consequence of these developments you can benefit from faster, higher performance spectral processing and interpretation, and apply absorption mode to new instruments which would not previously have been possible.

CHARACTERIZATION OF ISOMERS IN PETROLEUM INTERFACIAL MATERIAL BY ION MOBILITY MASS SPECTROMETRY

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Introduction
Petroleum interfacial material comprises the compounds that lie at the oil/water interface of petroleum emulsions. These species are believed to be responsible for emulsion stability; therefore, knowledge of their chemistry and structure helps to determine strategies to handle petroleum emulsions. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) has been used to characterize interfacial material isolated from petroleum samples at the molecular level and reveals that the species present at the oil/water interface are enriched in sulfur- and oxygen-containing functionalities. However, structural and isomeric information about the chemical functionalities is still unclear. Here, we demonstrate the use of ion mobility mass spectrometry and post-ion mobility collision-induced dissociation (CID) to complement FT-ICR MS analysis and help characterize isomers in petroleum interfacial material.

Methods
Interfacially active materials were isolated from Athabasca bitumen as previously described.¹ (-) ESI IM-MS experiments were performed with a Synapt G2Si HDMS (Waters Corp.) equipped with a time-of-flight (TOF) mass analyzer. Post-ion mobility CID experiments were performed in the transfer collision cell for selected ions. (-) ESI FT-ICR mass spectra were acquired with a custom-built 9.4 tesla FT-ICR mass spectrometer at an average mass resolving power of m/Δm50% > 600,000 at m/z 500 and 100-400 ppb mass error.

Preliminary Results
(-) ESI FT-ICR MS analysis of interfacial material isolated from Athabasca bitumen shows that it is enriched in O₃S₁ species, with O₃S₁ as the most abundant heteroatom class. (-) ESI IM-TOF MS was performed on the same sample and mobiligrams extracted for nominal m/z (∼ 0.2 Da window) centered on O₃S₁ class species of interest. The IM-TOF results reveal broad asymmetrical peaks that suggest multiple structures with the same m/z. Ultrahigh resolution FT-ICR MS confirmed that the multiple structures are isomers, not isobars that are insufficiently resolved by TOF MS. Post-ion mobility CID yields separate product ion mass spectra for each isomer separated by mobility (each drift time) and mobiligrams extracted for diagnostic fragment ions can be used to deconvolve mobility peaks from parent isomers that are not fully resolved: e.g., ions of m/z 325.18 (C₁₈H₃₀O₃S, DBE 4) exhibit a broad asymmetrical mobility peak. Mobiligrams extracted for fragment ions from losses of H₂O (m/z 307.17), CO₂ (m/z 281.19) and CO₂ + H₂O (m/z 263.18) give a single mobility peak at 4.25 ms. Therefore, a C₁₈H₃₀O₃S isomer containing a carboxylic group elutes at 4.25 ms. A mobiligram extracted for fragment ions of m/z 183.01, from loss of C₁₀H₂₀, shows two mobility peaks at 4.57 and 4.89 ms, indicating that two other C₁₈H₃₀O₃S isomers are present. The resulting fragment C₉H₁₀SO₃ (DBE 4) is consistent with a benzenesulfonate (linear alkyl benzenesulfonates (LAS) are chemicals commonly added to petroleum as surfactants). Analysis of standard LAS mixture confirmed that the isomers in the sample at 4.57 and 4.89 ms are LAS (same drift times and fragment ions). Therefore, IM-MS(/MS) is able to characterize isomers in petroleum interfacial material and distinguish between anthropogenic and naturally occurring surfactants.

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TOP DOWN CHARACTERIZATION OF OXIDATION DURING HYDROXYL RADICAL PROTEIN FOOTPRINTING

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The bioanalytical characterization of higher order structure in therapeutic proteins has seen increasing interest due to the burgeoning market for novel originator drugs as well as follow-on biologics, also known as biosimilars. Although the determination of amino acid constituency and primary sequence can be routinely performed in academia as well as industry, the means to determine three dimensional conformations by mass spectrometry has not matured into practice. The determination of critical quality attributes (CQAs) including higher order structure is essential for the pharmaceutical industry. The covalent labeling of proteins by hydroxyl radicals provides a robust approach but methods are required to quantify the oxidation levels.

The analysis and quantitation of oxidation in hydroxyl radical protein footprinting (HRPF) [1] typically relies on a bottom-up approach by monitoring multiple channels that are possible during the experiment and can vary based upon the oxidized amino acid and three dimensional protein conformation. Oxidation sites can be easily be determined to the peptide level by mass difference alone, but locating the modification site requires tandem mass spectrometry. Recently, electron transfer dissociation (ETD) has been demonstrated as an attractive method to both locate the oxidation site as well as provide relative quantitation regardless of precursor ion charge at the peptide level [2].

To avoid the limitations of a bottom-up approach such as peptide recovery and sequence coverage, we have utilized top down mass spectrometry [3] to characterize oxidation during HRPF. Protein standards and biopharmaceutical proteins were prepared and diluted in water with glutamine and hydrogen peroxide added before UV irradiation (248 nm, >60mJ/pulse) generated by a KrF laser (EX100, GAM Laser Inc). The reacted volume was collected and quenched in methionine amide and catalase. Each protein of interest was irradiated in triplicate with one control performed without laser activation. In the test case of ubiquitin, multiple charge states are observed with multiple oxidation states. For each ETD experiment performed on an Orbitrap Elite (Thermo Scientific), a single charge state and accompanying oxidation states are mass selected and fragmented to locate the oxidation site to the amino acid level and quantitation is based on ion intensity ratios. Current efforts are focused on applying this workflow to a series of pharmaceutical proteins. By applying this methodology, the selection of multiple ion chromatogram traces due to varying oxidation levels of a given peptide can be avoided and the relative number preparative steps reduced. With the recent developments in MS technology and data processing for top down protein analysis, this approach provides a new method to quantify both oxidation extent at the protein level and modification site in a single experiment.

COUNTING THE NUMBER OF UNOBSERVED PEAKS IN A MASS SPECTRUM: THE LOG-NORMAL DISTRIBUTION

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Fourier transform mass spectrometry (FT MS) requires that the maximum number of trapped ions be limited in order to avoid loss of ion packet coherence due to space charge effects. Thus, for compositionally complex samples, such as petroleum crude oil, the signal-to-noise ratio for low-abundance ions may fall below a specified threshold (e.g., 6σ of rms baseline noise), thereby limiting dynamic range and restricting sample characterization. Dynamic range may be extended by summing mass spectra from (say) 100 digitized time-domain transient signals. However, the question remains as to how many peaks are unobserved due to insufficient signal-to-noise ratio.

In 2011, Enke and Nagels proposed that the number of unobserved peaks could be predicted by fitting the data to a log-normal distribution, namely, number of peaks within a narrow range of peak heights vs. the logarithm of that peak height. However, we found that log-normal plots of ultrahigh-resolution FT-ICR mass spectra of petroleum crude oil typically span only about half of the predicted number of peaks.

Here, we further extend dynamic range by separately accumulating ions in multiple, overlapped, narrow m/z ranges, so that more ions are present across a narrow m/z range. The mass spectral segments, with increased signal-to-noise ratio and corresponding increase in dynamic range, may then be stitched together to form a composite broadband mass spectrum that spans the full range of peak heights and is well-fitted by a log-normal distribution. Thus, it may not be necessary to segment the mass spectrum to count all of the possible peaks, if the number of unobserved peaks can be predicted accurately by fitting just the high-magnitude peak heights to a log-normal distribution.

The question then becomes, why should mass spectral peak heights follow a log-normal distribution? One reason is that the number of possible elemental compositions doesn’t increase monotonically with increasing mass resolution, because the maximum possible number of elemental compositions for any given nominal mass is finite. Moreover, the mass spectrometer response function will also contribute. We are currently testing the range of validity of the log-normal description for chemically defined mixtures (e.g., various heteroatom classes from petroleum).

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FOUR YEARS AFTER THE DEEPWATER HORIZON SPILL: MOLECULAR TRANSFORMATION OF MACONDO WELL OIL IN LOUISIANA SALT MARSH SEDIMENTS REVEALED BY FT-ICR MASS SPECTROMETRY

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Gulf of Mexico saltmarsh sediments were severely contaminated by Macondo well oil (MWO) released from the 2010 Deepwater Horizon (DWH) oil spill. Detailed characterization of samples collected up to 48 months post-spill reveals molecular-level transformations that occur to MWO contamination deposited in coastal saltmarshes in Barataria Bay and exposes the chemical diversity of highly polar, oxygen-containing compounds that remain environmentally persistent. Biotic/abiotic oxidative transformation of the MWO resulted in a multiplicative increase in compositional complexity of the weathered oil that precluded direct analysis by conventional analytical methods. Thus, electrospray ionization (ESI) Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) was employed to expose compositional changes at a molecular-level. Bulk elemental analysis highlights an increase in total oxygen content as a function of time, while solid phase extraction (SPE) of carboxylic acid-containing compounds by aminopropyl silica (APS) reveals that carboxylic acid functionalities comprise 9.4 wt.% of oil extracted 48 mos post-spill. Selective ionization of acidic compounds by negative ESI revealed a two-fold increase in molecular complexity after 9 mos compared to the parent MWO, followed by a decrease in mass spectral complexity up to 48 mos. Higher-order oxygenated compounds (O₄-O₆) increase in abundance over 48 mos, and initial/continued oxidation occurs across a range of O/C and H/C ratios. Surprisingly, many aromatic Oₓ species detected in early samples were not detected after 41 mos. Here, we present the first molecular-level characterization of temporal compositional changes that occur in Deepwater Horizon derived oil contamination deposited in a saltmarsh ecosystem from 9 to 48 mos post-spill, and reveal the recalcitrant nature of highly oxidized Macondo well oil compounds that are not detectable by routine GC-based techniques.
TOP-DOWN PROTEOMICS REVEALS CONCERTED REDUCTIONS IN MYOFILAMENT AND Z-DISC PROTEIN PHOSPHORYLATION AFTER ACUTE MYOCARDIAL INFARCTION

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Heart failure (HF) is a leading cause of morbidity and mortality worldwide and is most commonly presented by myocardial infarction (MI). However, the molecular changes driving cardiac dysfunction immediately after MI remain poorly understood. Myofilament proteins, responsible for cardiac contraction and relaxation, play critical roles in signal reception and transduction in HF. We hypothesize that post-translational modifications (PTMs) of myofilament proteins, which afford a mechanism for the beat-to-beat regulation of cardiac function, act in concert to regulate early molecular events in the post-infarction myocardium. Herein, we have developed a novel liquid chromatography-mass spectrometry (LC/MS)-based top-down proteomics strategy to comprehensively assess the PTM changes of key cardiac proteins in the myofilament subproteome extracted from a minimal amount of myocardial tissue with high reproducibility and throughput. The entire procedure that includes tissue homogenization, myofilament extraction, and on-line LC/MS analysis takes less than three hours. Notably, enabled by this novel top-down proteomics technology, we discovered a concerted significant reduction in the phosphorylation of three crucial cardiac proteins in acutely-infarcted swine myocardium: cardiac troponin I and myosin regulatory light chain of the myofilaments and, unexpectedly, enigma homolog isoform 2 (ENH2) of the Z-disc. Furthermore, high-resolution top-down MS comprehensively sequenced these proteins and pinpointed their phosphorylation sites. For the first time, we have characterized the sequence of the ENH2 and identified it as a phosphoprotein. ENH2 is localized at the Z-disc which has been increasingly recognized for its role as a nodal point in cardiac signaling. Thus our proteomics discovery opens up new avenues for investigation of concerted signaling between myofilament and Z-disc in the early molecular events in cardiac dysfunction and progression to HF, which can significantly contribute to the field of cardiovascular disease research.
EMPIRICAL CHARACTERIZATION OF FT-ICR CELL HARMONIZATION UTILIZING EXTERNAL SHIM ELECTRODES

Jared B. Shaw, Aleksey V. Tolmachev, Tzu-Yung Lin, Errol W. Robinson, David W. Koppenaal, Ljiljana Paša-Tolić

Development of a trapped ion cell that maximizes utilization of the homogeneous field region and creates a trapping electric field which approaches that of an ideal quadrupolar, or harmonic, electric field is required to take advantage of the recent advances in superconducting magnet technology (i.e. high magnetic field strength and homogeneity). We have previously outlined criteria for FTICR cell harmonization by minimizing the change in the radial electric field divided by radius \( (E_r/r) \), metric that defines the magnetron frequency, to achieve radial electric field uniformity.\(^1\)\(^2\) This method was used to design and evaluate a new approach to FTICR cell harmonization which utilized external shim electrodes that are electrically de-coupled from the excitation and detection electrodes.\(^2\) Herein, we present the empirical characterization of an externally shimmed cell configuration in which the trapping electric field penetrates through rectangular windows (the window cell) in the excite/detect electrode cylinder. Performance metrics (i.e. mass measurement accuracy, resolving power, dynamic range etc.) for analytes of interest will be presented as a function of a number of cell operating parameters, such as external shim potential, excitation radius, and the number of ions. Initial results at 12T magnetic field include the observation of isotopic fine structure for the peptide MRFA from a single transient acquisition (\( m/\Delta m >2,000,000 \) at \( m/z =526, \) and S/N >15 for isotopic fine structure peaks). Preliminary characterization was performed at 12T and will be extended to 21T once the system is operational.

FT-ICR COMBINED WITH GAS PHASE HDX, IRMPD, AND IM TO DISTINGUISH BETWEEN ISOMERIC STRUCTURES OF PEPTIDE ION FRAGMENTS

Árpád Somogyi, Vicki H. Wysocki

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Studies on peptide fragmentation mechanisms have been an important area of fundamental mass spectrometry that lead to the better understanding the role of the mobile ionizing proton (mobile proton model [1]). Although initial work has been performed on low resolution (e.g., Q-type hybrid instruments), more recently FT-ICR instruments with ultrahigh resolution have extensively used to confirm and/or correct chemical compositions of (isobaric) fragment ions. (For example, the b2 ion from protonated G_nR peptides (C_{5n+1}H_{9n+2}N_{2n+1}O, m/z 115.05020) is “isobaric” with an R related fragment (C_{5n+1}H_{11n+2}N_{2n+1}O, 115.08659), see Ref. [2]).

FT-ICR instruments allow us not only to determine ion masses (chemical compositions) with sub ppm accuracy but also to perform MS/MS fragmentation and ion-molecule reactions by efficiently trapping ions in the ICR cell. A relatively simple hydrogen/deuterium exchange (HDX) method was implemented in our laboratory with only minor hardware and software additions. The degree and kinetics of HDX depend on the structure of the trapped ions, thus structural isomers can be distinguished in the gas phase. HDX kinetics can be controlled by using different reagents, such as D_2O (slower rates) or ND_3 (faster rates).

In our presentation, we show some selected examples for using FTICR HDX experiments to distinguish between oxazolone (up to 3 exchanges) and diketopiperazine (only one exchange) structures of b_2 and b_3 fragments [3, 4], amino acid (i.e., potential metabolites) fragments [5]. We will also demonstrate the usefulness of FTICR HDX reactions for studying sequence scrambling [6] and producing specific gas-phase “guest-host” chemistry that can be used to distinguish between larger cyclic or linear b_n type ions [7]. Related and supporting results from variable wavelength IRMP and ion mobility (IM) will also be presented.

TITAN’S THOLINS: HOW CAN WE DETERMINE THE COMPONENTS OF ORGANIC “PARADISE”? A COMPREHENSIVE FTMS (ICR AND ORBITRAP) STUDY

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Saturn’s largest moon, Titan, is often described as an “organic paradise”. The term is used not for catching the attention but mostly because Titan’s atmosphere resembles the early (reductive) atmosphere of Earth. The great success of the Cassini-Huygens mission allowed us to directly detect ions and neutrals in Titan’s atmosphere by using, e.g., the ion-neutral mass spectrometer (INMS) mounted on the Cassini spacecraft. Complex organic materials found on the surface and within the haze layers of Titan are attributed to chemistry occurring in Titan’s tick N2/CH4 (ca., 98:2) atmosphere.

Unfortunately, the “in-situ” measurements on Titan have limitations due to limited mass resolution or mass range. Thus, high resolution mass spectrometers that are necessary to reveal important details of chemical composition and reliably distinguish “isobaric” ions are still to be build and sent to orbit. This is an enormous technical and engineering (and costly) challenge that will be overcome easily in near future. Therefore, using model reactions and ultrahigh resolution mass spectrometers in laboratories on Earth are, currently, the only way to obtain detailed and comprehensive information on ion-molecule processes and their products.

In our presentation, we show some selected results from our long term collaboration projects. Tholin samples were generated in an airtight, recently designed HV ultrahigh vacuum chamber and were harvested anaerobically. Ions from tholin samples were generated by using laser desorption/ionization (LDI) and electrospray ionization in both the positive and negative modes. Two ultrahigh resolution instruments were used: a Bruker 12 T FT-ICR and a Thermo LTQ-Orbitrap.

Tholin components have a general formula of CxHyNz. More saturated compounds (amines, imines) are detected in the positive mode, while more unsaturated compounds indicating compounds rich in CN group contribution. These differences can be easily visualize on van Krevelen plots. Many of the CxHyNz can be hydrolyzed with water and/or ammonia/ice water that leads to CxHyNzO_n molecules. Hydrolysis kinetics was measured from which Arrhenius activation energies have been determined indicating that oxygen incorporation can occur in a 3,000-10,000 year time frame even at around 100 K (surface temperature) [1]. Our recent study [2] showed that, overall, both ESI and LDI resulted in complex negative ion MS spectra that contains several hundreds of ions in the m/z range of 50-300. LDI produced more CxNz- ions, such as C10N5-, which we assigned as the pentacyanide cyclopentadiene ion that was supported by MS/MS measurements (CID, QCID, SORI, and IRMPD). In addition to the ultrahigh resolution MS and MS/MS results we will show supporting information obtained by variable wavelength IRMPD experiments and quantum chemical calculations.

PHOTOCHEMICAL CHANGES IN WATER ACCOMMODATED FRACTIONS OF MC252 AND SURROGATE OIL CREATED DURING SOLAR EXPOSURE AS DETERMINED BY FT-ICRMS

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To determine the effects of photochemical weathering of petroleum, surrogate and Macondo MC252 crude oils were exposed to solar radiation during the formation of Water Accommodated Fractions (WAF) in sterile seawater. Samples were incubated in either unfiltered sunlight, with ultraviolet radiation blocked (Photosynthetically Active Radiation [PAR] only), or in darkness in a temperature controlled water bath. WAFs were collected after one and six days of exposure. Fourier Transform Ion Cyclotron Resonance Mass Spectrometry (FT-ICRMS) analysis of acidic species formed in WAFs during exposure to sunlight were compared for the different treatments. While photochemical differences were observed between MC252 and surrogate oils, microbial production in seawater responded similarly to both WAFs from both types of oils with the majority of the inhibition resulting from oil exposure to visible light. Heteratom compound distributions varied between the two oils under various treatments (dark, PAR and full sun). In general, surrogate oil was photo-oxidized across a wider carbon number range compared to MC252.
KENDRICK MASS ANALYSIS AIDS IN UNDERSTANDING IMPORTANT MICROBIAL
REACTION PATHWAYS FOR THE DEGRADATION OF DISSOLVED ORGANIC MATTER IN
PEATLANDS

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Peatlands are major repositories of global carbon, storing CO₂ from the atmosphere as organic carbon. Global climate change is triggering the thaw of permafrost in boreal regions and although the fate of that stored carbon is uncertain, it may have major implications for climate feedback and the global carbon cycle. If thawing permafrost stimulates the enhanced degradation of old peat into CO₂, the resulting increase in CO₂ flux to the atmosphere could act as a positive feedback loop. This effect could be enhanced by increased emission of CH₄, which is a stronger greenhouse gas than CO₂. Alternatively, increased primary production in thawing permafrost regions may be increasing the sequestration of CO₂ as newly formed peat. Thus, the balance between the increased CO₂ sequestration by peat accumulation and the increased CH₄ flux will likely determine the ultimate effect of permafrost thawing on climate. It is thought that the substrates for microbial production of methane are largely found within the dissolved organic matter (DOM) pool. DOM in peatlands is a complex mixture of compounds reflecting the combined influences of vegetation inputs and the microbial activity that degrades the fresh organic matter. DOM from depth within peatlands mostly represents the recalcitrant microbial leftovers that are resistant to degradation and make up the bulk of accumulated peat. In the surface, the DOM pool is likely much more dynamic, representing, in addition to the recalcitrant microbial leftovers, new inputs from surface vegetation and partially degraded products of microbial metabolism. In this poster, we will show how Kendrick Mass analyses can be used to understand the microbial pathways most important for DOM decomposition in peatlands. Further, we will show that changes in those pathways across a permafrost thaw sequence demonstrate differences in the lability of available compounds, which correlate with methane fluxes.
CHROMATOGRAPHIC PRE-SEPARATION COMBINED WITH FT-ICR MASS SPECTROMETRY FOR PETROLEOMIC APPLICATIONS

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High field FT-ICR mass spectrometry has changed the utility and expectations of complex mixture analysis over the past decade.1 The inherent high resolving power and high mass accuracy enable direct elemental composition determination of tens of thousands of individual components by mass measurement alone.2 Modern ionization methods facilitate the selective ionization of components based coarsely on chemical functionality, which combined with FT-ICR MS, reveals neutral, acidic, basic, and aromatic contributions to complex mixtures at a molecular level. Chromatographic pre-separation facilitates isolation of petrogenic species based on chemical functionality (i.e. acids and bases) or structural motifs (i.e. saturates vs. aromatics3, or within the aromatic class by the number of aromatic rings3,4). When combined (targeted pre-separation, selective ionization, and ultra-high resolution mass spectrometry), they allow for unprecedented molecular insight into petroleum composition.

Petroleomics seeks to predict the behavior of a complex mixture through the elucidation of all of the individual components that comprise it. However, many classes of compounds are ineffectively, or not ionized by modern atmospheric ionization methods. Other components are of low relative abundance and co-ionized with highly abundant classes that mask their presence. Thus, the success of Petroleomics largely depends on an ever-expanding toolbox of separation, ionization, and data analysis techniques that enable the widest possible detection / identification window. Here we demonstrate results from several sample fractionation methods combined with a collection of state-of-the-art analytical techniques for the analysis of crude oils to highlight the current state of modern petroleomic analyses. Specifically, we address advances in the speciation of sulfur, oxygen, and metal-containing species in petroleum. Advanced data analysis and visualization are provided by a custom, comprehensive petroleum software package, Petro-Org®.

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