NATIONAL HIGH MAGNETIC FIELD LABORATORY

Florida State University
1800 East Paul Dirac Drive
Tallahassee, Florida 32310
nationalmaglab.org

17 April 2023

Colleagues,

On behalf of the National High Magnetic Field Laboratory and Florida State University, we welcome you to the 13th North American FT MS Conference! We hope that your stay in Key West is both personally and professionally rewarding. Topics span a broad range of techniques and applications, and we have scheduled several mixers throughout the meeting, to encourage discussions.

The primary effort for organizing the conference has been provided by the conference coordinators, Kimberly Mozolic and Krista Jemmott. They have done an excellent job with the many required logistical and personal arrangements. Should you need any assistance or have any issues that need to be resolved while at the conference, please see Kimberly or Krista at the registration table so that we can ensure that your experience at this conference is a most enjoyable one.

Your registration fee covers only a portion of the expenses of the 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 graduate student virtual 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,

Christopher Hendrickson
Director, Ion Cyclotron Resonance Program, NHMFL

Christopher Hendrickson, Director, ICR Program 850.644.0711 | hendrick@magnet.fsu.edu

Operated by Florida State University, University of Florida, and Los Alamos National Laboratory
Supported by the U.S. National Science Foundation and the State of Florida

- 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 in the poster room.

- 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 at the top of every hour until 11pm. There are two stops. First stop is at 20 minutes past the hour at Fausto's Food Palace at 522 Fleming Street (last pick up every evening at 11:20pm). The second stop is at 25 minutes past the hour at the Butterfly Conservatory at 1316 Duval Street (last pick up every evening at 11:25pm).

THANKS TO OUR SPONSORS

Platinum Sponsors

National High Magnetic Field Laboratory
https://nationalmaglab.org

ExxonMobil
www.exxonmobil.com

Silver Sponsors

ThermoFisher Scientific
www.thermoscientific.com

Bronze Sponsors

Bruker Corporation
www.bruker.com

Spectroswiss
www.spectroswiss.ch

Catherine Costello

Catherine E. Costello is a Professor in the Dept. of Biochemistry, Cell Biology and Genomics and holds secondary appointments in Physiology and Biophysics at BUSM and Chemistry on the CRC. She earned her AB at Emmanuel College, Boston, and PhD at Georgetown Univ., Washington, DC, and was a postdoctoral fellow and Senior Research Scientist at MIT. She founded the BU School of Medicine Center for Biomedical Mass Spectrometry in 1994. Her research centers on development and application of MS-based methods to study glycobiology, protein post-translational modifications and folding disorders, cardiovascular and infectious diseases, and bioactive lipids. Major current development projects include Mass Spectrometry Imaging using Matrix-Assisted laser Desorption/Ionization, with emphasis on its application to Chronic Traumatic Encephalopathy (CTE) and Traumatic Brain Injury (TBI); Ion Mobility Coupled to Mass Spectrometry, particularly for the characterization of glycans, glycoconjugates and protein complexes; and exploration of the fragmentation pathways for glycans and glycoconjugates initiated by Electron-based Dissociation methods (ExD). Current collaborative studies include glycan mapping and structural determinations in cancer, signalling, infectious diseases and archaea, protein post-translational modifications that are evidence of oxidative stress, investigations of HLA-DR antigen presentation in Lyme Disease and Rheumatoid Arthritis, MS-based studies of CTE and TBI, the adaptation of ExD to Ion Mobility MS, applications of Ion Mobility and cross-linking to understanding protein misfolding in systemic amyloid and prion diseases, and RNA binding proteins of Trypanosomes.

She has authored ~350 scientific papers. She is President of the International Mass Spectrometry Foundation, a member of the Boards of Directors of US-HUPO and the Malta Conferences Foundation, a Councilor of the American Chemical Society, and a member of several editorial and advisory boards. She served as President of the American Society for Mass Spectrometry (2002-04) and President of the Human Proteome Organization (2011-12). She has received the HUPO Awards for Discovery in Proteomics and Contributions to Proteomics, the IMSF Thomson Medal and the ACS Field and Franklin Award, and is an ACS Fellow.

Group members include PhD students, postdoctoral fellows, visiting scientists and undergraduate researchers

13th North American FTMS Conference

KEY WEST MARRIOTT BEACHSIDE HOTEL
Key West, Florida
April 30 - May 4, 2023

Conference Program

Sunday April 30 5:00 p.m.-10:00 p.m. Registration - Flagler Foyer

Session I. Flagler Ballroom

7:25 p.m. Welcome: Alan Marshall
National High Magnetic Field Laboratory/Florida State University

7:30 p.m. Evan Williams
University of California, *Berkeley*

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

Monday May 1 7:45 a.m. - 8:40 a.m. Breakfast - Flagler Terrace/Ballroom
Registration Open - Flagler Foyer

Session II. Flagler Ballroom

8:45 a.m. Ying Ge
University of Wisconsin-Madison

9:20 a.m. Xin Ma
Georgia Institute of Technology

9:55 a.m. Chad Weisbrod
National High Magnetic Field Laboratory

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

Session III. Flagler Ballroom

10:45 a.m. Helen Cooper
University of Birmingham, UK

11:20 a.m. Carlos Afonso
Universite de Rauen Normandie

11:55 p.m. Caitlyn Tressler
Johns Hopkins

12:30-1:30 p.m. Lunch - Flagler Terrace/Ballroom

Session IV. Flagler Ballroom

1:30 p.m. Jared Kafader
Northwestern University

2:05 p.m. Vicki Wysocki
The Ohio State University

2:40 p.m. Petr Novak
Institute of Microbiology, Czech Republic

3:10 p.m. Break - Flagler Terrace

3:20 p.m. Ljiljana Pasa-Tolic
Pacific Northwest National Laboratory

3:55 p.m. Robert Young
New Mexico State University

6:30-7:30 p.m. Dinner- Flagler Ballroom

Virtual Poster Presentations. Flagler Ballroom

7:30-8:45 p.m. Lightning Talks (5 minutes each)

8:45-11:00 p.m. Mixer

Tuesday May 2 7:30 a.m. - 8:25 a.m. Breakfast - Flagler Terrace/Ballroom
Registration Open - Flagler Foyer

Session V. Flagler Ballroom

8:25 a.m. Francisco Fernandez-Lima
Florida International University

9:00 a.m. Pierre Giusti
TotalEnergies

9:35 a.m. Lydia Babcock-Adams
National High Magnetic Field Laboratory

10:10 a.m. Jeffrey Agar
Northeastern University

10:45 a.m. Conference Photo on the beach

Open afternoon to explore Key West

Wednesday May 3	9:00 a.m.-9:55 a.m.	Breakfast - Flagler Terrace/Ballroom Registration Open - Flagler Foyer

Session VII. Flagler Ballroom

10:00 a.m. Will Kew
Pacific Northwest National Laboratory

10:35 a.m. Kristina Hakansson
University of Michigan

11:10 a.m. Yury Tsybin
Spectroswiss

11:45 a.m. Jesper Olsen
University of Copenhagen

12:30-1:30 p.m. Lunch - Flagler Terrace/Ballroom

Session VIII. Flagler Ballroom

1:30 p.m. Mike Goodwin
ThermoFisher Scientific

2:05 p.m. Caroline DeHart
Frederick National Laboratory for Cancer Research

2:40 p.m. Jon Amster
University of Georgia

3:15 p.m. Break - Flagler Foyer/Terrace

3:30 p.m. David Goodlett
University of Victoria

4:05 p.m. Brianna Garcia
Woods Hole Oceanographic Institute

4:40 p.m. Roman Zubarev
Karolinska Institutet

Session IX. Flagler Ballroom

5:15 p.m. Pre-Dinner Mixer

6:00 p.m. Cathy Costello
Boston University

7:00-10:00 p.m. Conference Banquet and Final Mixer
Hammock Beach

Thursday
May 4

Checkout and Depart by 11:00 a.m.

SPEAKERS

EVOLVING FT MS APPROACHES ENABLE ELUCIDATION OF GLYCAN AND GLYCOCONJUGATE STRUCTURES

Chaoshuang Xia[1], Juan Wei[1,2], M. Maissa Gaye[1], Cheng Lin[1], Catherine E. Costello[1]

[1]Center for Biomedical Mass Spectrometry, Boston University Chobanian & Avedisian School of Medicine, 670 Albany St, Boston, MA 02118-2646 USA, [2]Shanghai Jiao Tong University, 800 Dongchuan Road, Shanghai, 200240, China.

A primary focus of our laboratory is exploration of the detailed structures of glycans, glycoproteins, and glycolipids. We continuously seek to advance the capability of mass spectral methods to enable these determinations. We are currently using a SolariX FT-ICR MS, a Fusion Lumos Tribrid Orbitrap MS equipped with a Booster, and an Omnitrap combined with a QE-HF Hybrid Orbitrap MS [1] for our studies. Examples of ExD performance data from these systems will be presented and the outcomes contrasted. Recent applications that resulted in site-specific assignments of glycopeptiforms will also be discussed, including investigations of the binding mechanisms and intracellular transport of wt and variant SARS-CoV-2 spike protein, [2,3] glycosylation changes in α2β1 integrins involved in mutation-induced Primary Myelofibrosis [4], and determination of *N*-linked glycans on a novel *O*-fucosyl transferase [5].

[1] J. Wei, *et al*., *ChemRxiv*, Cambridge, 2022.
[2] R. Amraei, *et al*., *ACS Cent. Sci.,* 2021, *7*, 1156-1165.
[3] R. Amraei, *et al*., *Proc. Natl. Acad. Sci.* USA, 2022, *119*(6):e2113874119.
[4] M. Gaye, *et al.*, *Mol. Cell. Proteomics*, 2022, *21*(4):100213.
[5] H. van der Wel, *et al.*, *Glycobiology*, *2023,* in press.

SINGLE ION FTMS: MASS AND DYNAMIC MEASUREMENTS OF LARGE MOLECULAR ASSEMBLIES

[1]Conner C. Harper, [1]Emeline Hanozin, [1]Zachary M. Miller, [1]Matthew S. McPartlan, [1]Veena Avadhani, and [1]Evan R. Williams

[1]Department of Chemistry, University of California, Berkeley CA 94720

Heterogeneous samples can often be challenging to analyze with mass spectrometry especially when the masses of individual components extend beyond 100 kDa. Individual charge states produced by electrospray ionization can be difficult to resolve due to overlap in *m*/*z* from other components or from adduction from salts or other non-specific molecular interactions. Charge-state resolution has been attained for highly purified samples of virus capsids as large as 18 MDa [1] but the masses of more heterogeneous native viruses are more difficult to effectively measure with MS owing to spectral overlap. One demonstrated solution to this problem of sample heterogeneity for high mass analytes is to measure the charge as well of the *m*/*z* of ***individual*** ions so that the mass of each ion can be determined without interference from other ions. Single ion mass measurements have been performed with a variety of instrument types, but charge detection mass spectrometry with electrostatic ion traps [2,3] have the advantage of virtually unlimited mass range, single charge accuracy, and the ability to make dynamic measurements [3]. A primary challenge is to acquire single ion data sufficiently rapidly in order to make this method practical. Simultaneous individual ion measurements are possible, but overlapping ion signals can limit the effectiveness of this method. A method to measure ion energy *in situ* makes it possible to decouple ion frequency and *m*/*z* and eliminates the use of energy filters [3]. This method significantly increases the rate at which individual ion measurements can be made and factors affecting the acquisition rate for common applications involving adeno associated virus (AAV) characterization will be presented. Effects of ion-ion interactions on measurement precision will be presented. The use of *in situ* energy measurements to obtain information about the dynamics of ion dissociation processes will be discussed and illustrated using measurements of the fission of aqueous nanodrops in the 100+ nm diameter range. This droplet size range corresponds to the initial droplet size formed by nanoelectrospray using emitters with tip diameters of several microns. Implications of these results for ion formation in electrospray will be discussed.

[1] Snijder, J.; Rose, R. J.; Veesler, D.; Johnson, J. E.; Heck, A. J. *Angew. Chem., Int. Ed.* **2013**, *52*, 4020-4023.
[2] Todd, A. R.; Barnes, L. F.; Young, K.; Zlotnick, A.; Jarrold, M. F. *Anal. Chem.* **2020**, *92*, 11357-11364.
[3] Harper, C. C.; Elliott, A. G.; Oltrogge, L. M.; Savage, D. F.; Williams, E. R. *Anal. Chem*. **2019**, *91*, 7458-7465.

UNDERSTANDING PROTEOFORM BIOLOGY ENABLED BY HIGH-RESOLUTION MASS SPECTROMETRY-BASED TOP-DOWN PROTEOMICS TECHNOLOGIES

Ying Ge[1,2,3]

[1]Department of Cell and Regenerative Biology, [2]Department of Chemistry, [3]Human Proteomics Program, University of Wisconsin-Madison, Madison, WI, 53706

High-resolution mass spectrometry (MS)-based top-down mass spectrometry proteomics is a premier technology for comprehensive characterization of proteoforms that arise from genetic variations, alternative splicing, and post-translational modifications (PTMs), but myriad challenges remain. We have been developing novel technologies to address the challenges in top-down proteomics in a multi-pronged approach including new cleavable surfactants for protein solubilization, new strategies for multi-dimensional chromatography separation of proteins, novel nanomaterials for enrichment of low-abundance proteins. Recently, we have developed a new comprehensive user-friendly software package for native top-down proteomics. Moreover, we have made major advances in top-down proteomics for analysis of intact proteins directly purified from heart tissue, blood, and human pluripotent stem cell-derived cardiomyocytes (hPSC). Importantly, we have linked altered cardiac proteoforms to contractile dysfunction in heart diseases using animal models and human clinical samples. Furthermore, we are harnessing the power of innovative top-down proteomics with patient specific hPSC-derived cardiomyocytes (CMs) in engineered cardiac tissue to understand proteoform biology in cardiac diseases.

Recently, we have developed a hybrid native and denaturing top-down mass spectrometry (MS) approach employing both ultrahigh-resolution Fourier transform ion cyclotron resonance (FTICR)-MS and trapped ion mobility spectrometry (TIMS) quadrupole time-of-flight. We report the complete structural elucidation of intact O-glycan proteoforms in S protein regional-binding domain (S-RBD). FTICR-MS/MS provides in-depth glycoform analysis for unambiguous identification of the glycan structures and their glycosites. A total of eight O-glycoforms and their relative molecular abundance are structurally elucidated for the first time. More recently, we have utilized ultrahigh-resolution FTICR-MS for the comprehensive characterization of the whole cTn complex purified from endogenous human heart tissue enabled by nanoproteomics.

[1] Smith, L. M.; Agar, J. N.; Chamot-Rooke, J.; Danis, P. O.; Ge, Y., Loo, J. A.; Pasa-Tolic, J.; Tsybin, Y. O.; Kelleher, N. L. *Science Adv. 2021, 375*, 411-418.

[2] Melby, J.A.; Roberts, D.S.; Larson, E.J.; Brown, K.A.; Bayne, E.B.; Jin, S.; Ge, Y. Novel Strategies to Address the Challenges in Top-Down Proteomics *J. Am. Soc. Mass Spectrom.*, 2021, *32*, 1278-1294.

[3] Brown, K.A.; Melby, J.A.; Roberts, D.S.; Ge, Y *Expert Rev Proteomics*, 2020, *17*, 719-733.

[4] Tucholski, T.; Ge, Y.; *Mass Spectrometry Reviews*, 2022, *41*, 158-177

[5] Tiambeng T, Roberts DS, Brown KA, Zhu Y, Chen B, Wu Z, Mitchell SD, Guardado-Alvarez TM, Jin S, Ge Y. *Nature Commun.* 2020, *11*, 3903.

[6] Aballo, T. J.; Roberts, D.S.; Bayne, E. F.; Zhu, W.; Walcott, G.; Mahmoud, A. I.; Zhang, J.; Ge, Y. *J. Mol. Cell. Cardiol.* 2023, 176, 33-40

[7] Roberts, D.S.; Mann, M.; Melby, J.A.; Larson, E.J.; Zhu, Y.; Brasier, A.R.; Jin, S.; Ge, Y. *J. Am. Chem. Soc*., 2021, *143,* 12014–12024.

[8] Tucholski, T.; Cai, W.; Gregorich, Z.; Bayne, E.; Mitchell, S.; de Lange, W.; McIlwain, S.; Wrobbel, M.; Karp, H.; Hite, Z.; Vikhorev, P. G., Marston, S. B.; Lal, S.; Li, A.; dos Remedios, C.; Kohmoto, T.; Hermsen, J.; Kamp, T.; Ralphe J. C.; Moss, R.L.; Ge, Y.; *Proc. Natl. Acad. Sci. U. S. A.* 2020, *117*, 24691-24700.

[9] Brown, K. A.; Chen, B.; Guardado-Alvarez, T.; Lin, Z.; Hwang, L.; Ayaz-Guner, S.; Jin, S.; Ge, Y. *Nature Methods,* 2019*, 16*, 417-420.

FT-MS SPACE- AND TIME-RESOLVED METABOLOMICS OF AN OVARIAN CANCER MOUSE MODEL.

Xin Ma[1], Samyukta Sah[1], Andro Botros[2], Sylvia R. Yun[2], Eun Young Park[2], Olga Kim[2], Soojin Park[2], Thu-Huyen Pham[2], Grace Grimsley[3], Ruihong Chen[4], Murugesan Palaniappan[4], Martin M. Matzuk[4], Richard R. Drake[3], Jaeyeon Kim[2], Facundo M. Fernández[1]

[1]School of Chemistry and Biochemistry, Georgia Institute of Technology, 901 Atlantic Dr NW, Atlanta, GA; [2]Department of Biochemistry and Molecular Biology, Indiana University School of Medicine, Indianapolis, IN; [3]Department of Cell and Molecular Pharmacology and Experimental Therapeutics, Medical University of South Carolina, Charleston, SC; [4]Pathology & Immunology Center for Drug Discovery, Baylor College of Medicine, Houston, TX

Ovarian cancer (OC) is one of the deadliest cancers among women with no effective screening tools available, especially for early-stage diagnosis. Due to the lack of symptoms at its early stage, only a small fraction of OCs is diagnosed for effective treatment.[1] Current diagnostic tools, including transvaginal ultrasound and CA-125 blood tests do not provide sufficient sensitivity and specificity.[2] Furthermore, the detailed mechanism of OC progression and metastasis remains unclear. Herein, we conducted spatially resolved lipid and N-glycan profiling of ovarian cancer tissues collected from two mouse models[3],[4] using ultra-high resolution MALDI-FTICR mass spectrometry imaging (MSI). We combined these imaging experiments with longitudinal UHPLC-MS serum lipidomic experiments on the same mice carried out in an Orbitrap MS system, allowing us to develop an in-depth metabolic profile of the disease. Certain lipids and N-glycans were identified and selected as potential OC biomarkers; the spatial distributions and alterations of these species in the mouse reproductive system and their temporal variations yielded unique signatures that could prove useful in early disease detection.[4]

A total of 228 lipid features and 137 N-glycan features were putatively annotated in MSI experiments. Univariate ROC analysis identified 151 lipid features and 33 N-glycans as discriminant. PCA models were developed using the selected features and tumors at different stages collected from different mice were distinguished from the healthy control reproductive systems. Further identification approaches—including MS/MS experiments, Endo F3 enzyme digestion, and amidation-amidation sialic acid stabilization reactions—were conducted to gain more detailed structural information of lipids and N-glycans. Time-resolved UHPLC-MS serum analysis showed specific temporal trends for 17 lipid classes, amino acids, and TCA cycle metabolites, associated with HGSC progression. Altogether, our results show that the remodeling of lipid and fatty acid metabolism, amino acid biosynthesis, TCA cycle, and ovarian steroidogenesis are critical components of HGSC onset and development. These metabolic alterations were accompanied by changes in energy metabolism, mitochondrial and peroxisomal function, redox homeostasis, and inflammatory response, collectively supporting tumorigenesis. Co-registration of lipids and N-glycan images in the tissues is now being conducted to discover correlations between specific lipid(s) and N-glycan(s) and their impact on OC development.

[1] Dilley, J.; Burnell, M.; Gentry-Maharaj, A.; Ryan, A.; Neophytou, C.; Apostolidou, S.; Karpinskyj, C.; Kalsi, J.; Mould, T.; Woolas, R.; Singh, N.; Widschwendter, M.; Fallowfield, L.; Campbell, S.; Skates, S. J.; McGuire, A.; Parmar, M.; Jacobs, I.; Menon, U. *Gynecol. Oncol.* **2020**, *158*, 316–322.
[2] Kamal, R.; Hamed, S.; Mansour, S.; Mounir, Y.; Abdel Sallam, S. *Brit. J. Radiol.* **2018**, *91*, 20170571.
[3] Paine, M. R. L.; Kim, J.; Bennett, R. V.; Parry, R. M.; Gaul, D. A.; Wang, M. D.; Matzuk, M. M.; Fernández, F. M. *PLoS One* **2016**, *11*, e0154837.
[4] Sah, S. M., X.; Botros A.; Gaul D. A.; Yun S. R.; Park E. Y.; Kim O.; Moore S. G.; Kim J.; Fernández, F. M. *Cancers* **2022**, *14*, 2262.

A NOVEL QUATERNARY HYBRID Q-ORBITRAP-IT-FT-ICR MASS SPECTROMETRY PLATFORM

Chad R. Weisbrod[1], Jesse D. Canterbury[2], John P. Quinn[1], Lissa C. Anderson[1], Amy M. McKenna[1], Greg T. Blakney[1], Michael W. Senko[2], and Christopher L. Hendrickson[1]

[1]National High Magnetic Field Laboratory, 1800 East Paul Dirac Drive, Tallahassee, FL; [2]Thermo Fisher Scientific, 355 River Oaks Pkwy, San Jose, CA 95134

The current MagLab 21 T FT-ICR instrument is a hybrid dual linear RF ion trap coupled with a custom ion cyclotron resonance mass spectrometer. This flexible instrument configuration has empowered our users with unique combinations of mass resolving power, mass accuracy, dynamic range, speed, and ion structural characterization. However, more sensitive ion optics, more selective precursor ion isolation, improved ion capacity, and a more flexible programming interface are available to replace our aging Thermo Scientific LTQ Velos front-end. Here we describe the first quaternary hybrid mass spectrometry platform coupling a Thermo Fisher Scientific Orbitrap Eclipse Tribrid mass spectrometer with the MagLab's 15 and 21 T FT-ICR instruments (quadrupole, dual cell linear RF ion trap, Orbitrap, FT-ICR MS).

The Eclipse instrument hardware was used as received from the vendor. An auxiliary vacuum chamber port located on the dual linear RF ion trap chamber allows for direct coupling to our 15 and 21 T FT-ICR systems. A quadrupole ion guide reaches into the Eclipse vacuum chamber to shuttle ions from the ion trap to a multipole storage device and eventually the ICR cell. Customization of the Eclipse instrument control software was required to enable ion ejection from the ion trap to the newly added ICR hardware and to support communication between the Eclipse and the MagLab Predator data acquisition systems. The 21 T Eclipse hybrid system is for research purposes only.

The initial goal of this updated instrument platform is to improve performance of the MagLab 15 and 21 T systems with respect to sensitivity, mass selection for MS/MS, and the ability to multiplex data acquisition between the available mass analyzers. This will also provide our users the entire suite of modern data acquisition capabilities (e.g., real-time search, TMT SPS MS3). The Eclipse ion source is far more sensitive compared with our current Velos hardware, as demonstrated by independent LC-MS analysis of the Pierce Intact Protein Standard (IPS) mixture (IGF-1 LR3, thioredoxin, protein G, carbonic anhydrase II, protein AG, exo klenow) on the current Velos 21 T platform compared to the Eclipse stand-alone. We observed a 4-5x improvement in the source sensitivity as measured by the injection times with a fixed AGC target.

The mass selection capabilities of the Velos 21 T platform are ultimately limited by space charge within the dual linear RF ion trap, leading to a loss of efficiency for isolation windows below 5 Da and unwanted transmission of neighboring proteoforms close in m/z. Mass selection using the Ecilpse's hyperbolic quadrupole mass filter results in faster and more efficient ion isolation compared with the current Velos 21 T platform. In stand-alone experiments with the Eclipse, we demonstrate highly efficient ion isolation down to 1 Da on intact proteins without the incursion of proximal proteoforms. We will present data from this updated instrument platform exhibiting these and other performance improvements over the current Velos 21 T platform, including application to intact proteins and complex environmental mixtures.

NATIVE AMBIENT MASS SPECTROMETRY FOR *IN SITU* ANALYIS OF PROTEINS AND THEIR COMPLEXES

[1]Oliver J. Hale, [1]Emma K. Sisley, [1]James W. Hughes, [1]Peter D.E. Macey, [1]Yuying Du, and [1]Helen J. Cooper

[1]School of Biosciences, University of Birmingham, Edgbaston, Birmingham B15 2TT, UK

Native ambient mass spectrometry (NAMS) combines native mass spectrometry, an established technique in structural biology, and ambient mass spectrometry, in which biological substrates such as thin tissue sections are analysed directly with little or no sample preparation. The combined benefits of NAMS for analysis of protein assemblies and protein-ligand complexes include measurement of accurate mass and stoichiometry, identification of both protein and non-covalently bound ligands, together with information on spatial distribution. Recent efforts using liquid extraction surface analysis (LESA) and nanospray desorption electrospray ionization (nano-DESI), have advanced NAMS for the analysis of fresh frozen issue, allowing the spatial distribution of protein assemblies to be mapped. Endogenous protein assemblies and their constituents (including small molecule ligands) can be identified by top-down dissection of assemblies in the gas phase, potentially allowing the discovery of new protein-ligand interactions.

In this presentation, recent developments in NAMS will be discussed, including the analysis of integral membrane proteins and membrane-associated proteins, characterisation of protein-drug complexes formed *in vivo*, and application of NAMS for understanding the molecular mechanisms involved in the fatal neurodegenerative disease amyotrophic lateral sclerosis.

NEW FTICR BASED METHODS FOR THE CARACTERISATION OF PYROLYSIS OILS

Charlotte Mase[1,2,3], Julien Maillard[2,3], Christopher Rüger[3,4], Pierre Giusti[1,2,3], Carlos Afonso[1,3]

[1]Université de Rouen, INSA de Rouen, CNRS, IRCOF, Mont Saint Aignan Cedex, France
[2]TotalEnergies OneTech, TotalEnergies Research and Technology Gonfreville TRTG, BP 27, 76700 Harfleur, France
[3]International Joint Laboratory – iC2MC: Complex Matrices Molecular Characterization, TRTG, BP 27, 76700 Harfleur, France
[4]Joint Mass Spectrometry Centre / Chair of Analytical chemistry, University of Rostock, Dr.-Lorenz-Weg 1, 18059 Rostock, Germany

In the context of energy decarbonization, for the production of liquid fuels and circular economy for plastics recycling, the production of pyrolysis oils is an essential step in industrial processes. These pyrolysis oils are highly complex organic mixtures that present many differences compared to petroleum. Processing them to produce liquid fuels and new plastics requires perfect knowledge of their chemical composition. Different analytical approaches involving FTICR to characterize these pyrolysis oils will be presented, including the use of different ionization sources such as MALDI and APCI and hyphenation of FTICR with gas chromatography with an APPI interface. The focus is on the complementary nature of these different approaches in order to selectively ionize specific molecular families or to have an exhaustive view of the sample molecular content. For instance, we recently evidenced the interest of APCI for the characterization of lignocellulosic biomass based biooils that appeared to be particularly efficient to detect energy rich aliphatic molecules not detected by ESI and APPI.

[1] C. Mase, M. Hubert-Roux, C. Afonso and P. Giusti, Journal of Analytical and Applied Pyrolysis 2022, 167.
[2] C. Mase, J. F. Maillard, B. Paupy, M. Farenc, C. Adam, M. Hubert-Roux, C. Afonso and P. Giusti, Energy & Fuels 2021, 35, 14828-14837.

IDENTIFICATION OF COVID-19 N-GLYCOSYLATION AT 21T

Caitlin M. Tressler[1], Nicole Jenkinson[1], Karl Smith[2], Chad Weisbord[2], David Nauen[3], and Kristine Glunde[1,4,5]

[1]The Russell H. Morgan Department of Radiology and Radiological Science, [2]National High Magnetic Field Laboratory, [3]Department of Pathology, [4]The Sidney Kimmel Comprehensive Cancer Center, [5]Department of Biological Chemistry The Johns Hopkins University School of Medicine, Baltimore, MD, United States

Fatal COVID-19 disease, as a result of SARS-CoV-2 infection, results in specific lung pathology, namely, large amounts of immune cell infiltrate with diffuse alveolar damage consistent with acute respiratory distress syndrome (ARDS). This phenotype often outlasts the presence of the virus in patients. To date, no specific treatments are available for ARDS and only supportive care is available. Despite well reported pathology, the molecular mechanisms behind this pathology are poorly understood. In order to better understand the pathology behind COVID-19 disease, we have begun working with an age and sex matched set of post-mortem patient lungs examining hematoxylin and eosin (H&E) staining, N-glycan MALDI imaging, and MALDI immunohistochemistry (IHC).

Post-mortem COVID-19 patient lung samples (n = 10) and post-mortem controls without any major lung involving illness (n = 10) were selected for study with one COVID-19 patient and one control per slide. Tissues were digested with PNGase F and sprayed with 5 mg/mL CHCA matrix prior to imaging. All tissues were imaged on a Bruker RapifleX in positive reflectron mode with 200 laser shots per pixel with 50-micron spatial resolution. A mass range of m/z 600 to 3200 was collected. Glycans were identified using tandem MS experiments on a 21T FT-ICR with a spectroglyph source. Adjacent sections were H&E stained for co-registration. Data was analyzed in FlexImaging, GlycoWorkbench, and SCiLS Lab software.

Hematoxylin and eosin (H&E) stained tissue sections show typical COVID-19 pathology as previously observed while control uninfected samples show typical lung pathology. N-glycan imaging revealed an increase in N-glycans associated with COVID-19 infection. Observation was expected due to the increase in immune cell infiltrate associated with lung pathology. We have identified a number of N-glycans from the data generated on the 21T FT-ICR previously associated with inflammatory pathways including other infections and cancer. In general, these N-gylcans are increased in men more than women. Most notably we have identified four high mannose and complex N-glycans by high mass resolution and tandem MS which are increased in male patients. Female patients, despite having similar features by H&E stain, showed overall fewer N-glycans and many were similar to the amount observed in uninfected patients.

ORBITRAP ANALYZERS: TURNING AN M/Z-SPECTROMETER INTO A MASS-SPECTROMETER

Jared O. Kafader[1], John P. McGee[1], Pei Su[1], Kevin JooB[1], Benjamin J. Des Soye[1], Michael W. Senko[2], Philip D. Compton[3], Neil L. Kelleher[1]

[1]Norhtwestern University, Proteomics Center of Excellence, Evanston, IL; [2]Thermo Fisher Scientific, San Jose, CA; [3]Integrated Protein Technologies Inc., Evanston, IL

A close 6-year collaboration between the Kelleher group at Northwestern University and Thermo Fisher Scientific has fostered an important investigation about the use of individual ions for direct mass spectrometry analysis. It has been determined that detecting individual ion signals, rather than traditional packets of ions, advances Orbitrap-based resolution and charge detection capabilities. To this end, individual ion mass spectrometry alters spectral outputs directly into the Dalton domain to deconvolute complex protein mixtures and determine mass distributions for large virus-like particles ranging from 8 kDa to 6 MDa. Advanced applications for individual ion analysis include coupling MASS readouts with various front-end solutions encompassing direct tissue imaging, time-based separations, and automated sample handling. Direct mass technologies coupled with easy-to-handle analysis software advances charge detection capabilities to the scientific community at large.

ELECTRON CAPTURE-SID-CDMS CHARACTERIZATION OF PROTEIN COMPLEXES

[1]Vicki Wysocki, [1]Marius Kostelic, [1]Chen Du, [1]Andrew Arslanian, [1]Sophie Harvey

[1]Department of Chemistry and Biochemistry, Resource for Native MS Guided Structural Biology, Ohio State University, Columbus, OH 43210

Characterization of the overall topology and inter-subunit contacts of protein complexes, and their assembly/disassembly and unfolding pathways, is critical because protein complexes regulate key biological processes, including processes important in understanding and controlling disease. Tools to address structural biology problems continue to improve. Native mass spectrometry (nMS) and associated technologies such as ion mobility are becoming an increasingly important component of the structural biology toolbox. When the mass spectrometry approach is used early or mid-course in a structural characterization project, it can provide answers quickly using small sample amounts and samples that are not fully purified. Integration of sample preparation/purification with effective dissociation methods (e.g., surface-induced dissociation, SID), ion mobility, and computational approaches provide a MS workflow that can be enabling in biochemical, synthetic biology, and systems biology approaches. Native MS can determine whether the complex of interest exists in a single or in multiple oligomeric states and surface induced dissociation can provide characterization of topology/intersubunit connectivity, and other structural features. Examples will be presented to illustrate the coupling of SID to electron capture charge reduction and charge detection mass spectrometry on the Orbitrap UHMR for the characterization of protein and nucleoprotein complexes, including glycoproteins and adeno associated virus capsids. Complementarity with other structural tools will be illustrated.

[1] Snyder, Harvey, Wysocki, V. H., Chemical Reviews, 2022, 122, 8, 7442–7487.
[2] Karch, Snyder, Harvey, Wysocki Annu. Rev. Biophys. 2022. 51, 157–79.

UTILIZATION OF REACTIVE OXYGEN SPECIES AND TOP-DOWN MASS SPECTROMETRY FOR STRUCTURAL CHARACTERIZATION OF PROTEINS AND PROTEIN COMPLEXES

Marek Polak[1,2], Ghazaleh Yassaghi[1], Petr Halada[1], Daniel Kavan[1], Jan Fiala[1,2], Dmitry Loginov[1], Zdenek Kukacka[1], Michael Volny[1,2], Petr Man[1,2], Petr Novak[1,2]

[1]Institute of Microbiology of the Czech Academy of Sciences, Vestec, Czech Republic
[2]Faculty of Science, Charles University, Prague, Czech Republic

Protein footprinting coupled to mass spectrometry is commonly applied for protein structural studies, providing information on protein conformations and dynamics. [1] Traditional mass spectrometry approaches for structural elucidation include hydrogen deuterium exchange, chemical cross-linking, ion mobility and covalent labeling. Among these, hydroxyl radicals or singlet oxygen are perspective probes for the fast protein footprinting. There are different methods to generate them, and bottom-up mass spectrometry is the dominant method to identify modified amino acids and determine the solvent accessible area of proteins. Here, we present utilization of the Top-down sequencing for localization of modified residues within the protein structure.
For protein footprinting experiment, an excimer (248nm KrF) or a diode (650nm) laser was used to generate hydroxyl radicals or singlet oxygen in a quench flow set-up, respectively. The extent of the hydroxyl radical or oxygen incorporation was checked by high-resolution mass spectrometry (solariX XR 15T, Bruker Daltonics). When a significant oxidation of proteins (ubiquitin, CytC, AsLOV2, aMyo and hMyo, apoFOXO4 and holoFOXO4) was observed, intact, singly and doubly modified protein ions were isolated in quadrupole and the ions of interest were fragmented by a broad repertoire of techniques. CID and ETD were performed in the hexapole, while ECD and IRMPD in the ICR cell. Ms2links algorithm was used for annotation, and the lab-built software (OxIntComp) to calculate the extent of modification. [2] Detail quantification of modifications obtained from fragment spectra allowed to determine a) amino acids more exposed to the solvent when the heme (myoglobin) or DNA (FOXO4) is removed; b) the migration pathway of hydrogen peroxide-induced radicals from the heme site to the protein surface (CytC); and c) the oxidation damage of AsLOV2 and ubiquitin. [3-5]

[1] X. R. Liu, M. M. Zhang and M. L. Gross, *Chem Rev.,* 120, 4355-4454 (2020)
[2] G. Yassaghi, Z. Kukačka, J. Fiala, D. Kavan, P. Halada, M. Volný and P. Novák, *Anal Chem.*, 94, 9993-10002 (2022)
[3] M. Polák, G. Yassaghi, D. Kavan, F. Filandr, J. Fiala, D. S. Loginov, P. Halada, and P. Novák, *Anal Chem.*, 94, 3203-3210 (2022)
[4] N. Tomášková, P. Novák, T. Kožár, M. Petrenčáková, D. Jancura, G. Yassaghi, P. Man and E. Sedlák, *Int J Biol Macromol.*, 174, 413-423 (2021)
[5] M. Petrenčáková, F. Filandr, A. Hovan, G. Yassaghi, P. Man, T. Kožár, M. S. Schwer, D. Jancura, A. Plückthun, P. Novák, P. Miškovský, G Bánó and E. Sedlák, *Sci Rep.*, 10, 4119 (2020)
L

RECENT ADVANCES IN SPATIAL TOP-DOWN PROTEOMICS

Ljiljana Paša-Tolić[1], Kevin J. Zemaitis[1], Dušan Veličković[1], Mowei Zhou[1], James M. Fulcher[1], Yen-Chen Liao[1], Marija Veličković[1], Sarah M. Williams[1], David J. Degnan[1]; Lisa M Bramer[1], William R. Kew[1], Ying Zhu[2]

[1]Pacific Northwest National Laboratory, Richland, WA, USA
[2]Genentech Inc., South San Francisco, CA

Spatial biology holds a promise to unlock the mechanisms responsible for tissue and cellular organization. Proteins and proteoforms are of particular interest in establishing cellular identities because they are the primary effectors of biological function, and their modification state and/or abundance cannot be easily inferred from transcriptomics. Yet, measuring proteins in a few cells or a single cell remains a major challenge and key analytical objective. Current spatial proteomic approaches typically rely on the use of labels or antibodies, which limits multiplexing and requires *a priori* knowledge of protein targets. Conventional bottom-up proteomics has been recently demonstrated for proteome-wide analysis of small tissue sections. However, this approach cannot characterize proteoforms, the main determinants of cell phenotype. To address this challenge and globally map proteoforms within tissues, we integrated nanoPOTS top-down proteomics (TDP) and MALDI mass spectrometry imaging (MSI).

Fresh frozen tissue sections were analyzed using an elevated pressure MALDI source (Spectroglyph) coupled with a Q Exactive HF Orbitrap MS upgraded with ultra-high mass range (UHMR) boards.[1] Laser capture microdissection (LCM) was used to cut tissue microstructures (or functional tissue units, FTUs) from serial sections for TDP analyses. LCM nanoPOTS TDP was accomplished in parallel with MALDI imaging of tissues (e.g., human kidney, pancreas). The MALDI UHMR HF Orbitrap platform allowed us to complete high-throughput sampling at high mass (~70k at *m/z* 11306, 1.024 s transients) and spatial (15-30 µm) resolution. Confident TDP proteoform identifications served as a lookup table for MSI peak annotations using custom software (i.e., IsoMatchMS). Proteoform maps revealed several known gene/protein markers of FTUs (e.g., islet and acinar in pancreas) and cell type specific markers (somatostatin, glucagon, insulin, etc.), but also suggested unique spatial distributions of their specific proteoforms. For targeted validation of heavily modified proteoforms (e.g., histones), we are implementing UVPD TDP directly from tissue.[2] Our goal is the creation of a 3D proteoform images of FTUs and their integration with other maps (e.g., metabolites, lipids, transcripts) into a comprehensive biomolecular atlas providing a deeper understanding of human biology in health and disease.

[1] K.J. Zemaitis, D. Veličković, W. Kew, K.L. Fort, M. Reinhardt-Szyba, A. Pamreddy, Y. Ding, D. Kaushik, K. Sharma, A.A. Makarov, M. Zhou M, and L. Paša-Tolić L, *Anal Chem*. 94, 12604-12613 (2022).
[2] K.J. Zemaitis KJ, M. Zhou M, W. Kew W, and L. Paša-Tolić, *JASMS*, 34, 328-332 (2023).

EXPLORING THE LIMITS OF PFAS ANALYSIS: SUSPECT AND NONTARGETED SCREENING WITH ULTRAHIGH RESOLUTION 21T FT-ICR MS

Robert B. Young[1], Nasim E. Pica[2], Hamidreza Sharifan[3], Huan Chen[4], Holly K. Roth[5], John J. Kornuc[6], Christopher P. Higgins[7], Thomas Borch[5,8], Amy M. McKenna[4], Jens Blotevogel[9]

[1]Chemical Analysis & Instrumentation Laboratory, New Mexico State University, Las Cruces, NM; [2]Weston Solutions, Inc., Lakewood, CO; [3]Department of Natural Sciences, Albany State University, Albany, GA; [4]National High Magnetic Field Laboratory, 1800 East Paul Dirac Drive, Tallahassee, FL; [5]Department of Chemistry, Colorado State University, Fort Collins, CO; [6]NAVFAC EXWC, 1100 23rd Avenue, Port Hueneme, CA; [7]Department of Civil and Environmental Engineering, Colorado School of Mines, Golden, CO; [8]Department of Soil & Crop Sciences, Colorado State University, Fort Collins, CO; [9]CSIRO, Adelaide, South Australia

Comprehensive characterization of per- and polyfluoroalkyl substances (PFAS) is critical for environmental monitoring and remediation applications. PFAS are a large family of thousands of chemicals that have been extensively analyzed using nontargeted time-of-flight and Orbitrap mass spectrometry methods. 21T Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS) offers the highest available mass resolving power for complex mixture analysis, and sub-ppm mass errors across a wide molecular weight range. To explore its application to suspect and nontargeted screening, we developed a 21T FT-ICR MS method to screen for PFAS in an aqueous film-forming foam (AFFF) sample (9 247 detected ions), using a dissolved organic matter (DOM) sample from a high-altitude stream (27 229 detected ions) as a negative control. The developed method included suspect screening for known PFAS (±0.2 ppm mass error), molecular formula assignment with a targeted database (C, H, Cl, F, N, O, P, S; ≤ 865 Da; ±0.2 ppm mass error), CF_2 homologous series, ^{13}C, ^{34}S, and ^{37}Cl isotopologues, and Kendrick-analogous mass difference networks (KAMDNs). KAMDNs make it possible to visualize multiple characteristic mass differences simultaneously, facilitating the identification of isotopologues, substituted elements, CF_2- homologous series, and other homologous series in one connected network of related ions. When some of the ions can be tentatively identified through suspect screening, related ions can also be tentatively identified using the characteristic mass differences. Negative mass defects were common in the AFFF sample, and rare in the DOM sample, demonstrating that negative mass defects can be used to screen for some PFAS. False positive PFAS identifications in the DOM sample suggested that a minimum length of 3 should be imposed when annotating CF2-homologous series, and conflicting formula assignments at sub-ppm errors indicated the importance of using isotopologues and homologous series during formula assignment. We putatively identified 163 known PFAS and 47 known surfactants in the AFFF sample during suspect screening, and 134 more PFAS during additional nontargeted analysis. These results demonstrate that FT-ICR MS-based suspect and nontargeted screening methods can provide unique insights into complex PFAS compositions often encountered at AFFF-impacted sites.

[1] R. B. Young; N. E. Pica; H. Sharifan; H. Chen; H. K. Roth; G. T. Blakney; T. Borch; C. P. Higgins; J. J. Kornuc; A. M. McKenna; J. Blotevogel, Environ. Sci. Technol. 2022, 56 (4), 2455–2465 (2022).
[2] D. Tziotis; N. Hertkorn; P. Schmitt-Kopplin, European Journal of Mass Spectrometry, 17 (4), 415–421 (2011).

DESCRIPTION OF DISSOLVED ORGANIC MATTER TRANSFORMATIONAL NETWORKS USING TIMS-FT-ICR MS/MS AND GRAPH-DOM

Dennys Leyva[1], Muhammad Usman Tariq[2], Rudolf Jaffé[1], Fahad Saeed[2], and Francisco Fernandez-Lima[1,*]

[1]Department of Chemistry and Biochemistry, Florida International University, Miami,Florida 33199
[2]School of Computing and Information Science, Florida International University, Miami,Florida 33199

Dissolved Organic Matter (DOM) is an important component of the global carbon cycle. Unscrambling the structural footprint of DOM is key to understand its biogeochemical transformations at the mechanistic level. Although numerous studies have improved our knowledge of DOM chemical makeup, its three-dimensional picture remains largely unrevealed. The introduction of ultrahigh-resolution mass spectrometry (e.g., Fourier transform ion cyclotron mass spectrometry, FT-ICR MS) has significantly advanced our understanding of the DOM molecular makeup. Improvements in dynamic range, signal-to-noise ratio, ultrahigh mass resolving power, mass accuracy, and mass selection have distinguished FT-ICR MS as a unique technique for the analysis of complex mixtures.

A fundamental aspect for the structural DOM characterization is the need to separate and identify isomeric components, as well as to better understand the DOM transformation pathways. In 2020, trapped ion mobility spectrometry (TIMS) coupled to FT-ICR MS44 allowed for the first-time isomeric separation followed by chemical formula level fragmentation of a DOM sample, and potential isomeric structures were proposed[1]. The integration of a score system from in silico MS/MS fragmentation and structural screening based on experimental vs theoretical ion-neutral collisional cross sections (CCS) allowed for a structural assignment with reduced ambiguities. Despite the unique advantages of this methodology, its application to a large-scale study (e.g., thousands of chemical formulas) could be challenging and ultimately impractical for routine characterization. In 2022, a more feasible approach based on the neutral loss patterns of nominal mass isolated precursors using continuous accumulation of selected ions (CASI) followed by collision induced dissociation (CID) and FT-ICR MS detection was proposed[2-4].

In the present work, we further interrogate the DOM structural space by identifying the transformational networks common to four signature ecosystem derived DOM samples. We implement for the first time a comparison across DOMs based on isomeric mobility separation and neutral loss-based MS/MS fragmentation strategies for the identification of common and unique structural families. This unsupervised structural classification workflow allows for the structural footprint across aquatic DOMs. Examples are shown for the case of SPE-DOM samples from a Suwannee River fulvic acid standard (SRFA), a wetland sample from Brazil (Pantanal), and two end-member samples from a marsh-to-estuary transect in the Harnery River (Florida Everglades; HR-1 and HR-5).

[1] D. Leyva, R. Jaffe, F. Fernandez-Lima, Structural Characterization of Dissolved Organic Matter at the Chemical Formula Level Using TIMS-FT-ICR MS/MS, Analytical Chemistry, 92 (2020) 11960-11966.
[2] D. Leyva, M.U. Tariq, R. Jaffé, F. Saeed, F. Fernandez-Lima, Unsupervised Structural Classification of Dissolved Organic Matter Based on Fragmentation Pathways, Environmental Science & Technology, 56 (2022) 1458-1468.
[3] M.U. Tariq, D. Leyva Bombuse, F. Fernandez-Lima, F. Saeed, Graph Theoretic Approach for the Analysis of Comprehensive Mass-Spectrometry (MS/MS) Data of Dissolved Organic Matter, 2021.
[4] D. Leyva, R. Jaffé, J. Courson, J.S. Kominoski, M.U. Tariq, F. Saeed, F. Fernandez-Lima, Molecular level characterization of DOM along a freshwater-to-estuarine coastal gradient in the Florida Everglades, Aquatic Sciences, 84 (2022) 63.

MOLECULAR CHARACTERIZATION IN LITHIUM-ION BATTERIES: FROM ELECTROLYTES TO ELECTRODES.

Julien F. Maillard[1,4], Julien Demeaux[2], Charlotte Mase[1,3,4], Antonin Gajan[2], Cécile Tessier[2] Patrick Bernard[2], Carlos Afonso[1,4*] and Pierre Giusti[1,3,4*]

[1]Normandie Université, COBRA, UMR 6014 et FR 3038, Université de Rouen-Normandie, INSA de Rouen-Normandie, CNRS, IRCOF, Mont Saint Aignan Cedex.
[2]Saft, Corporate Research, 33074 Bordeaux, France
[3]TotalEnergies OneTech R&D, Total Research & Technology Gonfreville, BP 27, 76700 Harfleur, France.
[4]International Joint Laboratory - iC2MC: Complex Matrices Molecular Characterization, TRTG, BP 27, 76700 Harfleur, France.

The performances of lithium-ion batteries (LIBs) are closely related to the control of the electrolyte composition and solid-electrolytes interface (SEI) stability [1-2]. To decrease the capacity fading electrolyte formulation has been continuously optimized over years and much attention has been paid to identifying the SEI composition. However, deciphering the molecular diversity of both liquid electrolytes and solid interphase remains extremely challenging because few analytical techniques show the appropriate sensitivity and spatial resolution without modifying their composition. Here, Fourier transform ion cyclotron resonance mass spectrometry (FTICR-MS) was used for the first time to characterize at the molecular level both parts of LIBs. The liquid electrolytes were characterized using atmospheric pressure chemical ionization to cover a broad polarity range. Surprisingly, obtained mass spectra revealed hundreds of signals even if the initial electrolytes cocktail was prepared using 10 species. Using mass defect molecular maps, it was possible to delimit regions of interest for each component and easily perform reverse engineering. Two anodes coming from LIBs with different performances were analyzed using laser desorption ionization operated in imaging mode. After the subtraction of species coming from the carbon reference electrode, the unique contribution of each distinct SEI was observed and known reaction products of electrolytes were researched and identified.

[1] Larcher, D.; Beattie, S.; Morcrette, M.; Edstroem, K.; Jumas, J.-C.; Tarascon, J.-M. J. J. o. M. C. Recent findings and prospects in the field of pure metals as negative electrodes for Li-ion batteries. 2007, 17 (36), 3759-3772.
[2] Liu, Y.; Zhang, R.; Wang, J.; Wang, Y. J. I. Current and future lithium-ion battery manufacturing. 2021, 24 (4), 102332.

MASS SPECTRAL CHARACTERIZATION OF CU(II) TETRAPYRROLE LIGAND COMPLEXES PRODUCED BY A MARINE DIATOM

Lydia Babcock-Adams[1], Jingxuan Li[2], Amy McKenna[1,3], Christopher Hendrickson[1], and Daniel Repeta[2]

[1]National High Field Magnetic Laboratory; [2]Woods Hole Oceanographic Institution, Department of Marine Chemistry and Geochemistry; [3]Colorado State University, Department of Soil and Crop Science

Copper (Cu) is a required micronutrient for a variety of key enzymes. When concentrations of Cu are low it can limit essential cellular processes. However, if Cu concentrations are too high it is toxic, causing decreased cell growth and even death for many microbes. In laboratory culture experiments, it has been shown that a diverse array of microbes produces organic ligands that complex Cu[1]. These ligands may function as chalkophores, ligands used to acquire Cu under limiting conditions, or they may be used as a strategy to decrease Cu toxicity when concentrations are elevated. In seawater, dissolved Cu is almost entirely bound by organic ligands[2]; however, the identities of these compounds remain largely unknown. To better characterize Cu ligands, we grew cultures of the marine diatom, *Phaeodactylum tricornutum*, and analyzed the Cu ligand complexes using liquid chromatography (LC) coupled to two mass spectrometers (MS), an inductively coupled plasma (ICP) MS and an electrospray ionization (ESI) MS[3]. These analyses revealed that *Phaeodactylum tricornutum* produces a suite of Cu ligands. Using Fourier transform ion cyclotron resonance (FT-ICR) MS at 21T, we were able to obtain the high mass resolution and accuracy needed to assign molecular formulae to the Cu ligand complexes. Elemental composition analysis indicates that these compounds are tri- and tetra-pyrroles. High resolution MS/MS fragmentation experiments reveal that the Cu ligands are structurally related with labile carboxylic acid moieties.

[1] J. S. Lopez, L. Lee, and K. M. Mackey, *Front. Mar. Sci.*, 5 (2019).
[2] K. W. Bruland, R. Middag, and M. C. Lohan, “Controls of trace metals in seawater”, 19-51 (2013).
[3] R. M. Boiteau and D. J. Repeta, *Metallomics*, 7, 877-884 (2015).

SPECTRAL SIMULATION AND PEAK ASSIGNMENT STRATEGIES THAT ACCOUNT FOR THE PREPONDERANCE OF INTERNAL FRAGMENTATION IN TOP-DOWN MASS SPECTROMETRY (TDMS).

Novera Alam[1], Somak Ray[1], Jeffrey N. Agar[1]

[1]Chemistry and Chemical Biology and Barnett Institute, Northeastern University

We present a first principles model for simulating TDMS spectra based upon successive fragmentation events. This model accounts for, among other things, the collision energy dependence of the relative intensities of TDMS spectra. We also present a program, “FragMaps,” for assigning internal fragments, superimposing assignments upon the protein backbone, and even providing difference maps between two experiments. Internal fragments have unique ambiguities that affect their assignment. FragMaps disambiguates internal fragments using a variety of strategies, including fragmentation propensity, probabilities of successive events, frameshift alignment, and mass accuracy. TDMS spectra typically have few terminal fragment ions (e.g., b- and y-type) originating from the interior (i.e., middle) portion of proteins, yet abundant small terminal fragment ions. This “missing middle” was thought to result from a lack of fragmentation within protein’s interiors. By incorporating internal fragment assignment and our novel spectral simulator, we show that the relative intensities (e.g., abundant terminal fragments) typically observed in TDMS spectra can be reinterpreted as resulting from abundant fragmentation throughout a protein- specifically, as combination of successive fragmentation events and fragmentation propensity.

[1] Increasing Top-Down Mass Spectrometry Sequence Coverage by an Order of Magnitude through Optimized Internal Fragment Generation and Assignment.”ND Schmitt, JM Berger, JB Conway, JN Agar *Analytical Chemistry* 93 (16), 6355-6362 (2021).

ULTRA HIGH-RESOLUTION MASS SPECTROMETRY AND MASS SPECTROMETRY IMAGING FROM 7 TO 21 TESLA FT-ICR LEVERAGING FEQUENCY MULTIPLE DETECTION AND ABSORPTION MODE PROCESSING

William Kew[1], Gregory Vandergrift[1], Kevin Zemaitis[1], Dušan Veličković[1], Christopher Anderton[1], Ljiljana Paša-Tolić[1]

[1]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, WA, USA.

Mass spectrometry is always in pursuit of faster measurements at higher resolutions. This demand is driven a perennial need for improved throughput, faster measurements, and the characterization of complex samples. Conventional approaches to improve speed and resolution include stronger magnetic fields, frequency-multiple detection schemes, and absorption mode (aFT) signal processing. Here, we present results from three distinct instruments – two custom built FT-ICR mass spectrometers (21T with 1ω detection, 12T with 2ω detection), and a commercial 7T instrument (scimaX, Bruker Daltonics, with 2ω). Additionally, we explore the practical gains of aFT spectra through either post-acquisition processing or hardware-enabled phase corrected transients via the FTMS Booster X3 (Spectroswiss) coupled to the 21T and, for the first time, the 7T instruments.

As a demonstration of these capabilities, first we compare direct infusion characterization of complex environmental samples, highlighting the needs and challenges in this domain – including spectrum artifacts arising from aFT or 2ω detection. Additionally, we explore the complexities of mass spectrometry imaging (MSI), including a multimodal characterization of the mammalian lipidome by complementary ionization techniques (MALDI, NanoDESI) coupled to 21 Tesla with aFT processing. In this effort, we also explore data analysis challenges and novel opportunities associated with such rich, high-resolution spectra. With 21T MSI we achieve the theoretical performance gains of aFT (~2x resolution enhancement), yielding mass resolving powers greater than 600k at *m/z* 800 with just 1.5s transients.

CHEMICAL AND PHYSICAL STRATEGIES FOR ENABLING PROTEOMIC ANALYSIS OF TYROSINE SULFATION

Eunju Jang[1], Steven A. DeFiglia[1], Carson W. Szot[1], Menatallah M. Youssef[1,2], Kristina Håkansson[1]

[1]Department of Chemistry, University of Michigan, Ann Arbor, MI
[2]Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

Tyrosine sulfation is an acidic, highly labile posttranslational modification, introduced by Golgi-resident tyrosylprotein sulfotransferases (TPSTs) and playing key roles in, e.g., inflammation, immunity, and protein-protein interactions. However, characterization of tyrosine sulfation has been challenging due to partial or complete sulfonate (SO_3) loss during positive ion mode ionization and tandem mass spectrometry (MS/MS). We found that tryptic or basic sulfopeptides appear more stable than standard, acidic sulfopeptides. Additionally, we observed that electron capture/transfer dissociation (ECD/ETD) of arginine- or lysine-containing peptides show partial sulfate retention following activation without metals or other stabilizing adducts, which have previously been shown to be required, thus allowing sulfation site confirmation/determination in some cases.

Here, we describe chemical derivatization strategies to improve positive ion mode sulfopeptide stability and to maximize sequence coverage/sulfate retention in ECD/ETD MS/MS, including sulfopeptides with multiple tyrosine residues discovered in proteomic analysis of rat liver Golgi. We further describe alternative MS/MS activation methods, including negative ion free radical initiated peptide sequencing (nFRIPS) and negative ion ECD (niECD) for sulfopeptide anion analysis, which shows higher sulfate stability. In particular, only negative ion mode MS/MS allowed differentiation of isomeric tryptic TPST 1 singly sulfated tryptic peptides with three tyrosine residues.

IN-HARDWARE PHASED TRANSIENTS IN FTMS

Anton N. Kozhinov,[1] Konstantin O. Nagornov,[1] and Yury O. Tsybin[1]

[1]Spectroswiss, 1015 Lausanne, Switzerland

The FTMS data quality depends on the ion detection systems. Ion detection in FTMS instruments is achieved through induced current sensing followed by analog-to-digital conversion in data acquisition (DAQ) systems. These DAQ systems output the digitized time-domain transients.

Hardware architectures of current built-in DAQ systems often remain sub-optimal in performance, including phase distortions requiring post-acquisition phase correction, reduced resolution and duty cycle, and compromised S/N and sensitivity. A direct consequence of these limitations is restricted access to the absorption mode FT (aFT) mass spectra, even in modern FTMS instruments. Furthermore, the current generation of built-in DAQ systems, accompanied by rigid data acquisition control software, may inhibit experimental flexibility, for instance, by prohibiting the stable acquisition of ultra-short or ultra-long time-domain transients in broadband measurements.

Previously, we demonstrated that the state-of-the-art field-programmable-gate-array (FPGA) technology enables a new-generation DAQ architecture with powerful in-line digital signal processing helping to overcome the said disadvantages. The new-generation DAQ systems, now known as the FTMS Boosters, can directly yield the phased transients, which can be readily converted into the equally-informative aFT mass spectra. The flexibility of the external and in-parallel interfacing of the high-performance DAQ systems to commercial and custom FTMS instruments provides additional benefits. Here, we overview recent advances in the external phased transient acquisition and processing technology and its use in selected applications [1-6].

[1] K. Nagornov, M. Zennegg, A. Kozhinov *et al., JASMS*, 31, 2, 257-266 (2020)
[2] J. Bills, K. Nagornov, A. Kozhinov, *et al., JASMS*, 32, 5, 1224-1236 (2021)
[3] K. Nagornov, N. Gasilova, A. Kozhinov, *et al., Anal. Chem.,* 93, 38, 12930-12937 (2021)
[4] P. Kösling, C. Rüger, J. Schade, *et al*., *Anal. Chem.,* 93, 27, 9418–9427 (2021)
[5] G. Vandergrift, W. Kew, J. Lukowski, *et al., Anal. Chem*., 94, 8, 3629-3636 (2022)
[6] A. Kozhinov, A. Johnson, K. Nagornov *et al*., *Anal. Chem.,* 95, 7, 3712–3719 (2023)

PROTEOME-WIDE INFERENCE OF PEPTIDE COLLISIONAL CROSS-SECTIONS IN AN ORBITRAP MASS ANALYZER

Ulises Hernandez Guzman[1], Konstantin Ayzikov[2], Kyle L. Fort[2], Martin Rykaer[1], Jeppe Madsen[1], Ana Martinez Del Val[1], Alexander A Makarov[2]; Jesper V. Olsen[1,3]

[1]Novo Nordisk Foundation Center for Protein Research, University of Copenhagen, Denmark
[2]Thermo Fisher Scientific, Bremen, Germany
[3]Presenting author

The coupling of ion mobility devices to mass spectrometers (IM-MS) has facilitated accurate measurements of peptide collisional cross sections (CCS) adding an extra dimension to proteomics investigations. A method that does not require a dedicated ion mobility cell was recently described for determining CCS values of protein ions based on their decay rates in the time-domain transient signal in an Orbitrap mass analyzer [1,2]. Here, we present an extension of this strategy to infer CCS of peptides from complex samples in a high-throughput manner by introducing a minor hardware modification to a Thermo Scientific™ Orbitrap Exploris™ 480 MS. The hardware change enable us to control the pressure in the ultra-high vacuum (UHV) region within an order of magnitude. This strategy enables the inference of CCS values of peptides in complex mixtures without a dedicated ion mobility cell by using the observed decrease in full-scan resolution of individual peptide ions at high UHV pressures at high MS1 resolution or by the determination of the decay rate of the time-domain transient signal from an Orbitrap mass analyzer. Our data indicates that changes in the UHV pressure enable reproducible CCS inference, (median CV less than 1.3 %) using full-scan MS acquisition methods with 120,000 resolution setting typically used in shotgun proteomics. From HeLa cell digests, we have determined CCS values for >20,000 unique peptides including unique charge states and side chain modifications (Nt-acetylation and Methionine oxidation) at a globally controlled false discovery rate (FDR) of less than 1%. The comparison of inferred CCS values with both strategies and previous published CCS measurements determined by IM-MS showed strong linear correlation (Pearson coefficient around 0.9) between data sets and comparable separation of different peptide charge states in the CCS vs m/z space. The observed variation in the correlation between the inferred CCS values compared to corresponding IM-MS measurements is likely due to the different nature of ion collisions between the different methods.
Our results suggest that the conformation of peptides is likely to be determined by the balance between hydrophobic interactions driving compressed conformations and charge repulsion promoting extended conformations. Phosphopeptides have smaller CCS in general compared to their unmodified counter parts; this trend is particularly evident for higher charged peptides. Contrastingly, methylated and acetylated peptides have higher CCS values compared to their unmodified counterparts, probably due to the hydrophobicity increase and charge loss. We envision that this strategy can benefit the understanding and utility of CCS in proteomics workflows contributing to increased confidence in peptide, PTM site and protein identification.

[1] J. D. Sanders, D. Grinfeld, K. Aizikov, A. Makarov, D. D. Holden, J. S. Brodbelt. *Anal Chem*. 90(9):5896-5902 (2018)
[2] V. K. James, J. D. Sanders, K. Aizikov, K. L. Fort, D. Grinfeld, A. Makarov, J. S. Brodbelt. *Anal Chem*. 94(45):15613-15620 (2022)

IMPROVED SIGNAL PROCESSING FOR ORBITRAP CHARGE DETECTION MASS SPECTROMETRY

Michael Goodwin[1], Michael Senko[1], Kyle Bowen[1], Ping Yip[1], Dmitry Grinfeld[1]

[1]Thermo Fisher Scientific, 355 River Oaks Parkway, San Jose, CA

Charge Detection Mass Spectrometry (CDMS) has evolved as a powerful tool for studying large, heterogeneous species which are unresolvable by conventional mass spectrometry. The STORI (Selective Temporal Overview of Resonant Ions) concept was developed to better discern charge for CDMS by measuring the rate at which an ion accumulates signal over a specified time interval. Conventional STORI processing presumes a fixed frequency signal. If an ion changes mass during the detection period (e.g. from loss of non-covalently bound solvent inside the Orbitrap), the resultant frequency drift can lower the charge estimate by upwards of 20 percent. Since many large species may change mass during detection, various approaches are being taken to enhance STORI processing to accommodate variable frequency.

TOP-DOWN MS REVEALS AN EXPANDED RAS PROTEOFORM LANDSCAPE

Robert A. D'Ippolito[1], Kanika Sharma[1], Nicole Fer[1], Brian Smith[1], Mackenzie Meyer[1], Scott Eury[1], Abigail Neish[1], Katie Powell[1], Vanessa Wall[1], William Burgan[1], Dominic Esposito[1], Anna E. Maciag[1], Frank McCormick[1,2], Dwight V. Nissley[1], and Caroline J. DeHart[1]

[1]NCI RAS Initiative, Cancer Research Technology Program, Frederick National Laboratory for Cancer Research, Frederick, MD, 21702
[2]Helen Diller Family Comprehensive Cancer Center, University of California, San Francisco, CA, 94158

The RAS family of GTPases (HRAS, KRAS4A, KRAS4B, NRAS) are the most frequently mutated proteins in human cancer. However, due to their low endogenous abundance and high sequence identity, deriving associations between oncogenic N-terminal mutations and key C-terminal post-translational modifications (PTMs) on a given RAS isoform can be formidably challenging by peptide-based proteomic workflows. Conversely, top-down mass spectrometry (TDMS), which measures intact and modified protein forms (proteoforms), can precisely identify and confidently localize mutations and PTMs within each RAS isoform sequence. Moreover, by combining immunoprecipitation with TDMS (IP-TDMS), endogenous RAS proteoforms can be isolated to near-homogeneity from malignant cell lines, detected at high signal-to-noise ratios, and targeted for optimized MS2-based characterization to provide molecular detail simply unachievable by other proteomic methods.

To capitalize on this, the NCI RAS Initiative has developed an optimized RAS proteoform assay incorporating IP-TDMS analysis on an Orbitrap Fusion Lumos mass spectrometer. We initially employed recombinant versions of each RAS isoform to determine the optimal targeted MS2 fragmentation (tMS2) parameters for sequence characterization and localization of endogenous PTMs. We next isolated FLAG-tagged KRAS4B -WT, -G12D, and -G12C expression constructs from HeLa cells to refine our liquid chromatography and tMS2 parameters while targeting stoichiometrically abundant proteoforms within each population. We then isolated FLAG-tagged HRAS-, KRAS4A-, KRAS4B-, and NRAS-G12V proteins from HeLa or Panc1 cells for further refinement of isoform-specific tMS2 parameters with emphasis on capturing unusual or labile PTMs (e.g. palmitoylation). This allowed us to visualize and directly compare the most abundant RAS isoform-specific proteoform populations within these cellular contexts. Complementary peptide-based analyses were performed on all samples to validate RAS isoform-specific PTM landscapes and facilitate detection of their respective endogenous versions. Finally, we applied our optimized IP-TDMS workflow to a panel of well-characterized malignant and *RAS* mutant cell lines with the goal of expanding our knowledge of the endogenous RAS proteoform landscape.

In doing so, we identified a wealth of novel HRAS, KRAS4A, KRAS4B, and NRAS proteoforms bearing stoichiometrically abundant PTMs differing markedly from the established literature. We also performed direct comparisons between isoform- and mutation-specific RAS proteoforms, revealing clear indications of context dependence. These initial results further underscore the potential for top-down proteomics to broaden our understanding of RAS-dependent signaling within a range of cancer contexts. They also reveal that extensive investigations remain to be performed to elucidate the roles of RAS PTMs and proteoforms in normal and oncogenic signaling.

TOP-DOWN AND BOTTOM-UP FT-ICR-MS FOR STUDYING SITE-TO-SITE CROSSTALK IN PROTEIN GLYCOSYLATION

JONATHAN AMSTER[1], ROBERT V. WILLIAMS[1], JAMES H. PRESTEGARD[2], KELLEY W. MOREMEN[2]

[1]Department of Chemistry, University of Georgia, Athens, Georgia 30602
[2]Complex Carbohydrate Research Center, University Georgia, Athens, Georgia 30602

N-glycosylation is a common posttranslational modification of secreted proteins in eukaryotes. This modification targets asparagine residues within the consensus sequence, N–X–S/T. While this sequence is required for glycosylation, the initial transfer of a high-mannose glycan by oligosaccharyl transferases A or B (OST-A or OST-B) can lead to incomplete occupancy at a given site.[1] Factors that determine the extent of transfer are not well understood, and understanding them may provide insight into the function of these important enzymes. Here, we use top-down FT-ICR mass spectrometry (MS) to simultaneously measure relative occupancies for three N-glycosylation sites on the N-terminal IgV domain of the recombinant glycoprotein, hCEACAM1. We demonstrate that addition is primarily by the OST-B enzyme and propose a kinetic model of OST-B N-glycosylation. Fitting the kinetic model to the MS data yields distinct rates for glycan addition at most sites and suggests a largely stochastic initial order of glycan addition. The model also suggests that glycosylation at one site influences the efficiency of subsequent modifications at the other sites, and glycosylation at the central or N-terminal site leads to dead-end products that seldom lead to full glycosylation of all three sites.[2]

References
[1] P. Stanley, N. Taniguchi, M. Aebi, “N-Glycans” in *Essentials of Glycobiology*, A. Varki et al., Eds. (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY, 2017), chap. 9.
[2] Williams, R. V.; Huang, C.; McDermott, C.; Columbus, L.; Moremen, K. W.; Prestegard, J. H.; Amster, I. J. “Site-to-Site Crosstalk in OSTB Glycosylation of hCEACAM1-IgV”, *Proc. Natl. Acad. Sci. USA* 2022, *119*, e2202992119.

STRUCTURAL ELUCIDATION OF LIPOPOLYSACCHARIDES USING FAIMS AND KENDRICK MASS DEFECT PLOTS

Abanoub Mikhael[1,2], Darryl Hardie[2], Derek Smith[2], Helena Petrosova[1,2] and David Goodlett[1,2]

[1] Biochemistry and Microbiology, University of Victoria, Victoria, BC V8W 2Y2, Canada
[2] Genome BC Proteomics Centre, University of Victoria, Victoria, BC V8Z 7X8, Canada

Introduction. Lipopolysaccharides (LPS) are a hallmark virulence factor of Gram-negative bacteria consisiting of lipid A, core oligosaccharides, and O-antigen. LPS extracts represent complex, structurally heterogenous mixtures of conjoiners due to variations in number, type, and position of fatty acids and sugars. For these reasons LPS structural characterization by mass spectrometry (MS) is challenging due to the mixture of isomers and/or isobars. To improve LPS structural characterization, we describe the benefits of Field Asymmetric Ion Mobility Spectrometry (FAIMS) for LPS characterization. Additionally, classifying and sorting structurally related LPS molecules by Kendrick mass defect (KMD) plots will be presented.
Methods. Negative ion mode electrospray ionization was performed on an Orbitrap Fusion ETD Tribrid Mass Spectrometer with a FAIMS pro interface. Rough LPS of E. coli J5 Rc mutant (1 µg/µL) was directly infused into the FAIMS source. Spray voltage was set to 2.5 kV, and capillary temperature to 275 °C. Pseudo-MS3 was accomplished with source-induced dissociation (SID) at a potential difference of 100 V. Mass spectra at different FAIMS compensation voltages were viewed and deconvoluted using the Xtract tool in FreeStyle software. KMD plots were created by the Constellation online software.
Results. Use of FAIMS showed several benefits for the structural characterization of LPS. In the absence of FAIMS voltages, intensities of LPS multi-charged ions were suppressed in favor of singly-charged fatty acid and phospholipid contaminants. Additionally, mass spectra were complicated to interpret due to overlapped isotopic distributions. When FAIMS compensation voltages (CV) were applied, the singly charged contaminant ions were eliminated, allowing LPS ions of interest to be characterized post FAIMS fractionation.
Fractionation of LPS by varying CV enhanced LPS ion intensities needed for more accurate tandem MS characterization and increased the number of detected LPS species by approximately 1.3 fold as compared to characterization of same sample in absence of FAIMS voltages. Moreover, careful manipulation of the FAIMS CV allowed separation of one of the major LPS isobaric ions at *m/z* 1085.17 and 1085.20, leading to the tandem MS structural characterization of each ion separately. Interestingly, both ions have the same lipid A structure but a completely different inner oligosaccharide composition.
Besides separation and signal enhancement provided by FAIMS, extra fractionation and sorting of LPS ion families were accomplished using custom Kendrick mass defect (KMD) plots. The FAIMS-MS showed that LPS ions were detected mainly as deprotonated ions with the formula $(M - zH)^{-z}$ along with some minor sodiated clusters with the formula $[M - zH + n(Na - H)]^{-z}$, where n =1,2,3. These sodiated ion clusters created a highly organized KMD plot when [Na-H] was used as the base unit instead of the traditional CH2 unit. The latter KMD plot deciphered important structural relationships and/or mass differences between LPS species. Lastly, other custom KMD units that are known to differentiate between LPS molecules, such as phosphate and phosphoethanolamine, some of which involve antibiotic resistance, allowed novel structures to be detected.

CHEMICAL DERIVATIZATION CAPTURES ELUSIVE ENVIRONMENTAL BIOMOLECULES FOR DETECTION BY ULTRAHIGH PERFORMANCE LIQUID CHROMATOGRAPHY TANDEM MASS SPECTROMETRY (UHPLC-MS/MS)

Brianna M. Garcia[1], Cynthia C. Becker[1], Laura Weber[1], Krista Longnecker[1], Melissa C. Kido Soule[1], Gretchen J. Swarr[1], Kathryn Halloran[1] Amy Apprill[1], Elizabeth B. Kujawinski[1]

[1]Woods Hole Oceanographic Institution, Department of Marine Chemistry and Geochemistry, Woods Hole, MA, 02543 USA

Chemical cues constitute much of the language of life among marine organisms from microbes to mammals.[1] These chemical cues shape foraging and feeding strategies, symbiotic interactions, selection of mates and habitats, and the transfer of energy and nutrients within and among ecosystems.[1],[2] However, the full scope and magnitude of the chemicals involved in these processes are unknown, largely due to insufficient methods and analytical challenges. Ultrahigh-resolution mass spectrometry provides the capability to measure low-concentration mixtures of metabolites in complex matrices. However, environmental samples, such as seawater, can be particularly challenging due to the wide range of physicochemical properties and high levels of inorganic salts (mM) relative to the analytes of interest (µM to fM) resulting in decreased ion formation and spectral quality.[3] Common approaches for isolating dissolved organic matter (DOM) from seawater include tangential flow filtration, reverse osmosis/dialysis, and solid-phase extraction.[4],[5] Such methods alleviate the complications from inorganic salts but suffer from large volume requirements and a significant bias that varies by matrix and compound class. Current PPL SPE extractions of DOM in seawater result in extraction efficiencies <1% for many small polar biomolecules, which form the majority component of labile DOM.[3],[6] To overcome these challenges, we demonstrate how pre-extraction derivatization methods, targeting amine- and alcohol-containing functional groups, and ultrahigh-performance liquid chromatography coupled with electrospray ionization tandem mass spectrometry can be used to detect and quantify small, polar biomolecules dissolved in seawater and demonstrate an application of this method to elucidate previously undetected metabolites associated with the coral reef holobiont.[3]

[1] M. E. Hay, “Marine chemical ecology: chemical signals and cues structure marine populations, communities, and ecosystems,” *Ann. Rev. Mar. Sci.*, vol. 1, pp. 193–212, 2009.

[2] L. Wegley Kelly *et al.*, “Molecular Commerce on Coral Reefs: Using Metabolomics to Reveal Biochemical Exchanges Underlying Holobiont Biology and the Ecology of Coastal Ecosystems,” *Frontiers in Marine Science*, vol. 8, 2021, doi: 10.3389/fmars.2021.630799.

[3] B. Widner, M. C. Kido Soule, F. X. Ferrer-González, M. A. Moran, and E. B. Kujawinski, “Quantification of Amine- and Alcohol-Containing Metabolites in Saline Samples Using Pre-extraction Benzoyl Chloride Derivatization and Ultrahigh Performance Liquid Chromatography Tandem Mass Spectrometry (UHPLC MS/MS),” *Anal. Chem.*, vol. 93, no. 11, pp. 4809–4817, Mar. 2021.

[4] T. Dittmar, B. Koch, N. Hertkorn, and G. Kattner, “A simple and efficient method for the solid-phase extraction of dissolved organic matter (SPE-DOM) from seawater,” *Limnol. Oceanogr. Methods*, vol. 6, no. 6, pp. 230–235, Jun. 2008.

[5] M. A. Moran *et al.*, “Microbial metabolites in the marine carbon cycle,” *Nat Microbiol*, vol. 7, no. 4, pp. 508–523, Apr. 2022.

[6] W. M. Johnson, M. C. Kido Soule, and E. B. Kujawinski, “Extraction efficiency and quantification of dissolved metabolites in targeted marine metabolomics,” *Limnol. Oceanogr. Methods*, vol. 15, no. 4, pp. 417–428, Apr. 2017.

ISOTOPES IN FT MS: THE TIME IS RIPE, RIGHT? MAYBE

[1]Hassan Gharibi, [1]Xuepei Zhang, [1]Yaroslav Lyutvinskiy, [1]Roman A. Zubarev

[1]Division of Chemistry I, Department of Medical Biochemistry & Biophysics, Karolinska Institutet, Stockholm, Sweden

Over the past few years it became abundantly clear that isotopic ratios (IRs) can be measured on a FT MS instrument with a precision comparable with that in routine IR MS analysis. In particular, the FT IsoR MS approach [1,2] provides IR measurements on immonium ions of 3-5 most abundant amino acids or residues in proteins for C, H, N and O. The method is particularly useful for samples with grossly deviating IRs compared to normal isotopic abundances [2,3], as for such samples no standards with similar IRs are available.

Here we review the state of the art of IR MS with FT for proteins and outline challenges for future development. The limitations of FT IsoR MS and the potential of the isotopic distributions of molecular ions will be discussed.

[1] Gharibi, H.; Chernobrovkin, A.; Saei, A. A.; Zhang, X.; Gaetani, M.; Makarov, A.; Zubarev, R. A. *Proteomics-compatible Fourier Transform Isotopic Ratio Mass Spectrometry of Polypeptides*, *Anal Chem* 2022, *94*, 15048–15056.
[2] Gharibi, H.; Chernobrovkin, A.; Eriksson, G.; Saei, A. A.; Timmons, Z.; Kitchener, A. C.; Kalthoff, D.; Lidén, K.; Makarov, A. A.; Zubarev, R. A. *Abnormal (hydroxy)prolines deuterium content redefines hydrogen chemical mass, J Am Chem Soc* 2022, PMCID# PMC8855419.
[3] Popovic, Z.; Anderson, L.; Zhang, X.; Butcher, D.; Blakney, G.; Zubarev, R.; Marshall, A. *Analysis of Isotopically-Depleted Proteins Derived from E. coli and C. elegans Cell Lines by Liquid Chromatography 21 Tesla Fourier Transform-Ion Cyclotron Resonance Mass Spectrometry, J Am Soc Mass Spectrom* 2023, *34*, 2, 137–144.

SPONSORS

THE USE OF MULTIPLE DETECTION ELECTRODES IN ELECTROSTATIC LINEAR ION TRAPS

[1]Michael W. Senko

[1]Thermo Fisher Scientific, San Jose, CA

Electrostatic Linear Ion Traps (ELIT) have grown in popularity in the mass spectrometry community due to the high performance that can be obtained with a low level of instrumental complexity [1]. ELIT's are also the basis for many Charge Detection Mass Spectrometers (CDMS) [2-4], which are of expanding importance due to the ability to easily characterize complex biopharmaceuticals and drug delivery vehicles like adeno-associated viruses (AAV).

Although ELIT's provide relatively high performance for a simple instrument, the achievable resolution falls short when compared to FTICR and Orbitrap instruments. To improve ELIT resolution, there have been attempts to use multiple detectors, which would allow the generation of a higher frequency signal than what is achieved with a single detector [5]. This is meant to mimic the successful use of multiple detectors with FTICR's [6,7].

Efforts with ELIT's have produced limited success, due to a fundamental difference of the ion motion and detector geometries of the two analyzers. While the use of multiple detectors in ICR can generate integer multiples of the cyclotron frequency, the detectors in ELIT's generate a high frequency modulation of the oscillation frequency. When using Fourier analysis, the modulation results in splitting, or sidebands of the fundamental, and not the desired multiplication that would provide higher resolution. ELIT's with multiple detectors may still prove to be beneficial if they are combined with non-FT based processing techniques [8,9].

[1] Zajfman, Daniel, et al. "High resolution mass spectrometry using a linear electrostatic ion beam trap." International Journal of Mass Spectrometry 229.1-2 (2003): 55-60.
[2] Benner, W. Henry. "A gated electrostatic ion trap to repetitiously measure the charge and m/z of large electrospray ions." Analytical Chemistry 69.20 (1997): 4162-4168.
[3] Keifer, David Z., Elizabeth E. Pierson, and Martin F. Jarrold. "Charge detection mass spectrometry: weighing heavier things." Analyst 142.10 (2017): 1654-1671.
[4] Elliott, Andrew G., et al. "Mass, mobility and MS n measurements of single ions using charge detection mass spectrometry." Analyst 142.15 (2017): 2760-2769.
[5] Dziekonski, Eric T., Robert E. Santini, and Scott A. McLuckey. "A dual detector Fourier transform electrostatic linear ion trap utilizing in-trap potential lift." International Journal of Mass Spectrometry 405 (2016): 1-8.
[6] Rockwood, Alan L., et al. "Resolution improvement in an ion cyclotron resonance mass spectrometer." U.S. Patent No. 4,990,775. 5 Feb. 1991.
[7] E. N. Nikolaev, M.V., Gorshkov, A.V., Mordehai, V.L. Talrose, USSR Inventors Certificate, No. 1307492, h01j 49/38 (1985).
[8] Ding, Li "Mass S[pectrometric Analyzer." U.S. Patent No. 8,294,085 23 Oct. 2012.
[9] Elliott, Andrew G., et al. "Single particle analyzer of mass: a charge detection mass spectrometer with a multi-detector electrostatic ion trap." International journal of mass spectrometry 414 (2017): 45-55.

FTMS DATA SIMULATIONS IN PROTEIN ANALYSIS

Konstantin O. Nagornov,[1] Anton N. Kozhinov[1], Yury O. Tsybin[1]

[1]Spectroswiss, 1015 Lausanne, Switzerland

Peptide and protein data analysis in FTMS typically begins with mass spectra deconvolution and deisotoping. The obtained monoisotopic or average mass lists are then searched against the suspect database by mass accuracy, retention time, fragments, and isotopic ratios. An alternative approach is to do the inverse, namely to start with the suspect database, targeted or large-scale, simulate the isotopic envelopes in diverse charge states, and identify compounds directly from the experimental data by their signals correlation with the simulated data. Here, we report on the development of the related algorithms and software tools, compare the outputs of both approaches and demonstrate the latter's benefits, focusing on a glycoprotein analysis.

Our approach utilizes the power of the mass spectra being accurately simulated for the employed Orbitrap and ICR mass spectrometers and their experimental settings. The FTMS mass spectra in profile mode are generated *in-silico* using the FTMS Simulator software tool (Spectroswiss) for the given experimental parameters and FTMS instrument type and model using user-supplied suspect databases containing peptide and protein sequences and possible modifications [1, 2]. The feature extraction and identification process is based on the similarity scoring between the experimental and simulated data's resolved or unresolved isotopic envelopes.

The presented approach and tool, supported with a graphical user interface, are implemented in our Peak-by-Peak Multiomics software package. Examples of application in protein and peptide modifications analysis demonstrate high analytical specificity, sensitivity, and quantitative precision. In particular, analysis of N- and O-glycosylated tryptic peptides from monoclonal antibodies demonstrated the identification and quantitation of modified species in a wide dynamic range using the MS-only data. The MS/MS data acts as an additional results validation filter. The approach intrinsically supports automation and provides transparent data processing and interpretation even for non-expert users.

[1] K. N. Nagornov, A. N. Kozhinov, N. Gasilova, L. Menin, Y. O. Tsybin, *J. Am. Soc. Mass Spectrom.,* 31, 9, 1927–1942 (2020)
[2] K. N. Nagornov, A. N. Kozhinov, N. Gasilova, L. Menin, Y. O. Tsybin, *J. Am. Soc. Mass Spectrom.,* 33, 7, 1113–1125 (2022)

STUDENT AWARDS

MALDI COUPLED WITH NEGATIVE ION ELECTRON CAPTURE DISSOCIATION (ECD) FOR CHARACTERIZATION OF LABILE ACIDIC BIOMOLCULES

Steven A. DeFiglia[1], Kristina Håkansson[1]

[1]Department of Chemistry, University of Michigan, 930 N. University Avenue, Ann Arbor, Michigan

Acidic chemical groups, e.g., phosphorylation/sulfation, are common constituents of biomolecules, including peptides, lipids, and glycans. To maximize precursor ion signal in tandem mass spectrometry (MS/MS) experiments, negative mode ionization is preferred; however, collision induced dissociation often shows poor structural information including preferential loss of such labile groups. Thus, alternative MS/MS activation approaches are needed. niECD [1] has been proposed to require gaseous zwitterionic structures and is most compatible with singly charged anions. The latter characteristic suggests that MALDI may be superior to electrospray ionization (ESI) for niECD implementation; however, the former characteristic suggests that MALDI may not be appropriate. Here, we show that niECD indeed proceeds for MALDI-generated gaseous anions for structural characterization of, e.g., isomeric sulfopeptides.

Experiments were performed on a Bruker SolariX FT-ICR instrument with a MALDI source and a ParaCell analyzer. The ECD cathode was set at 1.6 A, - 6 V bias for a 5 second irradiation period. Peptides (sulfohirudin, β-casein phosphopeptide and synthetic sulfopeptide isomers of tyrosylprotein sulfotransferase and ST6 beta-galactoside alpha-2,6-sialyltransferase 1 were diluted from aqueous stock solutions to 10 μM in 30% acetonitrile/70% 0.1% aqueous trifluoroacetic acid. Peptides were combined with matrix (75% methanol: 25% isopropanol) and spotted in 0.5 μL aliquots on a stainless steel MALDI plate. Matrices included α-cyano- hydroxycinnamic acid, 2,5-dihydroxybenzoic acid, 1,8-diaminonapthalene, 9-aminoacridine, and 1,8-bis(diamino) napthalene (proton sponge). The sulfopeptide hirudin, previously shown to undergo efficient niECD for ESI-generated singly charged anions [2], was first used to explore whether MALDI-generated anions would be compatible with niECD. An original hypothesis was that the different matrices would yield different amounts of niECD-compatible zwitterionic gaseous structures with proton sponge being particularly problematic. However, surprisingly, all matrices tested yielded similar niECD spectra, in favor of the lucky survivor rather than the gas phase proton transfer MALDI mechanism. MALDI also allowed niECD of singly deprotonated β-casein phosphopeptide for a significantly improved niECD outcome compared with the doubly deprotonated species previously examined following ESI. A similar outcome was seen for the three TPST1 sulfopeptide isomers. The corresponding peptide sequence was observed with a single sulfate in a sulfoproteomic experiment, however the sulfation site could not be determined with any positive ion mode MS/MS technique [3]. MALDI yielded greater abundance of the niECD-favored singly charged anions from the same amount of sample as previous ESI-niECD experiments [3]. The latter experiments only allowed sulfation site determination for the first TPST1 sulfopeptide isomer with limited fragmentation observed for the other two isomers. By contrast, MALDI-niECD yielded rich fragmentation with accompanying sulfation site determination for all three isomers. In addition, MALDI-niECD allowed differentiation of the ST6GAL1 sulfopeptide isomers with adjacent tyrosines. Additional experiments will focus on MALDI-niECD of phosphoinositol lipids and glycosaminoglycans.

[1] Yoo, H.J.; Wang, N.; Zhuang, S.; Song, H. Håkansson, K.; *J. Am. Chem. Soc*. **2011**, *133*, 16790–16793.
[2] Hersberger, K.E.; Håkansson, K. *Anal. Chem*, **2012**, *84*, 6370–6377.
[3] Jang, E., DeFiglia, A. S.; Håkansson, K. Radical Driven Tandem Mass Spectrometry for Sulfotyrosine Site Determination in Cationic and Anionic Peptides with Multiple Tyrosine Residues. *Proc. 70th Annual ASMS Conference on Mass Spectrometry and Allied Topics*; Minneapolis, 2022.

MOLECULAR CHARACTERIZATION OF SOLUBLE ORGANIC MATERIAL FROM METEORITES BY 21T FT-ICR MS

Joseph Frye-Jones[1,2], Martha Chacón-Patiño[1,3], Ryan Rodgers[1,2,3,4], and Alan G Marshall[1,2]

[1]National High Magnetic Field Laboratory, Florida State University, 1800 E Paul Dirac Drive, Tallahassee, Florida, 32310
[2]Department of Chemistry and Biochemistry, Florida State University, 95 Chieftan Way, Tallahassee, Florida 32306
[3]International Joint Laboratory iC2MC: Complex Matrices Molecular Characterization, TotalEnergies Research & Technology, Gonfreville, 76700 Harfleur, France
[4]Université de Pau et des Pays de l'Adour, E2S UPPA, CNRS, IPREM, Institut des Sciences Analytiques et de Physico-chimie pour l'Environnement et les Materiaux, UMR5254, Hélioparc, 64053 Pau, France

Stony chondrite meteorites are the most common type of meteorites that fall to Earth. Of those, three percent are carbonaceous chondrites, which contain the highest concentration of organic material of all classifications of meteorites. Previous studies on their composition have primarily focused on bulk elemental composition and targeted analysis of specific compounds such as amino acids, carbohydrates, and other precursors to molecules essential to life.1,2 Non-targeted analysis is rarely performed as meteorites are complex mixtures of presumably tens of thousands of molecular formulas and thus, require ultrahigh-resolution techniques for characterization.3,4 Here, we utilize 21 tesla Fourier-transform ion cyclotron resonance mass spectrometry (FT-ICR MS) to characterize the soluble organic material from two meteorites, Murchison and Aguas Zarcas. In 1969, the Murchison chondrite fell in Murchison, Australia, and became one of the most studied meteorites to date. The rapidity at which it was collected minimized terrestrial contamination, and over 100 kilograms of material was recovered. Aguas Zarcas chondrite is a relatively new meteorite that hit a doghouse in rural Costa Rica in 2019, and as such, very little is known about this meteorite.

A range of solvents with diverse eluent strengths was used to extract the organic material from the two meteorites. Analysis was performed by positive- and negative-ion electrospray ionization (ESI) coupled to 21T FT-ICR MS. Formic acid and tetramethylammonium hydroxide were used as modifiers to aid during the ionization process. Over 30,000 peaks were detected within each mass spectrum, which were also assigned unique molecular formulas sorted in various heteroatom classifications such as CHNOS, CHOMg, and CHO, among others. As previously seen for terrestrial complex mixtures, the more polar, protic solvents extracted more material. Oxygenated compounds were detected in high relative abundances. More specifically, acidic O2 species were observed in high abundances, up to fifteen percent of assigned peaks, when the analysis was performed in negative-ion ESI assisted by a deprotonating modifier. Conversely, positive-ion ESI revealed a higher relative abundance of NOx and SOx species. Detected compounds overall show species with lower aromaticity, carbon numbers ranging from C10 – C60, as well as high oxygen content with up to O16 in a few molecular formulas.

[1] Monroe, A. A.; Pizzarello, S. Geochim. Cosmochim. Acta 2011, 75 (23), 7585–7595.
[2] Elsila, J. E.; Callahan, M. P.; Dworkin, J. P.; Glavin, D. P.; McLain, H. L.; Noble, S. K.; Gibson, E. K. Geochim. Cosmochim. Acta 2016, 172, 357–369.
[3] Schmitt-Kopplin, P.; Gabelica, Z.; Gougeon, R. D.; Fekete, A.; Kanawati, B.; Harir, M.; Gebefuegi, I.; Eckel, G.; Hertkorn, N. Proc. Natl. Acad. Sci. U. S. A. 2010, 107 (7), 2763–2768.
[4] Hertzog, J.; Naraoka, H.; Schmitt-Kopplin, P. Life 2019, 9 (2), 1–14.

REACTION MECHANISM DECONVOLUTION DURING WASTE-FED HYDROTHERMAL LIQUEFACTION

Heather O. LeClerc[1], Amy M. McKenna[2], Michael T. Timko[1], Andrew R. Teixeira[1]

[1]Worcester Polytechnic Institute, Department of Chemical Engineering, 100 Institute Rd. Worcester, MA
[2]National High Magnetic Field Laboratory, 1800 East Paul Dirac Drive, Tallahassee, FL

The use of hydrothermal liquefaction (HTL) technologies in the conversion of wet wastes to biocrude has been explored for nearly two decades.[1, 2] Despite the prevalence of research in this area, HTL remains a black-box process due to the inherent complexity of real-world waste feedstocks. Fourier transform ion cyclotron resonance mass spectrometry (FT-ICR MS) is an advanced characterization method capable of distinguishing between compounds at the ppb resolution level. Due to the complexity of biocrude samples, the enhanced resolution provided by FT-ICR MS is necessary for determination of chemical pathways to biocrude formation. In addition, the use of an ionization-based technique in place of volatility-based separation is imperative in painting a complete picture of biocrude composition due to the inherent molecular weight range of HTL biocrudes.

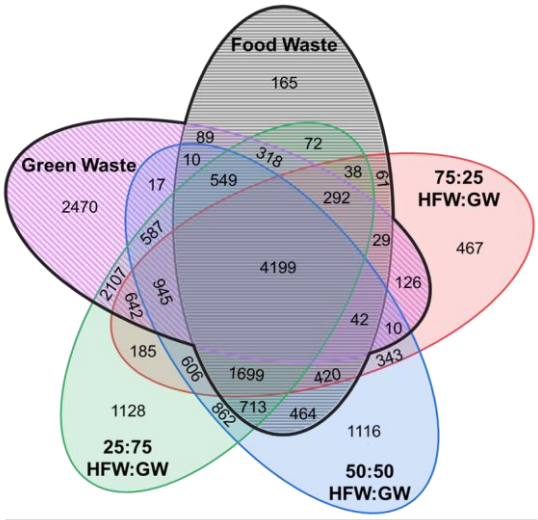

Figure 1. Venn diagram showcasing the number of unique and shared compounds identified through (+) APPI FT-ICR MS. Numbers outside the black border represent emergent compounds.

In this study, positive ion atmospheric pressure photoionization (+ APPI) FT-ICR MS was utilized to determine chemical pathway information from single-source and mixed-waste feedstocks. This work aims to develop relationships between feedstock composition and the resultant biocrude heteroatom distributions, allowing for additional coupling with further analytical techniques to determine chemical pathways. The results of this study indicate that mixed food and lignocellulosic waste results in emergent chemical behavior in hydrothermal liquefaction product yields as well as biocrude composition. FT-ICR MS reveals the occurrence of compounds not identified in either individual feedstock, as highlighted in **Figure 1**. Further analysis of these emergent compounds revealed Maillard-like reaction networks through changes in N_xO_y heteroatom classes.

Additionally, FT-ICR MS was employed for analysis of HTL biocrudes from various wood types to determine the effect on oxygen class abundance and molecular weight distribution. This work revealed the dependence of biocrude yield and oxygen class abundance on the syringol-guaiacol ratio of wood. Lastly, results from FT-ICR MS were corroborated with those obtained from GC-MS, FT-IR, and solid-state NMR to deconvolute the feedstock-dependent chemical pathways towards biocrude formation.

[1] Akhtar, J.; Amin, N. A. S., A review on process conditions for optimum bio-oil yield in hydrothermal liquefaction of biomass. Renewable and Sustainable Energy Reviews 2011, 15 (3), 1615-1624.
[2] Gollakota, A. R. K.; Kishore, N.; Gu, S., A review on hydrothermal liquefaction of biomass. Renewable and Sustainable Energy Reviews 2018, 81, 1378-1392.

HYDROXYL RADICAL FOOTPRINTING OF PROTEIN/DNA COMPLEX COUPLED TO HIGH-RESOLUTION MS

Marek Polák[1,2], Michael Volný[1,2], Daniel Kavan[1,2], Petr Novák[1,2]

[1]BioCeV – Institute of Microbiology, The Czech Academy of Sciences, Prague, Czechia; [2]Charles University, Faculty of Science, Prague, Czechia

The methods of structural proteomics have undergone a remarkable growth in recent years, which had a huge impact on the field of structural and molecular biology. One of these methods, radical covalent labelling, is one of the structural techniques that has showed to be an effective analytical tool for characterization of biomolecules. In this study, we adopted the Fast Photochemical Oxidation of Proteins (FPOP) approach to study the dynamics of FOXO4 transcription factor and its DNA partner, Insulin Response Element (IRE). To study such complex, FOXO4 in the absence or presence of dsIRE was oxidized by FPOP in a quench-flow capillary reactor. Irradiated samples were further analyzed by classical bottom-up approach. To investigate IRE-FOXO4 binding and to monitor the DNA damage caused by the hydroxyl radicals, IRE alone and in the complex with the protein was exposed to FPOP oxidation. FOXO4 was digested into dipeptides using proteinase-K and IRE fragments were analyzed by LC-MS using high-resolution FT-ICR mass spectrometer operated in negative ion mode.
Analysis of oxidized peptides enabled localization and quantification of residues directly involved in protein-DNA interaction. Analysis of separated IRE fragments revealed that hydroxyl radicals cleave the DNA nonspecifically, creating set of all possible 3'OH, 3'P, 5'OH and 5'P terminal fragment ions. Complementary fragment ions were found in the LC-MS trace and further quantified. Comparison of IRE fragment ions revealed significant protection of IRE by FOXO4 binding, predominantly in both major and minor groove of IRE. Obtaining detailed information about solvent accessibility for IRE and FOXO4 surfaces might enable ab initio design of FOXO4/IRE structural model. This is potentially valuable because the corresponding crystal structure is currently unclear.

This work was supported by the Czech Science Foundation (grant numbers 19-16084S), Grant Agency of Charles University (359521), European Commission H2020 (EPIC-XS- grant agreement ID: 823839 and EU FT-ICR MS - grant agreement ID: 731077), and, in part, by the Czech Academy of Sciences (RVO61388971).

AN INFRARED MULTIPHOTON DISSOCIATION MS^3 WORKFLOW FOR THE DISCOVERY AND MASS FINGERPRINTING OF NATURAL PRODUCTS

Josh Salem[1], Kristina Håkansson[1]

Department of Chemistry, University of Michigan 930 N. University Ave, Ann Arbor, MI, 48104

Infrared multiphoton dissociation (IRMPD) at 10.6 μm is a selective fragmentation technique for phosphate-containing molecules. This specificity is due to the inherent strong IR absorption of the P-O-R/P-O-H bond. Selective IRMPD has been demonstrated for phosphopeptide analysis and our group has also shown its utility for targeted analysis of peptides containing the phosphopantetheine (ppant) modification; a moiety characteristic of biosynthetic pathways for natural products such as pikromycin, curamycin, and precursors for other antibiotic and chemotherapeutic drugs. An Orbitrap Fusion Lumos tribrid and a Solarix Q-FT-ICR mass spectrometer were equipped with 10.6 μm CO_2 lasers with 60 W and 25 W power, respectively. We employed our previously developed large m/z window, high mass resolution data independent method for the identification of active site peptides, including peptides containing natural product metabolites and their intermediates. The active site ppant-containing peptide is selectively fragmented among other peptides from the pikromycin module 5 (PikAIII) biosynthetic enzyme at 30 W IR irradiation for 150 ms. Similar selectivity is observed following loading of methyl malonyl from methyl malonyl-coenzyme A or pentaketide from synthetic thiophenyl-pentaketide onto the ppant thiol group. This loading of pikromycin building blocks results in ppant peptide mass shifts, correlating with the different states of covalent modification. MS^3 scans of the ppant-conjugate fragments were collected in the low-pressure ion trap. Using CID MS^3 we observe several intermediate-associated neutral losses that are characteristic of the pentaketide. Understanding the correlations between neutral loss, and fragment ions will allow us to further interrogate the structure of natural products in a proteomics workflow.

HIGH TEMPERATURE FLUOROALCOHOL-FREE LIQUID CHROMATOGRAPHY-7T FT-ICR MASS SPECTROMETRY OF RNAS UP TO 100 kDA

Carson W. Szot[1], Elizabeth Tidwell[2], Ingrid Kilde[3], Markos Koutmos[1,2], Kristina Hakansson[1]

1Department of Chemistry, University of Michigan, Ann Arbor, MI
2Department of Biophysics, University of Michigan, Ann Arbor, MI
3Program in Chemical Biology, University of Michigan, Ann Arbor, MI

Liquid chromatography-mass spectrometry (LC-MS) analysis of oligonucleotides often utilizes fluoroalcohol-containing mobile phases although a shift to other solvent systems is gaining traction. Comprehensive characterization, including intact mass determination, identification and quantification of polymerase readthrough failures can inform biosynthesis. LC separation of longer RNAs (up to 652 kDa) with UV-based quantification has been demonstrated[1]; however, MS-based detection of such large RNAs remains elusive. Nevertheless high-end mass spectrometers are being increasingly applied in conjunction with LC to longer RNA, e.g., a 32 kDa single guide RNA.[2] Here, we demonstrate a high temperature, fluoroalcohol-free LC method enabling FT-ICR MS detection of up to 100 kDa RNA.

In vitro transcribed 112 and 300 nucleotide RNAs were purified by size exclusion chromatography to remove reaction components, free nucleotides, RNA polymerase, and DNA template. Samples were then desalted with MWCO filtration employing either ammonium acetate or ammonium formate at 100 mM. Ion pair reverse phase LC at a flow rate of 300 µL/min was performed with an Agilent 1200 HPLC coupled to a SolariX XR Q-FT-ICR (7T; Bruker) mass spectrometer. An Agilent PRLP-S polystyrene-divinylbenzene 2.1X50 mm, 5 µm, 1000 Å, stainless steel column was used with a 5 mM trimethylammonium bicarbonate (pH 8.5)/methanol gradient at 80 ˚C column temperature, 350 ˚C drying gas temperature, 10 L/min drying gas flow rate, and 3 bar nebulizer pressure. The LC runtime was 35 min. An Agilent ES standard nebulizer was used without modification. Data were acquired in serial mode with 4 scan averaging, 4 second external accumulation time, and 2 megaword acquisition size. Excitation was performed in broadband mode employing frequency sweep in either the increasing or decreasing direction with either excite calculation version 1 or version 2 in Bruker ftmsControl v2.3.0. The highest MS spectral quality was achieved with excitation calculation version 2 and the sweep in the decreasing direction. The electrospray charge state distribution showed 40 charge states, from - 120 to - 80, between m/z 800 and m/z 1200. The - 97, - 96, and - 95 charge states, centered at m/z 1000, were quadrupole isolated to decrease space charge and thus improve resolution. The resulting transient was sine apodized and processed with a magnitude mode FFT. The spectrum was then smoothed with a single Gaussian smoothing function over a 0.02 m/z width. The quadrupole isolated and apodized smoothed charge states were identified as peaks using the Bruker Apex peak finder. Non-isotopically resolved deconvolution of these charge states showed a measured molecular weight of 97 kDa for the 300 nucleotide RNA. Isotopically resolved data were acquired for the 112 nucleotide RNA with quadrupole isolation but no further processing, showing a measured mass of 37 kDa.

[1] L. Abhijeet, A. Chakravarty, T. Rades, D. Christensen, H. Franzyk, A. Thakur, C. Foged, *Int. J. Pharm.,* 626, 122171 (2022).
[2] C. Crittenden, M. B. Lanzillotti, B. Chen, *Anal. Chem.*, **95**, 3180-3186 (2023).

FIRE IMPACTS ON SOIL ORGANIC MATTER COMPOSITION AND MICROBIAL METABOLISM: A FOURIER TRANSFORM MASS SPECTROMETRY APPROACH

Jacob VanderRoest[1], Amy McKenna[2], Holly Roth[1], Corey Broekling[3], Julie Fowler[4], Timothy Fegel[5], Charles Rhoades[5], Claudia Boot[1], Mike Wilkins[4], Thomas Borch[1,4]

[1]Colorado State University, Department of Chemistry, Fort Collins, CO; [2]National High Magnetic Field Laboratory, 1800 East Paul Dirac Drive, Tallahassee, FL; [3]Colorado State University, Bioanalysis *and Omics Lab, Fort Collins, CO; [4]Colorado State University, Department of Soil & Crop Sciences, Fort Collins, CO; [5]USDA Forest Service, Rocky Mountain Research Station, 240 W Prospect Rd, Fort Collins, CO;*

Wildfire activity has increased in frequency and severity throughout the past decades which changes the physical, biological, and chemical characteristics of fire-impacted soils.[1] Specifically, fire exposure can significantly alter the structure and composition of soil organic matter (SOM). However, it is unclear how SOM in burned soils impacts microbial activity. Addressing this knowledge gap will help indicate what carbon sources (i.e., electron donors) and nutrients are available for microbial metabolism in burned soils. Herein, we identified the initial alterations in SOM composition that occurred during a simulated wildfire, tracked the subsequent changes during one month after the fire, and assessed how pre- and post-fire SOM composition influence microbial metabolism. We simulated wildfires by burning lodgepole pine fuel over 53 L steel containers packed with forest soil, and soil samples were collected over the course of 28 days. SOM composition was determined by analyzing soil-water extracts with 21 T Fourier transform-ion cyclotron resonance mass spectrometry (FT-ICRMS). Using both positive and negative electrospray ionization, burned SOM was found to contain more nitrogen-containing molecules and had lower molecular weights compared to unburned SOM. However, the $\Delta G_{oxidation}$ of organic compounds was similar between burned and unburned samples, indicating that wildfires may not alter SOM thermodynamics and that microbes may metabolize burned SOM to the same extent as unburned SOM.[2]

[1] McLauchlan, K. K.; Higuera, P. E.; Miesel, J.; Rogers, B. M.; Schweitzer, J.; Shuman, J. K.; Tepley, A. J.; Varner, J. M.; Veblen, T. T.; Adalsteinsson, S. A.; et al. Fire as a fundamental ecological process: Research advances and frontiers. *J Ecol* 2020, *108* (5), 2047-2069.
[2] Larowe, D. E.; Van Cappellen, P. Degradation of natural organic matter: A thermodynamic analysis. *Geochimica et Cosmochimica Acta* 2011, *75* (8), 2030-2042.

DIRECT DETERMINATION OF TYROSINE SULFATION SITES IN PROTEOMIC ANALYSIS

Menatallah M. Youssef[1, 2], Carson W. Szot[1]; Miriam F. Ayad[2]; Lobna A. Hussein[2]; Maha F. Abdel-Ghany[2]; Kristina Hakansson[1]

[1]Deparment of Chemistry, University of Michigan, Ann Arbor, MI; [2]Faculty of Pharmacy, Ain Shams University, Cairo, Egypt

Tyrosine sulfation is an understudied posttranslational modification (PTM) of crucial biological significance. Conventional liquid chromatography-tandem mass spectrometry (LC-MS/MS) methods have limited ability to directly identify sulfation sites due to the extreme sulfate lability and low sulfopeptide ionization efficiency in positive mode. Sulfated and phosphorylated tyrosines can be misassigned due to their isobaric nature; however, high-resolution mass spectrometers can differentiate between these two PTMs at the MS1 level. Furthermore, PTM-containing fragments are observed for phosphopeptides but not sulfopeptides in collision-induced dissociation (CID)/beam-type CID (HCD). Complete sulfonate elimination can also occur upon electron capture/transfer dissociation (ECD/ETD) depending on sulfopeptide sequence/basicity and charge state. Here, we report detection of sulfate-containing fragments from bottom-up LC-ECD MS/MS, thus allowing direct determination of tyrosine sulfation sites. Bovine fibrinogen and bovine plasma (Sigma-Aldrich) were digested overnight with sequencing grade modified trypsin (Promega) at 1:50 enzyme/protein ratio and 37 °C. Nanoflow LC (nLC)-HCD MS/MS was performed with an Orbitrap Fusion Lumos. The autosampler, column, and source gas were set to 4, 46, and 285 °C, respectively. The HCD collision energy was 32 %. nLC-HCD MS/MS analysis of tryptically digested bovine fibrinogen on an Orbitrap Fusion Lumos resulted in detection of the known sulfopeptide QFPTD**Y***DEGQDDRPK (**Y***=sulfotyrosine) from the N-terminus of the beta chain (residues 1-15), as identified by Proteome Discoverer. However, as expected, no sulfated fragments were observed. Proteome Discoverer also identified the doubly charged peptide QVGVEHHVEIE**Y**D from the C-terminus of fibrinogen gamma-B chain (residues 432-444) as sulfated or phosphorylated. Therefore, manual spectral interpretation was required. No PTM-containing fragments were observed in the HCD spectrum, supporting a sulfopeptide assignment. However, due to the complete sulfonate elimination, direct annotation of the sulfation site is not achieved. With the goal of generating sulfate-containing fragment ions, an alternative nLC method involving loss-triggered ETD was used. ETD was triggered upon neutral loss of either 79.9663 (phosphate) or 79.9568 (sulfate). However, neither sulfopeptide was detected. Instead, LC-ECD MS/MS was performed with an Agilent 1290 HPLC coupled to a 7 Tesla Solarix Fourier transform ion cyclotron resonance (FT-ICR) instrument (Bruker) via an Agilent InfinityLab Poroshell 120 EC-C18 column. The autosampler, column, and source gas temperatures were set to 6, 40, and 250 °C, respectively. The ECD heater was at 1.5 A. LC-ECD MS/MS was achieved with the gamma-B chain sulfopeptide added to the preferred list for fragmentation. This approach allowed this relatively low abundance peptide to be selected for ECD. Both intact and desulfated precursor ions were observed, however, sulfate- retaining fragment ions were detected, including sulfated z_8 and c_{12} ions. While higher sequence coverage is desired, ECD provided direct evidence of sulfation at tyrosine 443. This PTM is not annotated in UniProt; however, Tyr 443 sulfation has been previously reported from indirect evidence [1]. We herein report LC-ECD MS/MS for generation of sulfate-retaining fragments in a proteomic analysis, allowing direct determination of tyrosine sulfation.

[1] Y. Amano, H. Shinohara, Y. Sakagami and Y. Matsubayashi, *Analytical biochemistry*, 346.1, 124-131 (2005)

APPLYING AMBIENT IONIZATION TO FOURIER TRANSFORM ION CYCLOTRON RESONANCE MASS SPECTROMETERS

Kevin J. Zemaitis,[1] Carter C. Bracken,[1] Gregory W. Vandergrift,[1] William Kew,[1] Troy D. Wood,[2] and Ljiljana Paša-Tolić[1]

[1]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory, Richland, Washington, USA
[2]Department of Chemistry, University of Buffalo, Buffalo, New York, USA

Historically, FTICR detection has paired well with pulsed laser desorption/ ionization (LDI) sources, with matrix-assisted laser desorption/ ionization (MALDI) being implemented on most commercial FTICR instruments. Despite the extreme utility of MALDI for the direct detection of xenobiotics, metabolites, lipids, peptides, and intact proteins from samples; research over the past two decades has resulted in several dozen unique modes of ambient ionization. These modes have found traction within both rapid screening applications and mass spectrometry imaging (MSI) due to reduced sample preparation needs and the ability to desorb and ionize analytes at atmospheric pressure. However, many of these developments have focused on throughput and acquisition speed, with sparse reports of implementation on instrumentation capable of resolving the extreme complexity of environmental and biological samples.

Herein, we describe our efforts towards developing a highly customizable ambient ionization platform constructed via fused-deposition modeling (FDM; 3D printing) for commercial FTICR instruments. The source was designed to be highly modular and modifiable, where current capabilities include desorption electrospray ionization (DESI), and more recently, implementation of low-temperature plasma (LTP) ionization. Thus, on singular commercial Bruker Daltonics FTICR instrument (with field strengths ranging from 7T, 12T, 15T) we highlight the utility of having multiple analysis methods with plasma, laser, and electrospray-based ionization for the characterization of a broad subset of compounds. We demonstrate that the diversity of ionization expands the possibilities of MSI applications within metabolomic and lipidomic analyses and allows for the broad direct MS profiling of complex organic environmental samples.

POSTERS

SIMPLIFIED OPERATION OF THIN SURFACE-INDUCED DISSOCIATION DEVICES

Andrew J. Arslanian[1], Leon (Yu-Fu) Lin[1], Arpad Somogyi[1,2], Vicki H. Wysocki[1,2]

[1]Resource for Native MS Guided Structural Biology, The Ohio State University, Department of Chemistry and Biochemistry, Columbus, Ohio 43210
[2]Campus Chemical Instrument Center, Mass Spectrometry and Proteomics, The Ohio State University, Columbus, Ohio 43210

Surface-induced dissociation (SID) is a collisional activation strategy that involves ion-surface collisions instead of the ion-neutral collisions common to collision-induced dissociation (CID). Ion-surface collisions deposit significantly more energy into the ion which opens higher energy dissociation pathways that lead to fascinating dissociation behavior.[1] When native-like protein complexes are subject to SID the protein subunits will typically cleave along the weakest protein-protein interfaces, which provides information about the protein's subunit connectivity.[2] Early SID devices were complicated, possessing multiple electrodes for tuning, and bulky, with lengths between 1.5 – 3 cm, which often necessitated extensive instrument modification. The most recent Wysocki SID device, referred to herein as a 'thin device,' is 3 – 4 mm in length, depending on the targeted mass spectrometer. This device uses fewer tuning electrodes and occupies significantly less space than previous SID iterations.[3] This allowed the thin SID device to be incorporated into multiple Wysocki group instruments, including a Bruker solariX XR 15 T FTICR-MS and a Thermo Scientific Q Exactive UHMR Orbitrap, without requiring extensive instrument modification as was required for earlier SID devices. These developments also led to the first commercialization of SID, by Waters in the SELECT SERIES cyclic IMS instrument.[4] The current thin device iteration features three electrodes: a deflector that pushes ions towards the surface electrode for collision, and an extractor electrode that helps create favorable voltage potentials to extract product ion downstream to the rest of the instrument. While these electrodes were designed to be independently tuned to create favorable potentials for collision and extraction, the commercialized SID device operates the surface and extractor electrodes at the same voltage value. While this may simplify device operation, the intra- and post-SID potential is different than originally designed, which may affect ion transmission. Using a Bruker solariX XR 15 T FTICR-MS and a 3 mm thin SID device, experiments conducted on the homotetramer streptavidin (53 kDa) and homopentamer c-reactive protein (115 kDa) show that operating the surface and extractor electrodes at both different and the same voltage can produce similar protein dissociation patterns, if the deflector is properly tuned to push ions towards the surface. For streptavidin, which possesses a dimer-of-dimers topology,[2] the primary SID product is dimer formation, with some monomer formation at higher SID collision energies. The ratio of dimer product to residual tetramer precursor can be tuned based on the deflector electrode setting, with higher values leading to more abundant dimer signal. For c-reactive protein, the primary products included monomer/tetramer and dimer/trimer pairs. Ion transmission in either case improves if the mass spectrometer's post-SID potential is favorable for product ion transmission. We believe that the thin device's installation requirements and ease of use will allow broader adoption of SID technology.

[1] Snyder, D. T.; Harvey, S. R.; Wysocki, V. H., Surface-induced Dissociation Mass Spectrometry as a Structural Biology Tool. Chem. Rev. 2022, 122 (8), 7442-7487.
[2] Harvey, S. R.; Seffernick, J. T.; Quintyn, R. S.; Song, Y.; Ju, Y.; Yan, J.; Sahasrabuddhe, A. N.; Norris, A.; Zhou, M.; Behrman, E. J.; Lindert, S.; Wysocki, V. H., Relative interfacial cleavage energetics of protein complexes revealed by surface collisions. Proc. Natl. Acad. Sci. U.S.A. 2019, 116 (17), 8143-8148.
[3] Snyder, D. T.; Panczyk, E. M.; Somogyi, A.; Kaplan, D. A.; Wysocki, V., Simple and Minimally Invasive SID Devices for Native Mass Spectrometry. Anal. Chem. 2020, 92 (16), 11195-11203.
[4] Snyder, D. T.; Jones, B. J.; Lin, Y.-F.; Cooper-Shepherd, D. A.; Hewitt, D.; Wildgoose, J.; Brown, J. M.; Langridge, J. I.; Wysocki, V. H., Surface-induced dissociation of protein complexes on a cyclic ion mobility spectrometer. Analyst 2021, 146 (22), 6861-6873.

LIPIDOMICS PROFILING OF ZERO-VALENT IRON NANOPARTICLE-TREATED CYANOBACTERIA

Huan Chen[1], LaDonna Wyatt[2], Yuan Lin[1], Samson Gichuki[2], Ying Liu[3], Samuel Moore[3], David Gaul[3], and Viji Sitther[2]

[1]National High Magnetic Field Laboratory, Florida State University, 1800 East Paul Dirac Drive, Tallahassee, FL 32310-4005, USA.

[2]Department of Biology, Morgan State University, 1700 East Cold Spring Lane, Baltimore, Maryland 21251, USA.

[3]School of Chemistry and Biochemistry, Georgia Institute of Technology, 901 Atlantic Dr, Atlanta, GA 30332, USA

For biofuel production, *Fremyella diplosiphon,* is an ideal model cyanobacterium due to its lipid producing capacity, fast growth rate, and ability to adapt to varying wavelengths and intensities of light.[1,2] Nanotechnology has been applied to cyanobacteria and microalgae to enhance lipid accumulation by stimulating metabolism and cell growth.[3,4] In particular, the zero-valent iron nanoparticles (nZVIs) can inertly penetrate cells to form reactive oxygen species (ROS), which induce cell oxidative stress. stress.[3] Here, we present fatty acid methyl ester (FAME) and polar lipids profiles of nZVIs-treated *F. diplosiphon* strains. Total lipids in nZVI-treated and untreated *F. diplosiphon* were extracted and measured using gravimetric analysis.[4] Extracted lipids were converted to FAMEs via direct transesterification using a commercial multimode scientific microwave.[5] Detailed characterization and quantitation of FAMEs and other volatile organic matters such as alkanes and olefins were characterized using comprehensive two-dimensional gas chromatography- time-of-flight mass spectrometry (GC×GC-TOFMS) from LECO. The polar lipids from the strains were extracted followed by reverse-phase liquid chromatography coupled with positive and negative electrospray ionization (ESI) orbitrap and FT-ICR mass spectrometry. Data-dependent collision-induced dissociation MS/MS was acquired to obtain additional structural information. Assigned elemental compositions from MS/MS were searched against lipid libraries to confirm lipid identities. The impact of nZVIs on lipid profiles of *F. diplosiphon* strains was investigated. A significant increase in *F. diplosiphon* lipids yield and FAMEs production were observed due to oxidative stress induced by the optimal nZVI concentrations (0.2-1.6 mg L^{-1}). The resulting lipid profiles provide a detailed compositional comparison of the nanoparticle-treated and non-treated cyanobacterial cells. Molecular characterization of lipids profiles provides understandings of how iron nanoparticle-induced oxidative stress affects lipid domains in a model cyanobacterium. This work was supported by the NSF Division of Chemical, Bioengineering, Environmental, and Transport Systems through 1900966, Divisions of Materials Research and Chemistry through 16-44779, and the State of Florida.

[1] Montgomery, B. Shedding new light on the regulation of complementary chromatic adaptation. Open Life Sci 3, 351–358 (2008).
[2] Tabatabai, B. et al. Nanoparticle-mediated impact on growth and fatty acid methyl ester composition in the cyanobacterium Fremyella diplosiphon. Bioenergy Res 12, 409–418 (2019).
[3] Ševců, A., El-Temsah, Y. S., Joner, E. J. & Černík, M. Oxidative stress induced in microorganisms by zero-valent iron nanoparticles. Microbes Environ 1107220320 (2009).
[4] Floch, J. A simple method for the isolation and purification of total lipids from animal tissues. J. biol. Chem. 226, 497–509 (1957).
[5] Wahlen, B. D., Willis, R. M. & Seefeldt, L. C. Biodiesel production by simultaneous extraction and conversion of total lipids from microalgae, cyanobacteria, and wild mixed-cultures. Bioresour Technol 102, 2724–2730 (2011).

INTERNAL FRAGMENT ASSIGNMENT CHALLENGES IN TOP-DOWN ELECTRON CAPTURE DISSOCIATION TANDEM MASS SPECTROMETRY

[1]Neven N. Mikawy, Carolina Rojas Ramírez, Brandon T. Ruotolo, Kristina Hakansson

[1]Department of Chemistry, University of Michigan, Ann Arbor, MI 48109

Top-down tandem mass spectrometry (MS/MS) of intact proteins typically generates sequence-informative fragments from backbone cleavages near the termini. This lack of fragmentation in the protein interior is particularly apparent in native top-down MS/MS. Improved sequence coverage, critical for reliable annotation of posttranslational modifications (PTMs) and sequence variants, can be obtained from internal fragments (containing neither terminus) generated by multiple backbone cleavage events. However, internal fragment assignments can be error prone due to isomeric/isobaric fragments from different parts of a protein sequence. Here, we focus on improved internal fragment annotation in electron capture dissociation (ECD) following both native MS and liquid chromatography (LC)/MS via control of the number of electron capture events as well as MS^3 of internal fragment candidates. Bovine calmodulin was used intact or following trypsin digestion. LC/ECD MS/MS was performed using a Hamilton polystyrene-divinylbenzene PRP-3 or Agilent Poroshell 120 EC-C18 column with an acetonitrile:water/0.1% formic acid solvent system. Native ECD MS/MS was performed via a CaptiveSpray nanoelectrospray (nESI) ion source from 50 mM ammonium acetate following calmodulin purification with a Biospin gel filtration column. ECD was performed with either a Q-FT-ICR or ion mobility (IM)-Q-TOF mass spectrometer. MS^3 was performed via infrared multiphoton dissociation (IRMPD) inside the ICR cell following CHEF isolation. Previous native ECD experiments on an Agilent 6560c equipped with an e-MSion ExD cell produced three internal fragment candidates: a doubly charged ion (m/z 1336.7) and two singly charged ions (m/z 1490.7 and 1759.8). Fragments of identical m/z were also observed for the +9, +10, +15, and +16 charge states following LC-ECD-MS/MS on the SolariX FT-ICR instrument, suggesting they may not be unique to native ECD. In addition, two of these internal fragment candidates contain lysine 116, which can be trimethylated to alter the fragment mass. The combination of LC-CID-MS/MS, LC-IRMPD-MS/MS, and LC-ECD-MS/MS of intact calmodulin on the SolariX FT-ICR instrument provided high sequence coverage, including unambiguous assignment of N-terminal methionine loss+acetylation and Lys116 trimethylation. These PTMs were also verified through bottom-up LC/MS via detection of the acetylated peptide 2-14 (lacking the N-terminal Met) as well as the trimethylated peptide 108-116 on the FT-ICR. Alternative assignments for the potential internal fragments are c_{23}^{2+} (including Met loss and acetylation), y_{12}, and z'_{15} (i.e., an even-electron *z*-type ion), all expected outcomes from ECD. To further analyze the identity of these fragments in native ECD MS/MS, direct infusion nano ESI ECD was performed on the SolariX instrument. ECD of the +9 charge state showed evidence of up to 4 electron capture events, depending on electron irradiation time. Because more than one electron capture event is likely required for internal fragment generation, the 8+ charge reduced species was further isolated inside the ICR cell and subjected to IRMPD MS^3. The three fragments of interest were still observed, suggesting a terminal point of origin. Furthermore, the m/z 1336.7 ECD fragment was isolated and subjected to IRMPD MS^3, showing a complex fragmentation pattern, including *b*-type and internal fragments matching the c_{23}^{2+} assignment. However, even for this 23-mer, multiple possibilities exist for internal fragment assignment, including isomeric options.

[1] C. Lantz, M. A. Zenaidee, B. Wei, Z. Hemminger, R. R. O. Loo, and J. A. Loo, *J. Proteome Res.*, **20**, 1928-1935 (2021).
[2] M. A. Zenaidee, B. Wei, C. Lantz, H. T. Wu, T. R. Lambeth, J. K. Diedrich, R. R. O. Loo, R. R. Julian, and J. A. Loo, *J. Am. Soc. Mass Spectrom.*, **32**, 1752-1758 (2021).

Afonso, Carlos
University of Rouen-Normandy
Mont-Saint-Aignan 76130
France
carlos.afonso@univ-rouen.fr

Agar, Jeffrey
Northeastern University
Boston, MA 02115
j.agar@northeastern.edu

Agar, Natalie
Brigham And Women's Hospital
Boston, MA 02115
Nathalie_Agar@dfci.harvard.edu

Amster, Jon
University of Georgia
Athens, GA 30602
jamster@uga.edu

Arslanian, Andrew
The Ohio State University
Columbus, OH 43210
ajarslanian@gmail.com

Babcock-Adams, Lydia
National High Magnetic Field Laboratory
Tallahassee, FL 32310
babcock-adams@magnet.fsu.edu

Beu, Steve
S C Beu Consulting
Austin, TX 78729
steve@scbeuconsulting.com

Blakney, Greg
National High Magnetic Field Laboratory
Tallahassee, FL 32310
blakney@magnet.fsu.edu

Bouyssiere, Bryce
Université de Pau et des Pays de l'Adours
Pau, France 64000
brice.bouyssiere@univ-pau.fr

Butcher, David
National High Magnetic Field Laboratory
Tallahassee, FL 32310
dbutcher@magnet.fsu.edu

Chacón-Patiño, Martha L.
National High Magnetic Field Laboratory
Tallahassee, FL 32310
chacon@magnet.fsu.edu

Chen, Huan
National High Magnetic Field Labortary
Tallahassee, FL 32310
huan.chen@magnet.fsu.edu

Cooper, Helen
University of Birmingham
Birmingham B15 2TT
United Kingdom
h.j.cooper@bham.ac.uk

Costello, Catherine
Boston University School of Medicine
Boston, MA 02118
cecmsms@bu.edu

DeFiglia, Steven
University of Michigan
Ann Arbor, MI 48109
sdefigli@umich.edu

DeHart, Caroline
Frederick National Laboratory for Cancer Research
Frederick, MD 21701
caroline.dehart@nih.gov

Fernandez-Lima, Francisco
Florida International University
Miami, FL 33199
fernandf@fiu.edu

Fischer, Joshua
Bruker
Billerica, MA 01821
joshua.fischer@bruker.com

Frye-Jones, Joseph
National High Magnetic Field Laboratory
Tallahassee, FL 32310
jwfrye@fsu.edu

Garcia, Brianna
Woods Hole Oceanographic Institution
Woods Hole, MA 02543
brianna.garcia@whoi.edu

Ge, Ying
University of Wisconsin-Madison
Madison, WI 53705
ying.ge@wisc.edu

Giusti, Pierre
TOTAL
Harfleur, Seine-Maritime, 76700
France
pierre.giusti@totalenergies.com

Goodlett, David
University of Victoria
Victoria, BC V8Z 7X8
goodlett@uvic.ca

Goodwin, Michael
Thermo Fisher Scientific
San Jose, CA 95134
mike.goodwin@thermofisher.com

Hakansson, Kristina
University of Michigan
Ann Arbor, MI 48109-1055
kicki@umich.edu

Hendrickson, Christopher
National High Magnetic Field Laboratory
Tallahassee, FL 32310
hendrick@magnet.fsu.edu

Kafader, Jared
Northwestern University
Evanston, IL 60208
jared.kafader@northwestern.edu

Kew, William
Pacific Northwest National Laboratory
Richland, WA 99354
william.kew@pnnl.gov

Kusel, David
Thermo Fisher Scientific
San Jose, CA 95134
david.kusel@thermofisher.com

LeClerc, Heather
Worcester Polytechnic Institute
Worcester, MA 01609
hleclerc@wpi.edu

Lytle, Cory
Bruker
Billerica, MA 01821
cory.lytle@bruker.com

Ma, Xin
Georgia Institute of Technology
Atlanta, GA 30332
xin.ma@chemistry.gatech.edu

Mackay, Logan
University of Edinburgh
Edinburgh EH8 9YL, United Kingdom
lmackay@ed.ac.uk

Marshall, Alan
National High Magnetic Field Laboratory
Tallahassee, FL 32310
marshall@magnet.fsu.edu

McIntosh, Dan
National High Magnetic Field Laboratory
Tallahassee, FL 32310
mcintosh@magnet.fsu.edu

Mikawy, Neven
University of Michigan
Ann Arbor, MI 48109
nmikawy@umich.edu

Modi, Kushal
Bruker
Billerica, MA 01821
Kushal.Modi@bruker.com

Nielsen, Michael
University of Copenhagen
1165 København, Denmark
michael.lund.nielsen@cpr.ku.dk

Novák, Petr
Institute of Microbiology

14220 Prague, Czech Republic
pnovak@biomed.cas.cz

Olsen, Jesper
University of Copenhagen
1165 København, Denmark
jesper.olsen@cpr.ku.dk

Pasa-Tolic, Ljiljana
Pacific Northwest National Laboratory
Richland, WA 99354
ljiljana.pasatolic@pnnl.gov

Polak, Marek
Institute of Microbiology
14220 Prague, Czech Republic
marek.polak@biomed.cas.cz

Rabus, Jordan
Pacific Northwest National Lab
Richland, WA 99352
jordan.rabus@pnnl.gov

Rodgers, Ryan
National High Magnetic Field Laboratory
Tallahassee, FL 32310
rodgers@magnet.fsu.edu

Salem, Joshua
University of Michigan
Ann Arbor, MI 48109
salemjp@umich.edu

Senko, Michael
Thermo Fisher Scientific
San Jose, CA 95134
michael.senko@thermofisher.com

Szot, Carson
University of Michigan
Ann Arbor, MI 48109
cwszot@umich.edu

Tressler, Caitlin
Johns Hopkins University School of Medicine
Baltimore, MD 21287
ctressl3@jhmi.edu

Tsybin, Yury
Spectroswiss
Lausanne, VD, 1015 Switzerland
tsybin@spectroswiss.ch

VanderRoest, Jacob
Colorado State University
Fort Collins, CO 80523
Jacob.VanderRoest@colostate.edu

Weisbrod, Chad
National High Magnetic Field Laboratory
Tallahassee, FL 32310
weisbrod@magnet.fsu.edu

Williams, Evan
University of California, Berkeley
Berkeley, CA 94720
erw@berkeley.edu

Wysocki, Vicki
The Ohio State University
Columbus, OH 43210
wysocki.11@osu.edu

Young, Robert
New Mexico State University
Las Cruces, NM 88003
rbyoung@nmsu.edu

Youssef, Menatallah
University of Michigan
Ann Arbor, MI 48109
menaty@umich.edu

Zemaitis, Kevin
Pacific Northwest National Laboratory
Richland, WA 99354
kevin.zemaitis@pnnl.gov

Zubarev, Roman
Karolinska Institutet
Stockholm, Sweden 17177
roman.zubarev@ki.se