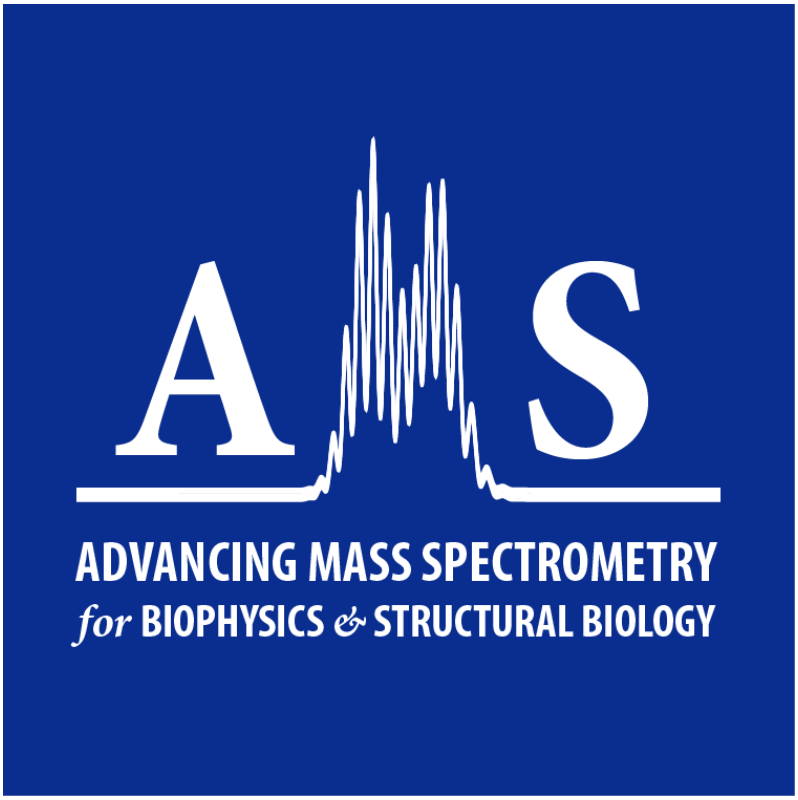

2nd Advancing Mass Spectrometry for Biophysics and Structural Biology Meeting

July 21st – July 25th, 2019
Amherst, MA

Organizer:
Richard W. Vachet, University of Massachusetts Amherst

AMS Board

Peter Armentrout
University of Utah

Matthew Bush
University of Washington

Valérie Gabelica
Univ. Bordeaux/CNRS/INSERM

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Keynote Speakers

Professor Michael T. Bowers is a Distinguished Professor in the Department of Chemistry and Biochemistry at the University of California Santa Barbara. He obtained his B.S. from Gonzaga University and his Ph.D. from the University of Illinois. After two years at the Jet Propulsion Laboratory, he joined the UC Santa Barbara faculty in 1968. His awards include the Nobel Laureate Signature Award of the American Chemical Society (1988), the Field and Franklin Award of the American Chemical Society (1996), the Thomson Gold Medal of International Mass Spectrometry Society (1997), a Guggenheim Fellowship (1995), and UCSB Faculty Research Lecturer (1994).

Professor Bowers' research is centered on determination of the structure and/or reaction dynamics and reaction mechanism of a number of exotic species in the gas phase. These studies all utilize state-of-the-art ion beam technologies and methodologies, most of which were developed in his lab. The work is a blend of experiment and theory, bring a chemical physics outlook to problems of chemical and biological interest.

At present, his group is involved in two broad areas: nanoclusters/materials and conformations, energetics and hydration of macromolecular systems. In the nanocluster/materials area, they investigate size-selected metal clusters, their binding energies to catalytically important ligands and the connection to the same chemistry on clean semiconducter surfaces. They are also interested in macromolecular systems of both industrial and biological importance. Industrial systems include primary and secondary structural/conformational mapping of both pure and copolymer systems. Of particular interest are polyhedral oligomeric silsesquioxanes (POSS), silicon/oxygen cages that greatly enhance polymer properties when attached. Biopolymer work has focused on the importance of conformational analysis, salt bridge formation and hydration of both peptides and oligonucleotides. Recent work has aimed at misfolding/aggregation phenomena that underlie many diseases, including Alzheimer's and various transmissible spongiform encephalopathies (TSE).

Keynote Speakers

Professor Lila M. Gierasch is a Distinguished Professor in the Departments of Chemistry and Biochemistry and Molecular Biology at the University of Massachusetts Amherst. She obtained her A.B. in Chemistry from Mount Holyoke College and her Ph.D. in Biophysics from Harvard University. She joined the faculty at the University of Massachusetts Amherst in 1994 after serving as a faculty member at Amherst College, the University of Delaware, and the University of Texas Southwestern Medical Center. Her awards include an A.P. Sloan Fellowship, the Vincent du Vigneaud Award for Young Investigators in Peptide Chemistry (1984), a Guggenheim Fellowship (1986), the Garvan-Olin Medal from the ACS (2006), an NIH Director's Pioneer Award (2006), the Dorothy Crowfoot Hodgkin Award from The Protein Society (2010), the Mildred Cohn Award from ASBMB (2014), and the Ralph F. Hirschmann Award in Peptide Chemistry from the ACS (2018). She currently serves as the Editor-in-Chief for the *Journal of Biological Chemistry*. She is also a fellow of the American Association for the Advancement of Science (1989) and an elected fellow of the American Academy of Arts and Sciences (2016). In 2019, she was elected a member of the National Academy of Sciences.

Professor Gierasch's principal research interests are in the area of protein folding. The protein folding problem, namely how amino acid sequence determines the three-dimensional structure of a protein, is not fully understood despite many years of effort. Her group is addressing this problem in a variety of ways. They study the conformational preferences of model peptides in order to explore how local sequence guides folding. They also carry out detailed studies of the *in vitro* folding of a predominantly β–sheet protein with a very simple topology. Methods used in their folding work include circular dichroism, fluorescence, mass spectrometry, and nuclear magnetic resonance.

Her group is also interested in how a protein folds *in vivo*. In recent years, a class of proteins called molecular chaperones has been found to facilitate protein folding *in vivo*. Professor Gierasch's group is addressing several questions concerning chaperones: How do they recognize and bind incompletely folded polypeptides? Do different classes of chaperones bind to their substrates in distinct ways? How do chaperones interact with their co-chaperones? Is the mechanism of chaperone-mediated folding different from that of the isolated protein?

Agilent Technologies Student Travel Award Winners

Meagan Gadzuk-Shea
University of Washington

Dante Johnson
University of Maryland, Baltimore

Marius Kostelic
University of Arizona

Carter Lantz
University of California, Los Angeles

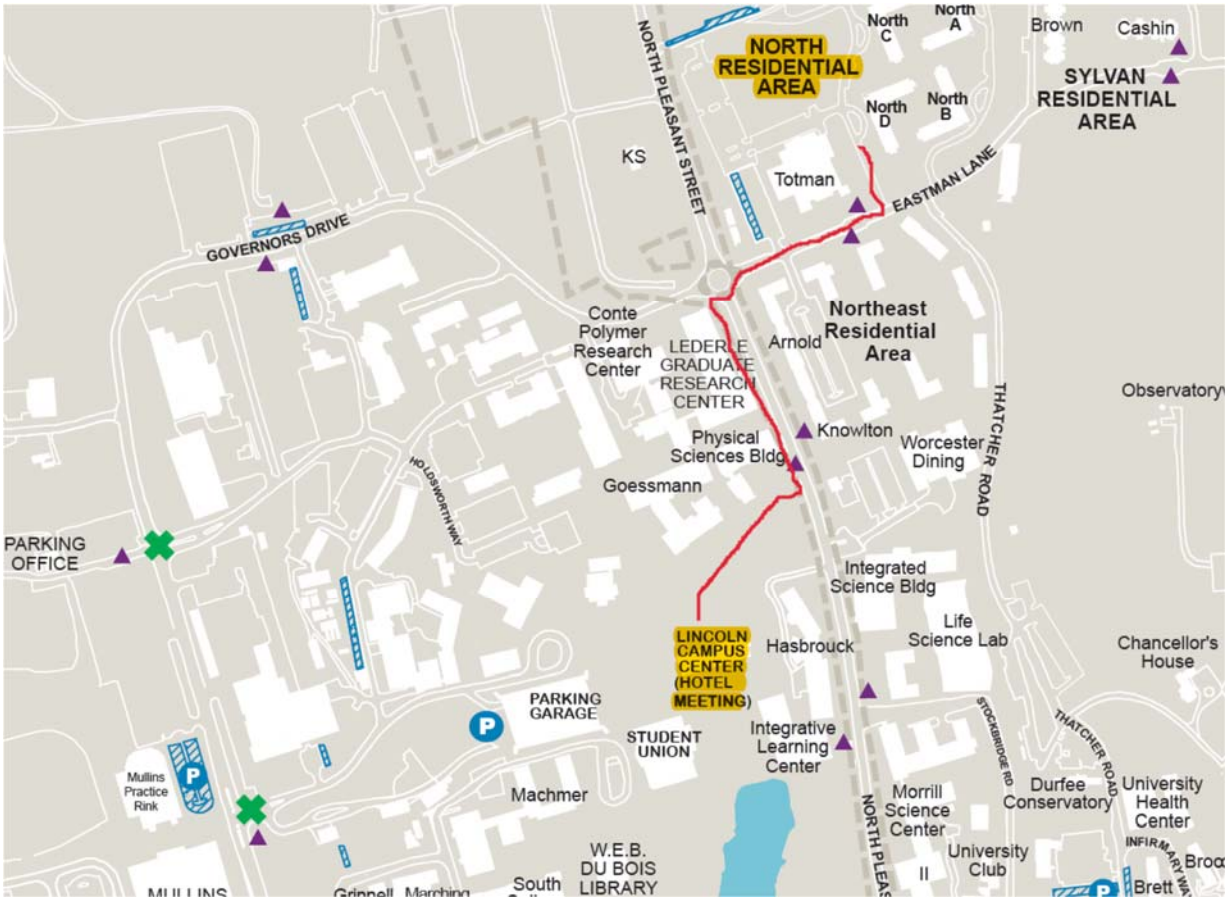
Carolina Rojas Ramirez
University of Michigan

Mehdi Shirzadeh
Texas A&M University

Sarah Sipe
University of Texas at Austin

Map

Map of UMass Amherst Campus, Conference Rooms, and Accommodations



PVTA bus stop

Stop light

walking directions from **North Residential Area** to **Lincoln Campus Center**

- **North Residential Area** is the where the on-campus residential apartments are located
- **UMass Hotel** is located in the **Lincoln Campus Center**
- **Meeting room (Room 163C)** is located down the escalators in the **Lincoln Campus Center**
- **Amherst** (10th floor) and **Marriott** (11th floor) Rooms are located in the **Lincoln Campus Center;** lunches and the Conference dinner will be located in these rooms

Lincoln Campus Center and UMass Hotel

Getting Around Amherst

More information about getting around campus and around Amherst can be found here: https://www.umass.edu/transportation/maps

Amherst Center, which has several bars, cafes, and restaurants, is approximately 1.5 miles from the Lincoln Campus Center. It is an easy walk to the Amherst Center, but there is also a bus system that connects the UMass Amherst campus and Amherst Center. More information about the PVTA bus system can be found here: https://www.umass.edu/transportation/find-my-bus

Buses 30, 31, 33, 36, 38, and 45 go through campus and through Amherst Center. On campus, these buses can boarded just outside the Physical Sciences Building (purple triangle on previous page map). **Bus drivers will not collect fares/passes.** Bus routes are fare-free for Five College students, faculty and staff. For the public, fares are on a proof-of-payment "honor system." Tickets and passes may be purchased online: http://www.pvta.com/faresPasses.php

UMass Amherst also participates in a bike share program called ValleyBike. Several bike-docking locations are found around campus. More information can be found here:
https://www.valleybike.org/
Bike-docking locations: https://www.umass.edu/sustainability/regional-bike-share-program

WiFi Instructions

Welcome to the University of Massachusetts Amherst!
Complimentary access to the UMass Wireless Network is available throughout campus, including Hotel UMass & the Campus Center/Student Union Complex.

Wireless Guest Account Access

Login instructions for the wireless network are below and on the blue sheet in your registration folder.

· In the WiFi settings on your phone/computer/tablet select the **UMASS** network

· Open up a web browser (IT suggests using Safari or Firefox)

· Manually type in the address bar: http://www.umass.edu

· Either a page telling you that this site is unsecure will present itself OR the UMass wireless login page will appear

· If it is a page telling you the website is unsecure, click "More Details" or "Advanced" at the bottom and click on the link to visit the page anyway

· You should then be on the UMass wireless network login page

· Enter the credentials below

Guest ID: ***07187113***
Password: ***69191704***

IT recommends that you save the guest ID and password in your browser to shorten the process for future logins

· The UMass home page should load and from this point you can type in any search query/website name you wish to visit

NOTE: If you close the web browser, shut the laptop, or change your location you will need to initiate the login session from the beginning.

Sunday July 21

9:00 – 16:00 (Optional) **Native MS Workshop** (Campus Center Room 163C)

16:00 – 18:00 **Registration** (Amherst Room, 10th Floor Campus Center)

17:00 – 18:00 **Welcome Reception** (Amherst Room, 10th Floor Campus Center)

Keynote Session (Amherst Room, 10th Floor Campus Center)

18:00 – 18:15 **Introductory Remarks** – Richard Vachet

18:15 – 19:00 **Michael Bowers** (UC Santa Barbara)
Assembly and Cross Talk in Amyloid Systems: ALS, Alzheimer's and T2D

19:00 – 19:05 Discussion

19:05 – 19:25 (Hot Topic) **Valérie Gabelica** (Univ. Bordeaux/CNRS/INSERM)
Circular dichroism ion spectroscopy to characterize the secondary structures of nucleic acid complexes

19:25 – 19:30 Discussion

19:30 – 21:00 **Reception** (Amherst Room, 10th Floor Campus Center)

Monday July 22

8:00 – 10:00 **Registration** (Campus Center 1st floor Concourse)

Session 1 – Gas-phase Biomolecules (Campus Center Room 163C)

Chair: Peter Armentrout

9:00 – 9:25 **Scott McLuckey** (Purdue University)
Ion Attachment to Enable Mass Measurement of High Mass Heterogeneous Complexes

9:25 – 9:30 Discussion

9:30 – 9:55 **Rachel Loo** (UCLA)
Ion Pairs and Entropy in Dissociating Complexes

9:55 – 10:00 Discussion

10:00 – 10:25 **Mike Guttman** (University of Washington)
Improving the reproducibility of solution-phase and gas-phase hydrogen/deuterium exchange with internal exchange reporters

10:25 – 10:30 Discussion

10:30 – 10:45 **Break** (Campus Center 1st floor Concourse)

Session 2 – Glycans (Campus Center Room 163C)

Chair: Ryan Julian

10:45 – 11:10 **Kevin Pagel** (Freie Universität Berlin)
Sugars in the Gas Phase - From Structure to Reaction Mechanisms

11:10 – 11:15 Discussion

11:15 – 11:40 **Catherine Costello** (Boston University)
Looking Forward: Thinking Big East & West of the Quabbin

11:40 – 11:45 Discussion

11:45 – 12:05 (Hot Topic) **Elyssia Gallagher** (Baylor University)
Characterizing solvated carbohydrates with rapid H/D exchange-mass spectrometry

12:05 – 12:10 Discussion

12:15 – 13:30 **LUNCH** (Amherst Room, 10th Floor Campus Center)

Session 3 – Proteomics (Campus Center Room 163C)

Chair: Igor Kaltashov

13:45 – 14:10 **Natalie Ahn** (University of Colorado Boulder)
Interrogating oncogenic B-RAF signaling through a microRNA network

14:10 – 14:15 Discussion

14:15 – 14:40 **Erin Baker** (North Carolina State University)
A Multi-Omic Investigation into the Molecular Signatures of Preeclampsia and Gestation Diabetes Mellitus

14:40 – 14:45 Discussion

14:45 – 15:10 **Eranthie Weerapana** (Boston College)
Chemical-proteomic strategies to investigate reactive cysteines

15:10 – 15:15 Discussion

15:15 – 19:00 **AFTERNOON FREE**

Session 4 – Proteins (Campus Center Room 163C)

Chair: Brandon Ruotolo

19:00 – 19:25 **Joshua Sharp** (University of Mississippi)
FOX: Laser-free sub-millisecond broadband Flash OXidation for hydroxyl radical protein footprinting

19:25 – 19:30 Discussion

19:30 – 19:50 (Hot Topic) **Steffen Lindert** (Ohio State University)

Utility of Covalent Labeling Mass Spectrometry Data in Protein Structure Prediction with Rosetta

19:50 – 19:55 Discussion

19:55 – 20:30 **Poster Previews**

Poster Session # 1 (Campus Center 1st Floor Concourse)

20:30 – 22:00 **Poster Session # 1 (Posters 1 – 31)**

Tuesday July 23

Session 5 – Gas-phase Biomolecules (Campus Center Room 163C)

Chair: Mary Rodgers

9:00 – 9:25 **Philippe Dugourd** (French National Center for Scientific Research (CNRS))
Electronic and structural dynamics of biomolecules

9:25 – 9:30 Discussion

9:30 – 9:55 **Etienne Garand** (University of Wisconsin - Madison)
Structure and microsolvation of protonated amino acids and peptides

9:55 – 10:00 Discussion

10:00 – 10:20 (Hot Topic) **Emily Chea** (University of Maryland)
Coupling FPOP with IM-MS for detailed structural characterization of the native ensemble of cytochrome c

10:20 – 10:25 Discussion

10:25 – 10:45 **Break** (Campus Center 1st floor Concourse)

Session 6 – Glycans (Campus Center Room 163C)

Chair: Matt Bush

10:45 – 11:10 **Tom Rizzo** (Ecole Polytechnique Fédérale de Lausanne)
Combining ultrahigh-resolution IMS with cryogenic ion spectroscopy for the analysis of glycans

11:10 – 11:15 Discussion

11:15 – 11:40 **Isabelle Compagnon** (Institut Lumière Matière, CNRS)
Spectroscopy-augmented mass spectrometry: towards a post isomeric barrier glycomics

11:40 – 11:45 Discussion

11:45 – 12:05 (Hot Topic) **Anouk Rijs** (Radboud University)

Making the invisible visible: IR photons as structural probe in mass spectrometry

12:05 – 12:10 Discussion

12:15 – 13:30 **LUNCH** (Amherst Room, 10th Floor Campus Center)

Session 7 – Nucleic Acids (Campus Center Room 163C)

Chair: Valérie Gabelica

13:45 – 14:10 **Renato Zenobi** (ETH Zürich)
Noncovalent Interactions Studied by Temperature-Programmed Native ESI-MS

14:10 – 14:15 Discussion

14:15 – 14:40 **Dan Fabris** (SUNY Albany)
Top-down approaches for the investigation of nucleic acids structure and dynamics

14:40 – 14:45 Discussion

14:45 – 15:10 **Kathrin Breuker** (University of Innsbruck)
Studying RNA interactions by mass spectrometry

15:10 – 15:15 Discussion

15:15 – 19:00 **AFTERNOON FREE**

Session 8 – Hot Topics (Campus Center Room 163C)

Chair: Rebecca Jockusch

19:00 – 19:20 (Hot Topic) **Nicholas Borotto** (University of Nevada, Reno)
Positive-Ion Mode Detection and Discovery of Tyrosine Sulfation via Alkylamine Adduction

19:20 – 19:25 Discussion

19:25 – 19:45 (Hot Topic) **Patrick Wintrode** (University of Maryland, Baltimore)
Molecular Dynamics Simulations as an Aid in Interpreting HDX-MS Data

19:45 – 19:50 Discussion

19:50 – 20:30 **Poster Previews**

Poster Session # 2 (Campus Center 1st Floor Concourse)

20:30 – 22:00 **Poster Session # 2 (Posters 32 – 61)**

Wednesday July 24

Session 9 – Lipids, Membranes, Proteins (Campus Center Room 163C)

Chair: Joseph Loo

9:00 – 9:25 **Michael Marty** (University of Arizona)
Stabilizing nanodiscs for native MS of membrane proteins and peptides in intact membranes

9:25 – 9:30 Discussion

9:30 – 9:55 **Jim Prell** (University of Oregon)
Gabor Transform Native IM-MS: From Membrane Toxins to ESI from Non-Volatile Salt Buffers

9:55 – 10:00 Discussion

10:00 – 10:25 **Art Laganowsky** (Texas A&M University)
Native mass spectrometry of mammalian potassium channels

10:25 – 10:30 Discussion

10:30 – 10:45 **Break** (Campus Center 1st floor Concourse)

Session 10 – Complex Macromolecules (Campus Center Room 163C)

Chair: Jennifer Brodbelt

10:45 – 11:10 **Igor Kaltashov** (University of Massachusetts Amherst)
New mass spectrometry-based approaches to characterization of highly heterogeneous macromolecules: focus on heparin

11:10 – 11:15 Discussion

11:15 – 11:35 (Hot Topic) **Derek Wilson** (York University)
Native Mass Spectrometry, Ion Mobility and Hydrogen Deuterium Exchange: A Powerful Toolkit for Accelerated Biopharmaceuticals Development in Vaccines and Neurodegenerative Disease

11:35 – 11:40 Discussion

11:40 – 12:00 (Hot Topic) **Joseph Gault** (University of Oxford)
Soft Landing Preparative Native MS of Soluble & Membrane Protein Complexes for High-Resolution Single Particle Imaging

12:00 – 12:05 Discussion

12:15 – 13:30 **LUNCH** (Marriott Room, 11th Floor Campus Center)

Session 11 – Advances in Instrumentation (Campus Center Room 163C)

Chair: Vicki Wysocki

13:45 – 14:10 **Ruwan Kurulugama** (Agilent Technologies)
Evaluation of a High-Resolution DeMultiplexing Method for Protein Structure Analyses

14:10 – 14:15 Discussion

14:15 – 14:40 **Roy Martin** (Waters)
Probing Protein structure by Cyclic Ion mobility and IMSn: Progress and Insights

14:40 – 14:45 Discussion

14:45 – 15:05 (Hot Topic) **David Russell** (Texas A&M University)
High Resolution FT-IMS-Orbitrap: Resolving What was Hidden

15:05 – 15:10 Discussion

15:10 – 18:00 **AFTERNOON FREE**

Conference Dinner (Marriott Room, 11th Floor Campus Center)

18:00 – 21:00 **Conference Dinner**

Thursday July 25

Session 12 – Proteins (Campus Center Room 163C)

Chair: Ian Webb

9:00 – 9:25 **Michal Sharon** (Weizmann Institute of Science)
Rapid Analysis of Recombinant Proteins

9:25 – 9:30 Discussion

9:30 – 9:55 **Tara Pukala** (The University of Adelaide)
Using structural mass spectrometry approaches to walk the pathways of protein misfolding

9:55 – 10:00 Discussion

10:00 – 10:25 **John Engen** (Northeastern University)
Multiprotein systems in protein folding and unfolding

10:25 – 10:30 Discussion

10:30 – 10:45 **Break** (Campus Center 1st floor Concourse)

Session 13 – Keynote Session (Campus Center Room 163C)

Chair: Richard Vachet

10:45 – 11:05 (Hot Topic) **Lynmarie Thompson** (University of Massachusetts Amherst)
Hydrogen exchange of chemoreceptors in functional complexes suggests protein stabilization mediates long-range allosteric coupling

11:05 – 11:10 Discussion

11:10 – 11:55 **Lila Gierasch** (University of Massachusetts Amherst)
A multi method approach to explore the allosteric mechanism of Hsp70 molecular chaperones

11:55 – 12:00 Discussion

12:00 – 12:10 **Closing Remarks**

Poster List

All posters will be located on the 1st Floor Concourse of the Campus Center
The maximum poster dimensions are 58 inches (147 cm) wide by 46 inches (117 cm) high

Posters 1 – 31 will be presented on Monday July 22 (Poster Session # 1) from 8:30 pm to 10:00 pm
Posters 32 – 61 will be presented on Tuesday July 23 (Poster Session # 2) from 8:30 pm to 10:00 pm

Posters should be set up by 6:30 pm the day of the poster session and removed at the end of the poster session.

POSTER SESSION # 1 – Monday July 22 – 8:30 pm to 10:00 pm

Poster Topic – Biomolecules in the Gas Phase

1. **Cytochrome P450s Captured within Lipid Nanodiscs Reveal Ligand-dependent Shifts in Gas-phase Stability** Kristine F. Parson, Colleen M. Riordan, Kathrine Gentry, Sarah M. Fantin, Carlo Barnaba, Ayyalusamy Ramamoorthy, Ryan C. Bailey, Brandon T. Ruotolo, *Department of Chemistry, University of Michigan*

2. **Towards Investigating Conformational Effects of DNA-drug Interactions by Gas-phase Förster Resonance Energy Transfer** JoAnn C. Chen Stephen V. Sciuto, Rebecca A. Jockusch, *Department of Chemistry, University of Toronto*

3. **Integration of high-resolution mass spectrometry with cryogenic ion vibrational spectroscopy** Evan Perez, Fabian S. Menges, Sean C. Edington, Chinh H. Duong, Nan Yang, Mark A. Johnson, *Department of Chemistry, Yale University*

4. **Making the invisible visible: IR photons as structural probe in mass spectrometry** Sjors Bakels, Sander Lemmens, Sebastiaan Porskamp, Oscar Janssen, Hidde Elferink, Thomas Boltje, Anouk M. Rijs, *Radboud University, Institute for Molecules and Materials, FELIX Laboratory, The Netherlands*

5. **Circular dichroism ion spectroscopy to characterize the secondary structures of nucleic acid complexes** Frédéric Rosu, Valérie Gabelica, *INSERM U1212, CNRS & Université de Bordeaux*

Poster Topic – Biotherapeutics

6. **Synergistic structural information about stressed therapeutic antibodies from hydrogen deuterium exchange and covalent labeling mass spectrometry** Catherine Y. Tremblay, Kong Limpikirati, Richard W. Vachet, *Department of Chemistry, University of Massachusetts Amherst*

7. **Collision Induced Unfolding Enables the Rapid Analysis of Stressed Monoclonal Antibodies and Biosimilars** Daniel D. Vallejo, Daniel A. Polasky, Jukyung Kang, Ruwan T. Kurulugama, John C.

Fjeldsted, Anna Schwendeman, Brandon Ruotolo, *Department of Chemistry, University of Michigan*

8. **A Robust MALDI-PSD Method for Site Specific Identification of Isomerized Aspartate Residue in Therapeutic Antibodies** John O. Hui, Tawnya Flick, Andrew Dykstra, Joseph A. Loo, Iain D. G. Campuzano, *Discovery Research, Amgen, Inc.*

9. **Analysis of NIST Monoclonal Antibody via a Quadrupole-Time-of-Flight Instrument Capable of Electron Capture Dissociation** Rebecca Glaskin, Cody Schwarzer, *Agilent Technologies*

Poster Topic – Covalent Labeling/Cross-Linking

10. **Energy Barriers to the Pre-amyloid Structural Change of β-2-microglobulin in the Presence of the Amyloidogenic Variant ΔN6 or Amyloid Inhibitors** Blaise G. Arden, Richard W. Vachet, *Department of Chemistry, University of Massachusetts Amherst*

11. **A New In Cell FPOP Platform for Studying Protein Folding** Dante T. Johnson, Benjamin Punshon-Smith, Anne Gershenson, Lisa M. Jones, *University of Maryland Baltimore School of Pharmacy*

12. **Comparing Gleevec's Drug Engagement in TNBC-African Ancestry and European Ancestry Cells using In-Cell Fast Photochemical Oxidation of Proteins** Emily E. Chea and Lisa M. Jones, *University of Maryland Baltimore School of Pharmacy*

13. **A Comparison of Crosslinker Length on Crosslinked Peptide Identification** Billy Samulak, *Fitchburg State University*

14. **Coupling FPOP with IM-MS for detailed structural characterization of the native ensemble of cytochrome c** Emily E. Chea, Daniel J. Deredge, Lisa M. Jones, *University of Maryland Baltimore School of Pharmacy*

15. **Covalent Labeling is Sensitive to Residue Microenvironment, Providing Improved Structural Analysis of Protein Higher Order Structure** Patanachai (Kong) Limpikirati, Xiao Pan, and Richard W. Vachet, *Department of Chemistry, University of Massachusetts Amherst*

Poster Topic – Hydrogen/Deuterium Exchange

16. **Understanding Signaling by Receptor Pseudokinases through Structures and Dynamics** Joshua B. Sheetz, Sebastian Mathea, Ketan Malhotra, Hanna Karvonen, Robert Perttilä, Niininen Wilhelmiina, Ravi Radhahkrishnan, Stefan Knapp, Daniela Ungureanu, Mark A. Lemmon, *Department of Pharmacology, Yale University School of Medicine*

17. **Reference Exchange Standards Enable Reproducible Measurements of Gas-Phase-Hydrogen-Deuterium Exchange Kinetics by Mass Spectrometry** Sanjit S. Uppal, Abhigya Mookherjee, Rick

Harkewicz, Sarah E. Beasley, Matthew F. Bush, Miklos Guttman, *Department of Medicinal Chemistry, University of Washington*

18. **HDX-MS Studies of CheA Domain Structure and Dynamic Changes to Control Kinase Activity** Aruni Karunanayake Mudiyanselage, Stephen J. Eyles, Lynmarie K. Thompson, *Department of Chemistry, University of Massachusetts Amherst*

19. **Characterizing solvated carbohydrates with rapid H/D exchange-mass spectrometry** Elyssia S. Gallagher, O. Tara Liyanage, H. Jamie Kim, Emvia I. Calixte, Amanda J. Pearson, Emily D. Ziperman, *Department of Chemistry and Biochemistry, Baylor University*

20. **Mass spectrometric-based methods for distinguishing isomeric protonated oligosaccharides utilizing the memory of their glycosidic bonds and more** Abhigya Mookherjee, Sanjit S. Uppal, and Miklos Guttman, *Department of Medicinal Chemistry, University of Washington*

Poster Topic – Ion Mobility Mass Spectrometry

21. **A New Platform for Multidimension Ion Mobility Mass Spectrometry of Native-like Ions Using Structures for Lossless Ion Manipulations (SLIM) Architecture** AnneClaire Wageman, Rachel M. Eaton, Benjamin Zercher, Matthew F. Bush, *Department of Chemistry, University of Washington*

22. **High-performance computing meets ion trajectory simulations: enabling the next generation of multidimensional ion mobility experiments** Ben Zercher, Rae M. Eaton, Matthew F. Bush, *Department of Chemistry, University of Washington*

23. **Ion Mobility-Mass Spectrometry Reveals α-Synuclein Conformational Changes Within Lipid Bicelles** Denise P. Tran, Joseph A. Loo, *Department of Chemistry and Biochemistry, University of California, Los Angeles*

24. **A Drift-Tube Ion Mobility-Mass Spectrometer for Native Mass Spectrometry: High Resolution Ion Mobility, Collision Induced Unfolding, and Electron Capture Dissociation** Varun V. Gadkari, Ruwan T. Kurulugama, John C. Fjeldsted, Brandon T. Ruotolo, *Department of Chemistry, University of Michigan*

25. **High Resolution FT-IMS-Orbitrap: Resolving What was Hidden** Jacob W. McCabe, Mehdi Shirzadeh, Art Laganowsky, David H. Russell, *Department of Chemistry, Texas A&M University*

Poster Topic – Native MS and Top-Down MS

26. **Comparison of native mass spectrometry in positive and negative-ion mode for proteins with varying isoelectric points** Alexis N. Edwards, Elyssia S. Gallagher, *Department of Chemistry and Biochemistry, Baylor University*

27. **Molecular mechanism of ISC Iron–Sulfur Cluster Biogenesis revealed by Native Mass Spectrometry** Cheng-Wei Lin, Jacob Macabe, David Russell, David Barondeau, *Department of Chemistry, Texas A&M University*

28. **Modelling Natural Bilayers with Mixed Lipid Nanodiscs for Native MS** Marius Kostelic, David Jurkowitz, Alex Ryan, Deseree Reid, Jibriel Noun, Michael Marty, *Department of Chemistry and Biochemistry, University of Arizona*

29. **Native Mass Spectrometry Guided Discovery and Characterization of Unknown Protein Machinery that Directs Plant Phenylpropanoid Synthesis and Stereochemistry** Mowei Zhou, Irina V. Novikova, Diana L. Bedgar, Laurence B. Davin, Callum J. Bell, John R. Cort, Jared Shaw, Norman G. Lewis, *Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory*

30. **Subunit interaction of glutathionylated human probed by surface-induced dissociation/ion mobility mass spectrometry** Monita Muralidharan, Amit Kumar Mandal, Vicki H. Wysocki, *Department of Chemistry and Biochemistry, Ohio State University*

31. **Structural Analysis of Gas-Phase Phosphoproteins** Carter Lantz, Rachel R. Ogorzalek Loo, Joseph A. Loo, *Department of Chemistry and Biochemistry, University of California, Los Angeles*

POSTER SESSION # 2 – Tuesday July 23 – 8:30 pm to 10:00 pm

Poster Topic – Biomolecules in the Gas Phase

32. **pH dependent binding affinity study of selected biologically active metal ions with a series of hexapepetides using ESI-IMMS** Ayobami Ilesanmi, Jack Williams, Tessa Moore, Laurence A. Angel, *Department of Chemistry, Texas A&M University-Commerce*

33. **The Effects of Metal Binding to the Primary Structure of Five Different Heptapeptides as Investigated Through Ion Mobility-Mass Spectrometry** Nayeli Fuentes, Laurence A. Angel, *Department of Chemistry, Texas A&M University-Commerce*

34. **Collision Induced Unfolding Captures Disease Relevant Differences in Stability and Ligand Binding for the Integral Membrane Peripheral Myelin Protein** Sarah M. Fantin, Kristine Parson, Pramod Yadav, Charles R. Sanders, Melanie D. Ohi, Brandon T. Ruotolo, *Department of Chemistry, University of Michigan*

35. **Carotenoid Profiling Analysis of Staphylococcus aureus by LC-MS^n in Different Growth Phases** Gerson-Dirceu López, Chad Leidy, Chiara Carazzone, *Laboratory of Advanced Analytical Techniques in Natural Products (LATNAP), Department of Chemistry, Universidad de Los Andes, Bogotá, Colombia*

36. **Positive-Ion Mode Detection and Discovery of Tyrosine Sulfation via Alkylamine Adduction** Nicholas Borotto, Phillip McClory, Brent Martin, Kristina Håkansson, *Department of Chemistry, University of Michigan*

Poster Topic – Covalent Labeling

37. **The Use of Chemical Penetration Enhancers to Increase Protein Modification by In Vivo Fast Photochemical Oxidation of Proteins** Jessica Arlett Espino, Zhihui Zhang, Lisa M. Jones, *University of Maryland Baltimore School of Pharmacy*

38. **In-Cell Fast Photochemical Oxidation of HCT116 Spheroids** Raquel Shortt, Jessica Lukowski, W. Temple Andrews, Amanda Hummon, Lisa M. Jones, *University of Maryland Baltimore School of Pharmacy*

39. **Covalent Modification via Ion/Ion Reactions with Ion Mobility/Mass Spectrometry Structural Analyses** Veronica Carvalho Sexton, Ian K. Webb, *Department of Chemistry and Biology, Indiana University – Purdue University Indianapolis*

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53. **Ion mobility spectrometry of proteins, nucleic acids and foldamers: advantages of high-level molecular dynamics to generate candidate structures** Frédéric Rosu, Valérie Gabelica, *CNRS UMS 3033, Inserm U01, IECB, Pessac, France*

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56. **Combining Native MS, Ion Mobility, Native Top-Down, and NMR Spectroscopy to study the Interaction of Roundabout1 with Arixtra** Robert V. Williams, Jeong-Yeh Yang, Kelley Moremen, James H. Prestegard, I. Jonathan Amster, *Department of Chemistry, University of Georgia*

57. **Enhanced characterization of membrane protein complexes using ultraviolet photodissociation** Sarah N. Sipe, John W. Patrick, Arthur Laganowsky, Jennifer S. Brodbelt, *Department of Chemistry, The University of Texas at Austin*

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59. **Top-down electron ionisation dissociation and internal fragment assignment of carbonic anhydrase II can enhance protein sequence coverage by mass spectrometry** Muhammad A. Zenaidee, Wonhyeuk Jung, Carter Lantz, Rachel R. Ogarzalek Loo, Joseph A. Loo, *Department of Chemistry and Biochemistry, University of California, Los Angeles*

60. **Application of omics and native mass spectrometry approaches to understand Salmonella pathogenesis** Angela Di Capua, Jikang Wu, Mikayla Borton, Anindita Sengupata, Anice Sabag-Daigle, Brian Ahmer, Venkat Gopalan, Kelly Wrighton, and Vicki Wysocki, *Department of Chemistry and Biochemistry, Ohio State University*

61. **Soft Landing Preparative Native MS of Soluble & Membrane Protein Complexes for High-Resolution Single Particle Imaging** Joseph Gault, Sabine Abb, Frank Sobott, Alexander Makarov, Klaus Kern, Carol Robinson, Stephan Rauschenbach, *Department of Chemistry, University of Oxford*

62. **Development of Gas-Phase Hydrogen Deuterium Exchange for Structural Characterization: Lessons from Enkephalin Variants** Cynthia Suarez, Rebecca Jockusch, *Department of Chemistry, University of Toronto*

63. **Detergents' Supercharging Effects on Soluble Proteins and Membrane Proteins** Wonhyeuk Jung, Muhammad Zenaidee, Janine Fu, Carter Lantz, Frederik Lermyte, Jennifer Lippens, Joseph A. Loo, Rachel Ogorzalek Loo, *University of California, Los Angeles*

64. **Evaluation of a modular atmospheric pressure drift tube coupled to an Orbitrap™ mass spectrometer with ultraviolet photodissociation for biomolecule analysis** James D. Sanders, Sarah Sipe, Tobias Reinecke, Brian Clowers, Jennifer S. Brodbelt, *Department of Chemistry, The University of Texas at Austin*

65. **Characterizing bond thermodynamics and dissociation dynamics in metal-containing cations**
Schuyler Lockwood, Ricardo B. Metz, *Department of Chemistry, University of Massachusetts Amherst*

Poster 1

Cytochrome P450s Captured within Lipid Nanodiscs Reveal Ligand-dependent Shifts in Gas-phase Stability

Kristine F. Parson[1], Colleen M. Riordan[1], Kathrine Gentry[2], Sarah M. Fantin[1,] Carlo Barnaba[2], Ayyalusamy Ramamoorthy[2], Ryan C. Bailey[1], and Brandon T. Ruotolo[1]
[1]Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109
[2]Biophysics Program and Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109

Introduction: Lipid nanodiscs (NDs) have proven to be highly informative environments for the study of membrane proteins (MPs), but many questions remain regarding the role of the ND lipid environment on MP structure. Furthermore, while native ion mobility-mass spectrometry (IM-MS) has emerged as an information-rich probe of MP structures within NDs, most studies focus on multi-pass MPs, leaving much unknown concerning monotopic and peripheral MPs. Here, we focus on the cytochrome P450s (CYPs), a centrally-important class of monotopic MP monooxygenases involved in drug metabolism, to both develop ND technology in combination with native MS, and answer long-standing questions surrounding CYP biophysics. Our collision induced unfolding (CIU) data, reveals that the lipid compositions of NDs can dramatically influence the stabilities of CYPs.
Methods: CYP isoforms were recombinantly expressed in E. coli, and purified. CYP isoforms were incorporated into lipid containing NDs using either peptide (4F) or membrane scaffold protein (MSP) scaffolding material and then purified using size exclusion chromatography. CYP incorporated peptide NDs were made using a shaker at 37 oC and MSP NDs were made using a microfluidic device. All samples were buffer exchanged into ammonium acetate prior to IM-MS acquisition on a Synapt G2 Q-IM-TOF instrument (Waters Corp, Milford, MA). When necessary, protein activation was performed in the trap region prior to ion mobility separation based on charge state, shape, and size. Data processing and analysis was performed using Waters MassLynx software and CIUSuite2.
Preliminary Data: We inserted CYP into nanodiscs comprised of purely POPC or DMPC for IM-MS analysis, yielding spectra indicative of native-like CYP monomers. Using IM-MS, we separated and removed chemical noise generated by the peptide, MSP scaffolding material, and free lipid clusters from the CYP-related signals detected. Our preliminary results show discrete shifts in CYP stability upon binding both ligands and lipids at various stoichiometries, as well as shifts in these overall stability manifolds when CYP complexes are housed within NDs of differing compositions. Initial IM-MS experiments using peptide NDs and MSP NDs illustrate differences in IM data suggesting the shape, size, and dynamics of the resulting ND plays a role in discovering the optimal ND environment for CYP isoforms and later for other membrane proteins. For the first time, we have demonstrated that CIU is capable of detecting stability differences in cytochrome P450, the first monotopic membrane protein analyzed in this manner, solubilized in NDs as a function of lipid composition and scaffolding belt. It is important to note that these differences are not apparent by IM-MS data alone, and are only revealed by employing CIU, where we observed significant differences in gas phase unfolding trajectories of CYP protein isoforms when liberated from POPC and DMPC NDs. In this presentation, we will cover our most recent data on CYP-ligand complexes, including: lipids, cholesterol, and pharmaceutically relevant drugs. We will also present our most recent work focusing on mixed lipid NDs, constructed in an attempt to mimic the endoplasmic reticulum from which CYP originates.

Poster 2

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

JoAnn C. Chen; Stephen V. Sciuto and Rebecca A. Jockusch*
Department of Chemistry, University of Toronto 80 St. George Street, Toronto, Ontario, Canada M5S3H6

Introduction

Mass spectrometry is a promising tool for structural characterisation of DNA-drug complexes. However, information on the desolvated DNA conformation (with or without drug binding) remains inconclusive. To probe the effects of solvent removal and drug binding on DNA conformation, we use Förster Resonance Energy Transfer (FRET) to examine mass-selected gaseous DNA and DNA-drug complex ions. FRET, a common condensed-phase spectroscopic technique, serves as a "molecular ruler" through nonradiative energy transfer from an electronically excited donor chromophore to an acceptor chromophore. Here, we employ a mass spectrometer that has been modified for fluorescence spectroscopy to probe conformational changes in DNA featuring terminal FRET chromophores *via* measurement of FRET efficiency. Development of an in-house fluorophore labelling and purification procedure will be discussed.

Methods

Two pairs of complementary 20-mer DNA sequences (5'-ATTCCGGCCGCTTAGGCAGT-3'/5'-ACTGCCTAAGCGGCCGGAAT-3' and 5'-TATACGCGAATTGGCCATAT-3'/5'- ATATGGCCAATTCGCGTATA-3') were covalently labelled with the donor/acceptor chromophores BODIPY FL/BODIPY 576 at their 5' ends by NHS ester-amine chemistry. Labelled single strands were melted at 60°C, then cooled slowly (-10°C/hour) into double stranded (ds) DNA's dsDNA1 and dsDNA2, respectively. FRET-labeled dsDNA samples were introduced into a modified quadrupole ion trap by nano-electrospray ionization (nanoESI), after which complexes with desired mass-to-charge ratios were isolated and stored. These complexes were irradiated with laser light tuned to donor absorption in the visible wavelength region. Steady-state fluorescence emission from the donor and acceptor chromophores were detected by dispersing light emitted from the complexes onto an electron-multiplying charge-coupled device to measure FRET efficiency.

Preliminary data

NanoESI mass spectra of the FRET-labelled dsDNA1 showed complexes in the 6-, 7-, 8-, and 9- charge states. Increase in charge state resulted in lower FRET efficiencies, suggesting a more extended dsDNA conformation consistent with greater electrostatic repulsion along the phosphate backbone. Measured FRET efficiencies for the 7- charge state were sensitive to adjustment of the nanoESI source conditions from "gentle" to "harsh." This was done by varying the capillary-skimmer voltage drop, with a gentleness scale pegged to retention of multiple ammonium adducts with a G-quadruplex. The measured FRET efficiencies from source harshness studies were indicative of dsDNA elongation as source conditions became harsher. The workflow described above was applied to dsDNA2, which show the same charge states as dsDNA1 in nanoESI mass spectra. After baseline characterisation, we will examine how mass spectra and FRET efficiencies change upon the addition of small-molecule drugs that interact with DNA through different modes of binding. Drugs of interest include amsacrine (intercalator) and cisplatin (covalent binder).

Poster 3

Integration of high-resolution mass spectrometry with cryogenic ion vibrational spectroscopy

Evan H. Perez, Fabian S. Menges, Sean C. Edington, Chinh H. Duong, Nan Yang, Mark A. Johnson
Department of Chemistry, Yale University, New Haven, CT 06520, USA

Introduction: Structural characterization of mass selected ions with vibrational spectroscopy is an increasingly valuable secondary analysis tool. This technique is most powerful when carried out with cryogenically cooled ions, typically exploiting the messenger gas "tagging" mode. The significant advantage of this method is that spectra are obtained for ions frozen at their global (or sometimes local) minima in a linear action regime that is directly comparable with calculated spectra of candidate structures. Here we describe the application of cold ion vibrational spectroscopy to the a_1, y_1 and z_1 fragment ions generated by collisional dissociation of a dipeptide precursor, PhenylalanylTyrosine (FY), in a commercial ThermoFisher Velos Orbitrap instrument that is paired with our custom cryogenic photofragmentation mass spectrometer based on time-of-flight (TOF).

Methods:

This new spectrometer was developed by interfacing this instrument to a cryogenic photofragmentation mass spectrometer based on time-of-flight (TOF) after three stages of mass-selection using two grid-free reflectrons. Collision induced dissociation was utilized to produce the fragment ions from the FY-H^+ ion. These fragments were then sent into the cryogen-cooled 3D quadrupole ion trap and tagged with N_2. After cooling, the tagged ions are transferred to a Wiley-McLaren TOF and the focused ion packet is intercepted by a tunable OPO/OPA IR source. Resonant excitation yields photoevaporative loss of the messenger tag, which is monitored by a second TOF mass separation stage as a function of laser excitation energy to generate the linear IR spectrum of the ion.

Preliminary data:

High throughput of the ions from the commercial orbitrap to the TOF laser interaction yields high quality spectra without extensive averaging. Preliminary IR spectra have been collected for the full FY-H^+ ion, the a_1 fragment, y_1 fragment, and the more controversial z_1 fragment. The c_1 and z_1 fragment are isobars, separated in mass by .05 amu, and thus relatively challenging to identify unambiguously. Thus, the high resolution afforded by the Velos Orbitrap (>100,000) was able to establish (with baseline accuracy) that the z_1 fragment is generated by CID. The z_1 vibrational spectrum was recorded found to be well matched with that calculated using density functional theory (DFT). The y_1 fragment also matches the calculation with reasonable accuracy. The a_1 fragment, on the other hand is more complex as the experimental spectrum contains a large broad (>100cm^{-1}) feature in the expected region of the NH_2+ fundamentals, more extensive (e.g., anharmonic) calculations are needed to achieve a compelling structure assignment. It is also the case that different isomers with correspondingly different intramolecular interactions could contribute to this broad envelope. Notably, the full FY-H^+ ion shows a strong interaction between the phenyl ring and one of the N-terminus hydrogens, shifting the peak about 150 cm^{-1} compared to no interaction. We address the present performance of the hybrid instrument as well as design features that would speed up the acquisition of the IR spectra as well as exploit the high resolution aspect of the Orbitrap directly in the isolation of the photofragments that yield the vibrational spectrum.

Poster 4

Making the invisible visible: IR photons as structural probe in mass spectrometry

Sjors Bakels, Sander Lemmens, Sebastiaan Porskamp, Oscar Janssen, Hidde Elferink, Thomas Boltje, Anouk M. Rijs. *Radboud University, Institute for Molecules and Materials, FELIX Laboratory, Toernooiveld 7-c, 6525ED Nijmegen, The Netherlands*

Introduction: To be able to control complex chemical reactions, to change the course of a disease at the molecular level or to direct the syntheses of functional biopolymers, it is necessary to fully understand the underlying molecular mechanism of these processes. This is often a major challenge as one needs to disentangle and probe molecular structures and transient intermediates hidden in heterogenic mixtures. What is necessary to reach this understanding is the ability to follow and structurally characterize the species present, i.e. to make the invisible processes visible by mapping the full molecular network.

Methods: Mass spectrometry is a vital tool for the identification and quantification of proteins, glycans, etc. and their non-covalent assemblies. However, despite its remarkable sensitivity, the structural resolution is limited. In contract, optical -and especially infrared- spectroscopy provides a diagnostic probe of molecular structure. Interfacing IR action spectroscopy to mass spectrometry has enabled us to add an extra dimension to mass spectrometric analysis, providing structural characterization of lowly populated intermediates in heterogenic mixtures.

Preliminary data: We probe the structure and structural changes of intermediates states s by advances in IR spectroscopy and mass spectrometry in order to unravel molecular mechanism of complex chemical reactions and biochemical/physical processes such as (1) self-assembly of peptides related to functional and pathological aggregate formation and (2) chemical glycosylation to predict and understand stereo selectivity. **Peptide-Aggregation – Keep on Growing**: The initial steps of peptide aggregation are often studied using mass spectrometry and ion mobility, and even combined with IR spectroscopy focusing on protonated aggregates. However, complementary studies on aggregates of neutral peptides remain elusive. The main reason for this gap is that it is experimentally challenging to form and to measure these large neutral peptide aggregate clusters. Here, we show that by advancing current technology we are able to make large peptide aggregates using laser desorption with molecular beam cooling. Moreover, we discuss here the mechanism of aggregation, including the competition between intramolecular interaction and intermolecular interaction, by probing their formation by a combination of infrared spectroscopy using the free electron laser FELIX and quantum chemical calculations [1-2]. **The mechanism of the glycosylation reaction:** A longstanding hurdle that prevents our understanding of the glycosylation reaction is the inability to characterize its elusive reaction intermediates. It is essential to characterize these intermediates and evaluate the influence of reaction parameters. We have recently shown that MS/MS can be used to generate reaction intermediates from the corresponding thioglycosides. Subsequent gas phase IR ion spectroscopy enabled the characterization of glycosyl oxocarbenium ions and dioxolenium ions using their highly diagnostic IR fingerprints. Currently, we are unraveling the role of remote participation to develop guidelines for the design of new stereoselective glycosylations [3-4].

References: [1] S. Bakels, E.M. Meijer, M. Greuell, S.B.A. Porskamp, G. Rouwhorst, J. Mahé, M.P. Gaigeot, and A.M. Rijs, Faraday Discussions (2019). [2] S. Bakels, S.B.A. Porskamp, and A.M. Rijs, *Angew.Chem.Int.Ed.* (2019) [3] H. Elferink, M. Severijnen, J. Martens, R. Mensink, G. Berden, J. Oomens, F. Rutjes, **A.M. Rijs**, and T. Boltje, *Journal of the American Chemical Society* **140**, 6034 (2018). [4] H. Elferink, R.A. Mensink, W.W.A. Castelijns, O. Jansen, J.P.J. Bruekers, J. Martens, J. Oomens, A.M. Rijs, T.J. Boltje, - *Angew.Chem.Int.Ed.* **58**, 8746–8751 (2019).

Poster 5

Circular dichroism ion spectroscopy to characterize the secondary structures of nucleic acid complexes

Steven Daly[1], Frédéric Rosu[2], Valérie Gabelica[3]

[1] Université de Bordeaux, INSERM & CNRS, Laboratoire Acides Nucléiques: Régulations Naturelles et Artificielles (ARNA, U1212, UMR5320), site IECB, 2 rue Robert Escarpit, 33600 Pessac, France. [2] CNRS, INSERM & Université de Bordeaux, Institut Européen de Chimie et Biologie (IECB, UMS3033, US001), 2 rue Robert Escarpit, 33600 Pessac, France

Introduction

To increase the power of native mass spectrometry in biophysics and structural biology, one needs new tools to characterize the ion structure. Ion mobility spectrometry gives information on shape, hence tertiary structure, but one lacks tools to characterize secondary structures. We present here the feasibility of circular dichroism ion spectroscopy to characterize nucleic acid complexes, for which the secondary structure is defined by the base stacking arrangements.

Methods: Nucleic acid complexes were prepared in 100 mM NH4OAc or in 100 mM trimethylammonium acetate and 1 mM KCl, sprayed in negative mode and trapped in a Bruker AmaZon quadrupole ion trap. The trap ring electrode has two opposite holes that allow the laser beam to pass through without reflections. A wavelength-tunable GWU Premiscan OPO laser and achromatic quarter wave plates were used to generate circularly polarized light. UV irradiation of DNA polyanions results in single photon electron detachment, which serves as a reporter. The CD effect is the difference of photodetachment efficiency between left and right circularly polarized light.

Results The first unambiguous demonstration of a statistically significant CD effect in the gas phase was made on the *d* and *l* enantiomers of the nucleic acid strand dTGGGGT, which forms the all-parallel G-quadruplex [(dTGGGGT)4.(NH4)3]. The *d* enantiomer forms a right-handed helix, and the *l* enantiomers form a left-handed helix. We observed opposite CD signals. Surprisingly, the magnitude of the CD effect in the gas phase was a few times higher than the magnitude of the effect in solution. This may be due to the delocalized nature of the electronic excited states responsible for the CD effect, combined with the fact that our reporter channel is electron detachment. We then investigated the CD signals of several oligonucleotides known to form 1) an antiparallel G-quadruplex, 2) a left-handed G-quadruplex formed by a sequence containing only the natural *d* enantiomers, and 3) a G-duplex stabilized by silver ions. In all cases, the CD spectra in the gas phase are analogous to the CD spectra in solution. In solution, the interpretation of CD spectra in terms of structure is based on the similarity with reference spectra, and our results suggest that the gas-phase spectra can use the solution reference spectra for their interpretation. For G-quadruplexes, we also can satisfactorily reproduce the experimental CD spectra by TD-DFT calculations, and this will provide us with a framework to interpret more unusual CD signals.

Poster 6

Synergistic structural information about stressed therapeutic antibodies from hydrogen deuterium exchange and covalent labeling mass spectrometry

Catherine Y. Tremblay, Kong Limpikirati and Richard W. Vachet
Department of Chemistry, University of Massachusetts Amherst

Introduction: Antibody based therapeutics are the fastest growing class of protein therapeutics. Robust approaches to accurately and quickly detect structural perturbations of protein therapeutics are necessary to ensure safety and efficacy. We are interested in studying the complementary structural information available for antibodies using both covalent labeling (CL) with diethylpyrocarbonate (DEPC) and hydrogen deuterium exchange (HDX) together with mass spectrometry (MS). HDX reports on the protein backbone structure and dynamics while covalent labeling reports on side chain accessibility. Because the kinetics of the two labeling techniques are on different timescales, we predict that there might be synergy between HDX and covalent labeling data when studying therapeutic antibody higher order structure (HOS).

Methods:DEPC CL and HDX were used to analyze structural perturbations of mAbs. Formulations of the therapeutic mAb, Rituximab, were heat stressed at 45, 55, and 65°C for four hours. After heat stress, both DEPC-CL and HDX were performed on the protein after cooling. CL took place at 37˚C for 5 minutes, then proteins were digested with trypsin and LC-MS was performed using a ThermoScientific nano-LC coupled to a ThermoScientific Orbitrap Fusion Tribrid. For HDX, Rituximab was incubated in deuterium at 10°C for various time points, then digested via pepsin column, and analyzed by LC-MS using the Waters HDX manager coupled with a Synapt G2 mass spectrometer. Both DEP-CL and HDX were compared to an unstressed control.

Preliminary data: DEPC CL and HDX experiments found no significant HOS changes at 45°C. However, HOS changes are detected at 55°C by DEPC CL, with increases in labeling extent clustering in the Fab region of the antibody. No HOS changes are detected at 55°C heat stress by HDX. Both methods were able to detect increases and decreases in exchange/labeling at 65°C. Increases in HDX exchange rates, due to unfolding or increased structural dynamics are observed primarily in the Fab region when heated at 65°C. Increases in DEPC covalent labeling, which are typical of residues becoming more solvent exposed due to unfolding, are also observed in only the Fab region. Decreases in both DEPC CL labeling extent and HDX uptake were observed throughout the protein. Overall, there are 21 residues detected by DEPC and 20 peptides detected by HDX that showed change after heat stress. Of those residues and peptides detected, 15 residues and 10 peptides had overlapping coinciding results. For instance, H439 decreases in DEPC labeling extent and falls within peptide 428-450, which shows a decrease in uptake. Three CL residues fall within peptides that show an opposite trend. For instance, K67 shows significant increase in labeling extent, however falls within peptide 35-70 that has a significant decrease in deuterium uptake. The remaining four CL residues and seven peptides do not overlap. However, three of these CL residues are structurally adjacent to peptides that show the same exchange trend. For the seven HDX peptides lacking corresponding DEPC CL data, two peptides reveal significant decreases in deuterium uptake, but have no changes in CL. We hypothesize that this is because these regions experience a loss of dynamics, yet no change to the solvent accessibility of the labelable side chains. These results highlight the synergistic data derived from HDX and DEPC CL.

Poster 7

Collision Induced Unfolding Enables the Rapid Analysis of Stressed Monoclonal Antibodies and Biosimilars

Daniel D. Vallejo[1], Daniel A. Polasky[1], Jukyung Kang[2], Ruwan T. Kurulugama[3], John C. Fjeldsted[3], Anna Schwendeman[2], Brandon Ruotolo[1]

[1]Department of Chemistry, University of Michigan, Ann Arbor, MI; [2]Department of Pharmaceutical Sciences, University of Michigan, Ann Arbor, MI; [3]Agilent Technologies, Santa Clara, California 95051, United States

Introduction: Many well-validated tools are currently in place to measure the critical quality attributes (CQA) of monoclonal antibody (mAb) based biotherapeutics. These tools are often employed in multiple attribute monitoring (MAM) methodologies in order to provide thorough analysis of mAbs throughout production. Many such MAM approaches require large amounts of purified protein, lengthy analysis times, and homogeneous protein populations. MS-based methods that measure the stability of gas-phase proteins, such as collision induced unfolding (CIU), have the potential to be orders of magnitude faster while providing greater information content than their solution phase counterparts. In this presentation, we study the capability of CIU to differentiate between innovator mAbs and their biosimilars under native and stressed conditions.

Methods: CIU data for innovator mAbs and their biosimilars were acquired either with a Synapt G2 quadrupole-ion mobility-mass spectrometry (Q-IM-MS) or an Agilent 6560 IM-Q-TOF instrument platform, by collisionally activating ions prior to IM separation. The resulting datasets were classified using a modified version of CIUSuite 2 software capable of handling charge multiplexed CIU datasets. Multiple lots of the pairs for Herceptin, Avastin, and Rituxan were stressed at 40°C with 250RPM orbital shaking for 4 weeks in formulation, and CIU data was analyzed to determine shifts in gas-phase protein stability, and similarly used to differentiate native and stressed mAbs. Unstressed Remicade, Inflectra, and Renflexis were analyzed similarly by CIU for 3-way classification workflows. All innovator and biosimilars mAbs were purchased commercially.

Preliminary Data: Typical CIU workflows involve the selection of a single m/z prior to collisional heating and subsequent CIU measurements. While this approach provides high precision CIU datasets, it can be slow, requiring 30-60 minutes to collect CIU data for each selected ion population. To improve analysis times, and make CIU more amenable for rapid biosimilar comparison workflows, we employ classification techniques that utilize charge multiplexed CIU datasets, typically collected in a few minutes. This data forms the foundation of our CIU assay which allows for the rapid characterization of mAb composition, stability and structure simultaneously. Our preliminary data reveals CIU differences for a range of stressed innovator/biosimilar pairs of Rituxan vs. Acellbia; Herceptin vs. HERtiCAD; and Avastin vs. Avegra. Specifically in the context of Herceptin, we are able to detect differences in the stability of mAb structure based on the collision energy required to reach unfold the antibody in CIU experiments. Generally, we observe that this CIU50 value for the first unfolding transitions across all charge states are similar, but subsequent transition values in stressed mAbs are destabilized by 5 V for the innovator, and 2 V for biosimilar samples. These differences in CIU datasets were used to train binary classifications schemes for individual charge states (23–28+), and each provided at least 80% probability for correctly classifying an unknown sample as either innovator or biosimilar. Our presentation will include CIU data demonstrating a modified CIUSuite 2 classification that incorporates all charge states of the mAb sample which generally improves upon single charge state classifications previously stated, and its application towards differentiating between innovator Remicade and its two biosimilars, Inflectra, and Renflexis.

Poster 8

A Robust MALDI-PSD Method for Site Specific Identification of Isomerized Aspartate Residue in Therapeutic Antibodies

John O. Hui[1], Tawnya Flick[2], Andrew Dykstra[2], Joseph A. Loo[3] and Iain D. G. Campuzano[1]
Discovery Research[1], Process Development[2], Amgen Inc., Thousand Oaks, CA 91320; Department of Chemistry and Biochemistry[3], UCLA, Los Angeles, CA 90095

Introduction Before a monoclonal antibody (mAb) can be developed for human therapeutic use, candidates are often evaluated after being subjected to various forced degradation studies and molecules which show chemical liabilities that negatively impact activity are rapidly identified. While methionine or tryptophan oxidation and asparagine deamidation can be easily detected by peptide mapping using LC-MS/MS, identification of *iso*Asp is challenging because both Asp and *iso*Asp are isomeric. Detection of *iso*Asp specific diagnostic ions using ECD, ETD and EThcD has been reported. However, depending on the peptide sequence, the resultant diagnostic ions can be of low intensity. Herein, we demonstrate Asp and *iso*Asp containing peptides from a temperature stressed mAb digest can be readily characterized by PSD using a MALDI-TOF/TOF mass spectrometer.
Methods A commercial mAb was employed as a model for this study because Asp102 of its heavy chain has been well documented to undergo isomerization with a concomitant loss in activity. The protein (1.26 mg/mL) was incubated in 0.1 M ammonium bicarbonate (pH 7.8) and at 40□C for 2 weeks. An aliquot was reduced and carboxyamidomethylated prior to proteolytic degradation. An unstressed control was treated in an identical manner. The digest was analyzed by reversed phase LC-MS/MS using a Q Exactive Plus mass spectrometer for peptide identification. Peptide elution was achieved by a linear gradient of acetonitrile in 0.1% formic acid. Another identical digest was chromatographically separated using the same LC system and the peptides collected for MALDI-PSD analysis.
Preliminary Data Identification of isomerized Asp residues in peptides is challenging because the MS/MS data is essentially identical to its unmodified counterpart. The earlier eluting peptide is usually assumed to contain the modified residue. In this study, we show that MALDI-PSD can be used to unequivocally identify *iso*Asp in peptides. MALDI-PSD was performed on synthetic tripeptides (LDA and L*iso*DA) and nonapeptides (WAGGDASGE and WAGG*iso*DASGE) and the results showed the bond N-terminal to the modified residue was preferentially cleaved. The method was tested on a commercial antibody that has been stressed at 40 °C for 2 weeks. After tryptic digestion, the peptide of interest with 26 amino acid residues W99GGDGFYAMDYWGQGTLVTVSSASTK124 was eluted in 2 major peaks at a ratio of approximately 3:2 in the stressed material. ESI-MS/MS was unable to distinguish the 2 peptides. However, MALDI-PSD of the 2 peptides clearly showed an enhancement of y23 in the later eluted peak; hence indicating this was the *iso*Asp containing peptide. Digestion of the protein with chymotrypsin again resulted in 2 major peaks encompassing heavy chain Asp102 with identical mass C96SRWGGDGFY104. PSD fragmentation of the 2 chymotryptic peptides showed the later eluted peak has an enhancement in the b6+H2O, thus concluding this was the peptide with the modified residue. It was also noticed even fragmentation after the *iso*Asp residue (the D-effect) was increased when compared with the unmodified peptide. It is unclear how PSD fragmentation of the singly charged species would lead to site specific fragmentation at the modified residue. Future study combining molecular dynamics and quantum mechanical calculations of di-or tripeptides would proceed to understand the subtle structural differences of the Asp and *iso*Asp containing peptide, resulting in the unique MALDI-PSD fragmentation patterns.

Poster 9

Analysis of NIST Monoclonal Antibody via a Quadrupole-Time-of-Flight Instrument Capable of Electron Capture Dissociation

Rebecca Glaskin and Cody Schwarzer
Agilent, Lexington, MA

Introduction:
Glycans and glycoconjugates have roles in several biological processes, from protein folding to molecular recognition, which makes it critical to determine the structures of these biomolecules. There are several tandem mass spectrometry (MS/MS) techniques developed for the structural determination of the sequences, compositions, linkages, branching patterns, and aglycons. Here we have modified a quadrupole-time-of-flight (Q-TOF) instrument with a radiofrequency- free electromagnetostatic (RF-free EMS) cell1 between the quadrupole and collision cell for the study of electron capture dissociation (ECD) of the resulting peptides and glycopeptides of the NIST monoclonal antibody (NIST mAb). ECD allows for the retention and characterization of post-translational groups that are typically lost via collision-induced dissociation (CID).
Methods: An Agilent 1290 Infinity II liquid chromatography system was coupled to a dual Agilent Jet Stream Electrospray Ionization Source (AJS ESI) into a 6545XT AdvanceBio LC/Q-TOF (Agilent Technologies, Santa Clara, CA). Prior to entering the mass analyzer, ions can be dissociated via ECD and/or CID without modification of the system once the RF-free EMS was installed. The modified Q-TOF included an RF-free EMS cell with the following assembled components (listed in order): two electrostatic lenses, a permanent magnet, heated wire filament within a holder, a permanent magnet, and two electrostatic lenses.
Preliminary Data: The resulting peptides and glycopeptides from the tryptic digest of NIST mAb were analyzed via both CID and ECD, with comparison of the resulting spectra. One feature of the RFfree EMS cell is the capability to reduce the current of the filament or alter lens potentials to stop the ECD fragmentation, enabling the system to perform CID without hardware modification and with results comparable to those obtained on nonmodified Q-TOFs. The ECD and CID glycopeptide spectra resulting from the tryptic digest of the NIST mAb demonstrated the additional information that can be obtained with the ECD cell. The CID spectra of the following glycopeptide, TKPREEQYN*STYR (N* is site of glycosylation), did not provide any significant cleavage of the peptide backbone but instead resulted in sequential loss of monosaccharide units from the glycopeptide. The ECD spectra of the same glycopeptide yielded much more informative fragments. Not only was peptide backbone fragmentation observed, but the modification was retained allowing for site localization.

References

1. Voinov., V. *et al*., *J. Am. Soc. Mass Spectrom*., **2015**, *26*, 2096-2104.

Poster 10

Energy Barriers to the Pre-amyloid Structural Change of β-2-microglobulin in the Presence of the Amyloidogenic Variant ΔN6 or Amyloid Inhibitors

Blaise G. Arden and Richard W. Vachet
Department of Chemistry, University of Massachusetts Amherst

Introduction

Dialysis related amyloidosis is caused by formation of β-2-microglobulin (β2m) fibrils, a protein subunit of the class one major histocompatibility complex. The amyloidogenesis of β2m involves a crucial structural conversion from the native to amyloid competent state. A truncated, amyloidogenic variant of β2m, ΔN6, is a structural mimic of β2m after this conversion. Coincubation of wild type (WT)β2m and ΔN6 has been shown to induce the structural conversion in the WT in a prion-like manner. Several small molecule drugs were discovered to inhibit the amyloid forming process in β2m. Using a Trp-specific covalent labeling mass spectrometry technique, we have elucidated the energy barrier to the pre-amyloid structural change of WT β2m when incubated with ΔN6 or inhibitors.

Methods

β2m and ΔN6 were dissolved in MOPS buffer at pH 6.8 or 7.4 and mixed. The mixture was then allowed to incubate for varying time points. Alternately, WT β2m was incubated with small molecule amyloid inhibitors doxycycline or Rifamycin SV for varying time points. At select time points, dimethyl(2-hydroxy-5-nitrobenzyl) sulfonium bromide (HNSB), which labels Trp residues, was added at a 25:1 molar excess (HNSB to protein) and reacted for 45 seconds. The change in extent of Trp labeling over time reflects the pre-amyloid structural change. Immediately following the labeling reaction, the solution was desalted and analyzed directly on a Bruker ion trap mass spectrometer. The experiment was performed at different temperatures and the activation energy determined.

Preliminary Data

When reacted with HNSB for 45 seconds, the total Trp labeling on ΔN6 does not exhibit a significant change over time, demonstrating that this variant is in fact a locked structural mimic of the amyloid competent state of β2m. When ΔN6 and WT β2m are incubated together, the extent of Trp labeling of the WT β2m drops over several days, indicating progression of the pre-amyloid structural change. This process is much slower when induced by interaction with ΔN6 than when induced by the addition of Cu(II), addition of trifluoroethanol, or low pH. In addition, over the first several hours (at 22°C) there is an increase in Trp labeling on ΔN6. This labeling increase suggests that the interaction of the two proteins is having an effect on the structure of ΔN6 as well as inducing the pre-amyloid structural change in the WT. The energy barrier to the structural switch in the presence of ΔN6 has been determined and will be presented. Small molecule drugs doxycycline and Rifamycin SV are known to inhibit the amyloid formation of β2m by altering the aggregation pathway towards formation of amorphous aggregates. Suramin is a small molecule drug that binds to β2m, but does not inhibit the amyloid forming process. Preliminary data indicates that when incubated with β2m and stoichiometric amounts of Cu(II), doxycycline eliminates the pre-amyloid structural change. Suramin was used as a control and preliminary data indicate that the pre-amyloid structural change proceeds unimpeded. The rates of the structural switch for each condition at different temperatures allow us to measure energy barriers using the Arrhenius Equation. To our knowledge, these are the first measurements of energy barriers to the pre-amyloid structural change of β2m as initiated by protein-protein interactions and energy barriers as affected by small molecule inhibitors.

Poster 11

A New In Cell FPOP Platform for Studying Protein Folding

Dante T. Johnson[1], Benjamin Punshon-Smith[2], Anne Gershenson[3] Lisa M. Jones[1]
[1]*University of Maryland Baltimore School of Pharmacy* [2]*University of Maryland Baltimore County*
[3]*University of Massachusetts Amherst*

Introduction: Traditionally, protein folding has been observed *in vitro* using full length protein sequences, but folding in the cell is quite different from folding studies in an isolated environment due to the interactions of chaperones and modifying enzymes. To overcome these limitations, our lab has begun development of a new method for protein folding studies. This method, pulse chase in-cell fast photochemical oxidation of proteins (pcIC-FPOP), couples pulse-chase technology with mass spectrometry-based in cell footprinting which will allow for fast analysis of short lived protein folding intermediates. Once developed, this method would fill a gap in knowledge in protein folding and its role in various diseases.

Methods: The new platform includes an incubation system, XY movable stage with controllers, peristaltic pumps, a 248nm KrF excimer laser, and optic mirrors. First, the incubation system which included a temperature and CO2 unit, humidifier, and monitoring system from OKOlabs was assembled. Next was the assembly of the custom six well plate OKOlabs incubator and MCL Madmotor drives. Perfusion line tubing was placed and flushed against the walls of each well with robust 3D printed rings to secure the tubing without disturbing the cells or getting in the way of the laser beam pulse. The system as a whole is called Platform Incubator with an XY movable stage (PIXY). Transient transfections were performed to assess and compare cell culture quality under standard incubator and stage top incubator conditions. A luciferase assay was performed to quantitate transfection efficiency. Manual FPOP experiments were also carried out to access comparability of technique and results.

Preliminary Data: The PIXY platform has been assembled and is currently being optimized to demonstrate its efficacy for pcIC-FPOP. The configuration and automation of the customized parts require a systematic approach for testing and optimizing the setup. The cell culture conditions in the stage top incubator were tested. For pcIC-FPOP, it is important that cells are incubated at optimal cell culture conditions so that normal cellular functions, particularly protein synthesis, can be maintained. To ensure the stage top incubator conditions are amenable for cell culture at the laser platform, we tested transient transfections in the incubator using the fluorescent protein GCaMP2. Transient transfections of GCaMP2 in HEK 293 cells were performed in two 6-well plates using Lipofectamine 3000. One well plate was incubated in the stage top incubator and the second well plate was incubated in a standard CO2 incubator. Two days after transfection, fluorescence imaging was performed on both plates for comparison of transfection efficiency. A luciferase assay was performed to quantitate transfection efficiency. Preliminary studies show that two days after transfection significant GCaMP2 fluorescence can be observed via fluorescence imaging. Since pcICFPOP protein folding will be studied after transient transfections, testing the efficiency of these transfections in the stage top incubator is an important benchmark. Results show PIXY significantly outperformed the standard incubator. This experiment provided a quantitative benchmark for preservation of cellular function during incubation in the stage top incubator. IC-FPOP was also performed with PIXY and compared to our flow system setup. For our standard IC-FPOP flow system 550 proteins were oxidatively modified while 770 proteins were modified in PIXY, the pcIC-FPOP platform.

Poster 12

Comparing Gleevec's Drug Engagement in TNBC-African Ancestry and European Ancestry Cells using In-Cell Fast Photochemical Oxidation of Proteins

Emily E. Chea and Lisa M. Jones
University of Maryland Baltimore School of Pharmacy

Introduction

For breast cancer (BC), the mortality disparity has elevated to 42-81% higher in young black women. This is especially true for triple negative breast cancer (TNBC), an aggressive form of BC that is more common in black women. To help close the gap in proven treatments for black women, we are comparing the effects of a kinase inhibitor, Gleevec, on TNBC from African ancestry (TNBC-AA) and European ancestry (TNBC-EA) cells using in-cell FPOP (IC-FPOP). We are developing IC-FPOP as a tool to study drug engagement in cells giving us information on both drug targets and off targets. With the use of IC-FPOP for Proteome-Wide Structural Biology (PWSB) we have observed a few major differences Gleevec has on TNBC-AA and TNBC-EA.

Methods

TNBC-AA and TNBC-EA were treated with 10 µM Gleevec or vehicle (water) for 24 hours. After 24 hours the cells were collected and subjected to IC-FPOP using a single cell flow system and collected in a cell permeable ROS quench. Cellular proteins were isolated and underwent a trypsin digest. Afterwards, peptides were separated offline using high pH reversed-phase chromatography and the fractions concatenated into 16 samples. All fractions were analyzed through tandem mass spectrometry on a Q Exactive HF and searched against the *Homo sapiens* database with possible hydroxyl radical modifications using Proteome Discoverer. Significant changes in FPOP modifications between Gleevec and vehicle treated cells are searched manually along with major differences of Gleevec's effects on TNBC-AA and TNBCEA.

Preliminary Data

TNBC-AA and TNBC-EA were treated with Gleevec and vehicle and oxidatively modified using IC-FPOP. Following treatment proteins underwent a trypsin digestion followed by a 2D high-low reverse phase separation with bottom-up MS/MS on a Q Exactive HF mass spectrometer. Using Proteome Discoverer, between 3000-5000 proteins were matched per sample against the *Homo sapiens* proteome with 970-2900 proteins with hydroxyl radical modifications. The extent of FPOP modification is compared first between the Gleevec and vehicle treated cells exposing several proteins with significant changes in oxidation alluding to possible effects caused by the Gleevec treatment. Then the differences between TNBC-AA and TNBC-EA cells show multiple changes in protein oxidation indicating varying affects from Gleevec. Using the same data set we can also localize multiple changes in phosphorylation. Following Gleevec treatment the TNBC-EA show a trend towards proteins with lower %phosphorylation while TNBC-AA have higher %phosphorylation. With the use of databases with known Gleevec targets and software observing protein signaling pathways, the observed differences for both oxidation and phosphorylation help characterize the drug engagement of Gleevec and why TNBC-AA and TNBC-EA are affected differently.

Poster 13

A Comparison of Crosslinker Length on Crosslinked Peptide Identification

Billy Samulak
Fitchburg State University

Introduction:

Chemical crosslinking combined with mass spectrometry has been increasingly used in the field of structural biology to develop models of proteins and complexes. This technique provides particularly valuable information for systems that cannot be detailed using traditional high resolution methods. Each crosslink observed places a distance restraint on an unknown protein model or suggests an interacting partner, therefore the identity of the crosslinker used in a crosslinked experiment is important. Here, I sought to compare the identity of crosslinked peptides observed, as well as partially quantify those peptides when crosslinkers of increasing length were applied to the same system.

Methods:

In separate experiments, the model protein aldolase was crosslinked using four crosslinkers of increasing length from 6 to 18 Angstroms. Additionally, the protein was crosslinked with increased ratios of crosslinker. After crosslinking, aldolase was digested with trypsin and then analyzed by LC/MS/MS on an Orbitrap-Fusion. Using an internal standard, both crosslinked and noncrosslinked peptides were quantified.

Data:

Crosslinkers used in this poster were all water soluble N-hydroxysuccinimide esters which react with primary amines. Crosslinking efficiency was verified using SDS-PAGE prior to MS analysis and samples using high ratios of crosslinker appeared highly crosslinked. Samples were successfully digested, also visualized using SDS-PAGE. A comparison of sequence coverage from unmodified aldolase to highly crosslinked aldolase did not result in percentage differences. However, the peptides observed were different. Regardless of length of crosslinker, sections of the protein that had not been observed prior to crosslinker were observed after crosslinking. Additionally, sections of the protein that were observed prior to crosslinking were not observed after crosslinking. It was expected that the longer the crosslinker, the more unique crosslinked peptides would be observed. For each crosslinked sample, the identity of crosslinked peptides was determined and then counted. Most peptides with primary amines were quantified, however, there was no general trend. The number of spectra that were unmodified remained constant for some amines, increased for others, and decreased for yet others.

Poster 14

Coupling FPOP with IM-MS for detailed structural characterization of the native ensemble of cytochrome c

Emily E. Chea, Daniel J. Deredge and Lisa M. Jones
Department of Pharmaceutical Science, School of Pharmacy, University of Maryland Baltimore

Introduction

Detailed biophysical characterization is essential for structure-function studies. Gold standard methods such as crystallography, NMR and cryo-EM achieve highest resolution but may lack detailed description of the conformational heterogeneity of solution conformational ensemble. Other approaches such as SAXS, AUC, fluorescence spectroscopy probe the structure of an ensemble in solution but report low resolution parameters averaged over the ensemble and/or time. Mass spectrometry based methods allow for both simultaneously. Fast Photochemical Oxidation of Proteins (FPOP) has been successful in structurally characterizing proteins at residue-level resolution. Ion mobility mass spectrometry (IM-MS) has been applied to delineate large conformational heterogeneity. Here, we used FPOP, IM-MS and an enzymatic assay in combination to characterize the native state ensemble of cytochrome c.

Methods

Bovine heart cytochrome c purchased from Sigma was prepared at 10 µM in 100 mM ammonium acetate and subjected to FPOP. Samples were split in three for intact mass analysis, bottom up FPOP and a native IM-MS/top down approach. Intact mass analysis was performed on a Synapt G2S. Bottom-up FPOP was performed on a Thermo orbitrap Fusion Lumos. The peptides and FPOP modifications are analyzed in Proteome Discoverer 2.1. For the IM-MS/top down, a Synapt G2S was used in MSe mode. Protein conformations on the +7 charge state were separated by arrival time distribution and subsequently subjected to CID fragmentation. The peroxidase activity of cytochrome c was probed by spectroscopically monitoring the oxidation of guaiacol at 470 nm.

Preliminary Data

Cytochrome c was oxidatively modified by FPOP and intact protein samples were run to observe and select samples with adequate levels of FPOP labeling. Bottom up FPOP analysis was performed to identify and quantify residues that were oxidatively modified. Overall, 26 residues were found to be modified but the extent of modification correlated poorly with calculated protection factors from cytochrome c structures in the PDB. However, native IM-MS revealed three distinct peaks at different drift times, pointing to distinct coexisting conformers. Furthermore, exposure to hydrogen peroxide resulted in the appearance and accumulation of a compact conformer. The exposure of cytochrome c to hydrogen peroxide has previously been linked to a change in heme coordination, concomitant with an increase in peroxidase activity leading to its role in apoptosis. Top-down analysis of mobility separated conformers was performed to assess conformer specific FPOP modifications. Of the 26 residues identified by the bottom-up approach, 16 were successfully identified. Of note, heme coordinating methionine 80 was observed to selectively populate two out of the three conformers in solution as well the hydrogen peroxide induced compact conformer. Time resolved IM-MS following exposure to hydrogen peroxide, performed in parallel with time resolved peroxidase activity assay, suggests that the accumulation of compact conformer is accompanied with an increased peroxidase active conformational ensemble of cytochrome c.

Poster 15

Covalent Labeling is Sensitive to Residue Microenvironment, Providing Improved Structural Analysis of Protein Higher Order Structure

Patanachai (Kong) Limpikirati, Xiao Pan, and Richard W. Vachet
Department of Chemistry, University of Massachusetts Amherst, Amherst, Massachusetts 01003, United States

Introduction: Covalent labeling (CL) along with mass spectrometry (MS) is increasingly being used for the structural analysis of proteins and protein therapeutics. Diethylpyrocarbonate (DEPC) is a simple to use, commercially-available reagent that can readily react with a range of nucleophilic residues in proteins. We find that in intact proteins weakly nucleophilic side chains (Ser, Thr, Tyr) can be modified by DEPC at significant abundances. Free peptides with these amino acids, however, rarely react with DEPC. We hypothesize that the microenvironment around these side chains, as formed by a protein's higher-order structure (HOS), tunes their reactivity such that they can be labeled. We have investigated this microenvironment effect to understand the structural features that tune the reactivity of these weak nucleophiles.
Methods: Model proteins (□-2 microglobulin [□2m], ubiquitin, and human growth hormone [hGH]) were prepared in a buffer at physiological pH. The DEPC modification reaction was performed at 37 ^{o}C for 1 to 5 minutes at 4:1 to 5:1 (DEPC : protein) molar ratio and was quenched by the addition of imidazole. The resulting samples were denatured, reduced, and/or alkylated before digestion with immobilized chymotrypsin or trypsin. Free peptides from the same proteins were produced via the same digestion conditions, and the N-termini of the peptides were subsequently acetylated via reactions with sulfo-NHS-acetate before labeling them with DEPC. DEPC-modified peptides from both cases were analyzed by LC-MS/MS on a Bruker AmaZon quadrupole ion trap mass spectrometer or a Thermo Orbitrap Fusion.
Preliminary data: Model peptides were used to test whether weak nucleophilic residues in free peptides, without the influence of microenvironment, can react with DEPC. We find that even when the other reactive site (e.g. N-terminus) was blocked in these peptides, free peptides with Ser and Thr rarely react with DEPC. In free peptides, Tyr residues have limited reactivity, compared to the reactive His and Lys at DEPC to peptide molar ratios of 5 to 1 while Ser and Thr residues are unreactive even under 50-fold molar excess of DEPC. In contrast, when intact proteins (□2m, ubiquitin, and hGH) are reacted with DEPC (at 4- to 5-fold molar excess), 18 Ser, Thr, and Tyr residues are labeled at levels ranging from 0.02% to 63.9%. Meanwhile, when the same proteins are first digested into peptide fragments and then reacted with DEPC at the same labeling conditions, almost none of these Ser, Thr, and Tyr residues are found to be modified. In these experiments the N-termini of the peptide fragments are blocked via acetylation before labeling them with DEPC because many new N termini are produced upon protein digestion and these new N-termini could potentially outcompete Ser, Thr, and Tyr residues. Even after reacting the N-terminally blocked peptides at DEPC to peptide molar ratios of 50 to 1 the reactivity of the Ser, Thr, and Tyr residues in free peptides are still lower than in the intact proteins. Together, these results indicate that weak nucleophilic side chains almost never react with DEPC in free peptides, supporting the hypothesis that a protein's local microenvironment tunes the reactivity of these residues. From a close examination of the structural features, we find that nearby hydrophobic residues are essential, suggesting that the enhanced reactivity of certain Ser, Thr, and Tyr residues occurs due to higher local concentrations of DEPC.

Poster 16

Understanding Signaling by Receptor Pseudokinases through Structures and Dynamics

Joshua B. Sheetz[1], Sebastian Mathea[2], Ketan Malhotra[1], Hanna Karvonen[4], Robert Perttilä[4], Niininen Wilhelmiina[4], Ravi Radhahkrishnan[3], Stefan Knapp[2], Daniela Ungureanu[4], Mark A. Lemmon[1]

[1.] Department of Pharmacology, Yale University School of Medicine, New Haven, CT 06520, USA; Yale Cancer Biology Institute, Yale University, West Haven, CT 06516, USA.

[2.] Institute for Pharmaceutical Chemistry, Johann Wolfgang Goethe-University, Max-von-Laue-Str. 9, D-60438 Frankfurt am Main, Germany and Buchmann Institute for Molecular Life Sciences, Structural Genomics Consortium, Max-von-Laue-Str. 15, D-60438 Frankfurt am Main, Germany; German Cancer Consortium DKTK Frankfurt/Mainz, Frankfurt, Germany. [3.] Graduate Group in Biochemistry and Molecular Biophysics, University of Pennsylvania Perelman School of Medicine, Philadelphia, PA 19104, USA; Department of Bioengineering, University of Pennsylvania, Philadelphia, PA 19104, USA. [4.] Cancer Signaling, Faculty of Medicine and Health Technology and BioMediTech, Tampere University, Tampere, 33014, Finland.

Introduction: Receptor tyrosine kinases (RTKs) are key regulators of critical cellular processes such as growth and proliferation, survival and differentiation, metabolism and migration. Phosphotransferase activity of the intracellular kinase domain is conventionally considered critical for signal initiation at the membrane. This convention is challenged by seven RTKs in humans, which bind protein ligands in their extracellular regions but lack robust catalytic activity needed for canonical RTK signaling. To understand the function of these highly conserved receptors and its mechanistic basis, we hypothesize that the RTK pseudokinase domains signal through 'conformational switching.' Rather than regulating enzyme activity, regulators of these receptors could promote switching between conformation states—possibly between the so-called active-like and (various) inactive-like conformations seen in *bona fide* kinases.

Methods: We have used X-ray crystallography, hydrogen-deuterium exchange-mass spectrometry (HDX-MS), chemical biology approaches, and cell signaling studies to investigate: 1) what range of conformation states can be adopted by RTK pseudokinase domains, 2) how particular conformational states may be controlled by small molecules, and 3) how control of pseudokinase conformations might regulate signaling outcomes.

Preliminary Data: Intriguingly, the Ror2, RYK, CCK4/PTK7 and ErbB3 RTK pseudokinases have all crystallized in conformations that resemble the inactive state of their closest ‘live’ relatives. For example, the Ror2, RYK, and CCK4/PTK7 pseudokinases display the same autoinhibitory interactions seen in the human insulin receptor kinase, suggesting that they may similarly undergo phosphorylation-driven conformational changes, and may even bind ATP under certain conditions (such as when phosphorylated). In addition to structures, our analyses of pseudokinase conformational dynamics using HDX-MS demonstrates conserved dynamics in solution compared to *bona fide* kinase relatives. We further identified small molecule binders of the pseudokinase Ror1 and demonstrated through co-crystallization and HDX-MS that occupation of the pseudo-ATP binding site can induce structural and conformational changes to promote a more ‘active-like’ state of the pseudokinase domain. We are now working to use these structural insights to guide functional investigations of the RTK pseudokinases.

Poster 17

Reference Exchange Standards Enable Reproducible Measurements of Gas-Phase-Hydrogen-Deuterium Exchange Kinetics by Mass Spectrometry

Sanjit S. Uppal[1], Abhigya Mookherjee[1], Rick Harkewicz[1], Sarah E. Beasley[1], Matthew F. Bush[2], Miklos Guttman[1]
[1]*Department of Medicinal Chemistry, University of Washington, Seattle WA, 98195*
[2]*Department of Chemistry, University of Washington, Seattle WA, 98195*

Introduction: Mass-spectrometry allows for high throughput studies in glycobiology. Gas-Phase hydrogen-deuterium exchange (gHDX) mass-spectrometry has been shown to resolve closely related isobaric carbohydrate ions. While the utility and immense potential of gas-phase HDX-MS has been demonstrated, it suffers from the critical limitation that instrumental conditions vary during day-to-day operation via several parameters that are nearly impossible to control; temperature, pressure in ion-source, detector performance, etc., all of which offset observed HDX-kinetics. We developed Reference Exchange Standards (R.E.S) for the purpose to correct for the observed HDX offsets. Secondly, we showed by using the R.E.S, intra-platform HDX datasets can be compared/standardized, since the experimental sample's HDX kinetics are consistent relative to the HDX of the R.E.S, regardless of intra platform variation.

Methods: HDX experiments were performed on a Waters SYNAPT-G1 quadrupole-time-of-flight (Q-TOF). The gas in the transfer traveling wave ion-guide was replaced with deuterated-ammonia, deuterated-water, or deuterated-methanol to enable deuterium exchange in the instrument. With all samples, the reference exchange standards (Tris, Bis-Tris-Methane and Bis-Tris-Propane) were infused through the secondary (lock-mass) inlet. A series of acquisitions were collected to sample a temporal-range of ~0.1 to 10 milliseconds (ms). Each standard was co-sampled with Galacto-N-biose, Myoglobin, Peptide P1, Arginine and Bis-2-hydroxy-amine under different HDX conditions. Data was batch converted using scripts in UniDec and analyzed using HX-Express-v2.

Preliminary Data: The mixture of Tris, Bis-tris-methane and Bis-tris-propane served as useful R.E.S to observe any offsets in HDX kinetics as each compound exchanges on a distinct timescale. Incorporation of the R.E.S effectively ensured instrumental reproducibility for HDX data sets. More importantly, by scaling data to the R.E.S it corrected for any offsets in instrument-conditions and enabled comparability studies using HDX-data obtained from different conditions. Secondly, any differences observed in HDX kinetics between closely related samples could now be attributed to the structural attributes of the ions in the samples and not to some type of experimental/instrumental variability. We also observed that the R.E.S ensured instrumental reproducibility for HDX-data sets in multiple deuterating agents, including but not limited to deuterated ammonia, deuterated methanol and deuterated water. HDX-data was obtained at different times from multiple classes of experimental samples, including peptides, glycans, proteins and bifunctional compounds. Purposeful HDX kinetic offsets were installed and the collected data was scaled to the R.E.S. Once scaled, the R.E.S indeed did correct for the purposeful HDX kinetic offsets. The data was then standardized for all experimental samples and different types of deuterating agents; indicative of instrumental reproducibility and allowing for comparability studies. Examination of the kinetics collected on different days over the course of several months showed excellent consistency, demonstrating the general reproducibility of gasphase HDX using R.E.S. With this new robust reference exchange standard, gas-phase HDX-MS can effectively be used to differentiate even very closely related carbohydrates and infer structural information about proteins, bifunctional-compounds and a host of other compounds.

Poster 18

HDX-MS Studies of CheA Domain Structure and Dynamic Changes to Control Kinase Activity

Aruni Karunanayake Mudiyanselage, Stephen J. Eyles, and Lynmarie K. Thompson
Department of Chemistry, University of Massachusetts, MA 01003

Introduction: Chemoreceptors in cells are organized in hexagonal arrays with the help of CheA kinase and CheW coupling proteins that interact at the membrane-distal cytoplasmic tip of the receptors. The central question that remains unanswered is how ligand binding at one end of the receptor molecule controls the activity of CheA bound to its other end ~300 Å away, and how CheA domain structure and dynamics change to control kinase activity. Domain-domain interactions among the five domains of CheA (P1-P5) are important for its function. Our HDXMS study aims to identify (1) changes in interactions and flexibility of domains of CheA that increase kinase activity, and (2) changes that are involved in receptor modulation of CheA activity between kinase-on and kinase-off complexes.
Method: Domain interactions and dynamics are being investigated using hydrogen deuterium exchange mass spectroscopy (HDX-MS) on CheA in solution and in native-like functional receptor arrays. Functional arrays are prepared by assembling the Asp receptor cytoplasmic fragment (CF), CheA, and CheW on vesicle surfaces, under conditions with limiting concentrations of CheA so that ~90% of CheA is bound in functional complexes. The exchange is compared for CheA incorporated into kinase-on complexes prepared with CF4Q and kinase-off complexes prepared with CF4Q.A411V mutant.
Preliminary data: We have identified peptides that span all domains and linkers of CheA with ≥90% coverage, and preliminary comparison of HDX for CheA alone and in functional complexes with CF4Q and CheW has produced interesting insights into CheA domain dynamics. For CheA alone (kinase-off), we observe slow initial uptake (at 3 min) on one face of domain P1, suggesting this face may be docked against another domain. Assembly of kinase-on complexes with CF4Q and CheW does not change HDX of this face of P1, but does increase uptake in the helix bearing the phosphorylation site. Increased flexibility of this helix may allow access of the phosphorylation site in P1 to the active site in P4. Fast initial uptake is observed throughout the P4 catalytic domain of CheA alone (kinase-off), suggesting P4 is more dynamic than all of the other domains. Formation of kinase-on complexes with CF4Q and CheW decreases initial HDX throughout P4, which may correspond to stabilization of this domain into its active conformation, especially since the greatest change in HDX occurs in the ATP lid. These observed changes in HDX provide insight into mechanisms of activating CheA. Comparison of HDX in kinase-on and kinase-off complexes will identify what changes are induced by the receptor to modulate activity of CheA, the central kinase in bacterial chemotaxis.

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Poster 19

Characterizing solvated carbohydrates with rapid H/D exchange-mass spectrometry

Elyssia S. Gallagher, O. Tara Liyanage, H. Jamie Kim, Emvia I. Calixte, Amanda J. Pearson, Emily D. Ziperman
Department of Chemistry and Biochemistry, Baylor University

Introduction: Glycans are structurally complex molecules that have been implicated in multiple diseases. H/D exchange mass spectrometry (HDX-MS) has become a standard method for analyzing conformations and binding interactions of solvated proteins. Carbohydrates, model systems for glycans, are susceptible to HDX since they contain labile hydrogens, primarily in the form of hydroxyls, which can be labeled with deuterium (D) upon exposure to deuterated solvents. However, compared to backbone amides, the functional group detected in traditional HDX-MS experiments for proteins, the exchange rate of glycan hydroxyls is two to eight orders of magnitude faster, depending on solution pH. This rapid exchange rate makes it unfeasible to monitor HDX of carbohydrate hydroxyls using traditional, bottom-up HDX methods.
Methods: We describe our ongoing efforts to characterize solvated carbohydrate structures. We perform rapid HDX reactions by introducing deuterating reagents to carbohydrates during ESI and/or ESI droplet evaporation. As with other MS analyses of carbohydrates, we detect carbohydrate-metal ion adducts following ESI in positive-ion mode. Experiments are performed on either a Waters Synapt G2-S or a Thermo Orbitrap Discovery MS. To describe how carbohydrate structure is altered during ESI, we perform molecular dynamics simulations in Gromacs using the CHARMM36 forcefield. We model melezitose, a nonreducing trisaccharide, in water droplets (TIP4P/2005) in the presence of sodium ions. We use the evaporation protocol previously described by the Konermann group (McAllister *et. al. J. Am. Chem. Soc.* **2015**, *137*, 12667).
Preliminary Data: Our previous work illustrates that these rapid, in-ESI HDX methods characterize solvated carbohydrates rather than gas-phase, metal-ion adducts (Liyanage *et. al. J. Am. Soc. Mass Spectrom.* **2019**, *30*, 235-247). However, during ESI, native (unmodified) carbohydrates must become desolvated and coordinate to metal ions to be detected by MS. Thus, we used simulations to examine when, and how, these processes occur, since they could alter the \ carbohydrate structures sampled by in-ESI HDX. Our simulations illustrate that native carbohydrates ionize by the charge residue model. Initially, ESI nanodroplets containing melezitose undergo solvent evaporation and Na+ are ejected when the Raleigh limit is reached. Melezitose remains solvated within the droplet and adducts to residual Na+ as solvent evaporates. When more than one melezitose is present in the droplet, the melezitose molecules and Na+ form a cluster with Na+. However, these clusters regularly decompose to form carbohydrate monomers adducted to Na+. These simulations are consistent with experimental results, in which the primary species that we detect following ESI-MS is the [melezitose-Na]+ adduct. These simulations illustrate that carbohydrates remain solvated until the late stages of the ESI process; therefore, in-ESI HDX methods primarily sample solvated carbohydrates. We have quantified how ESI source conditions alter the magnitude of HDX for carbohydrate model systems. We have developed an internal standard, maltoheptaose derivatized by reaction with Girard's T reagent, to control for daily humidity differences that alter back exchange, improving the measurement repeatability on different days. We have established methods to alter the HDX labeling time on the microsecond to millisecond timescale. Furthermore, we have measured differences in the relative D-uptake for trisaccharide isomers (melezitose and maltotriose), detected as sodium-adducts in the MS. This work illustrates the promise of utilizing HDX to characterize solvated-carbohydrate structures.

Poster 20

Mass spectrometric-based methods for distinguishing isomeric protonated oligosaccharides utilizing the memory of their glycosidic bonds and more

Abhigya Mookherjee, Sanjit S. Uppal, and Miklos Guttman
Department of Medicinal Chemistry, University of Washington

Introduction:
Carbohydrates are implicated in many biological processes, including in diseased conditions, which necessities our understanding of glycosylation. However glycans exhibit great structural diversity. They can exist as many potential isomers that differ in their linkages branching patterns that complicate structural characterization by mass spectrometry. MS data is further complicated with the increasing numbers of carbohydrate monomers. Here we utilize ion mobility (IM) and gas-phase hydrogen deuterium exchange (HDX) along with tandem MS for resolving structural and positional glycans. Differences in fragmentation patterns, ion mobilities, and deuterium uptake kinetics of fragment ions are used to differentiate isomeric glycans.

Methods:
Various oligosaccharides at 10 to 200 μM in 0.1% formic acid were examined by direct infusion on a Thermo Scientific LTQ where tandem MS (up to MS^4) was performed. IM was performed on a Synapt G2-Si Q-TOF mass spectrometer using different drift gases in the IMS cell. Gas-phase HDX experiments were performed on a Synapt G1 Q-TOF where ND3 was infused in to one of the traveling wave ion guides. Further, the anomers of each oligosaccharide were resolved over a HILIC LC-MS system and subjected to tandem MS and ion mobility, on the Thermo Scientific LTQ and Synapt G2-Si Q-TOF respectively.

Preliminary Data:
Targeted MS was performed on a set of protonated disaccharides differing in their linkage (e.g., β1-3 vs β1-6) and monosaccharide composition (e.g., GalNAc vs GlcNAc). MS^n revealed characteristic differences in the fragmentation patterns of the isobaric disaccharides differing only in their linkages. Distinct fragmentations were also observed for isomeric disaccharides differing in a single monosaccharide, e.g., GalNAc vs GlcNAc on the reducing end, and galactose vs. fucose on the non-reducing end. While LC-MS resolved the anomers of the isomeric saccharides and their respective fragmentation products, ion mobility when employed along with LC-MS helped in the identification of signature fragmentation patterns and distinct IM profiles of the diagnostic fragment ions formed from the LC-resolved anomers of the isomeric saccharide precursors. Further, gas-phase HDX performed on the isomeric disaccharides showed bimodal exchange spectra for some diagnostic ions symptomatic of the presence of more than a single family of structures for these ions, in turn adding an orthogonal identifier. These methods are being further used to develop universal metrics that could be used for identifying similar diagnostic ions formed from the fragmentation of larger oligosaccharides.

Poster 21

A New Platform for Multidimension Ion Mobility Mass Spectrometry of Native-like Ions Using Structures for Lossless Ion Manipulations (SLIM) Architecture

AnneClaire Wageman, Rachel M. Eaton, Benjamin Zercher, Matthew F. Bush
University of Washington, Department of Chemistry, Box 351700, Seattle, WA 98195-1700

Introduction

Native ion mobility mass spectrometry is a technique gaining popularity for use in structural biology and the analysis of protein complexes. Through the use of nanoelectrospray from native-like solution conditions, gas-phase size and shape measurements can provide insight about protein complex interactions in solution. Typical ion mobility experiments are performed by applying an electric field across a series of stacked ring electrodes in the presence of a neutral background gas, resulting in temporal separation of ions based on their size and shape. Here, we combine modular devices constructed using Structures for Lossless Ion Manipulations (SLIM) architecture that enable the injection, separation, selection, and trapping of native-like ions to enable higher-order experiments.

Methods

This instrument is comprised of 12 SLIM modules that are each constructed using a mirrored pair of 76 mm by 76 mm printed circuit boards. These modules are positioned between the ion source and the mass spectrometer. During transfer through the modules, ions pass through a first dimension separation path of 457 mm, followed by an orthogonal transfer region of 76.2 mm, and finally a second dimension separation path that is 381 mm. Depending on the time-dependent potentials applied to electrodes on the modules, this instrument can enable longer, single dimension ion mobility separations as well as many operating modes of tandem ion mobility.

Preliminary data

The potential energy surfaces experienced by ions within the SLIM instrument are created by applying a monotonically decreasing set of voltages to all of the rung electrodes. After ions are trapped in the first printed circuit board “module”, they are released into the first dimension for separation. By keeping all applied voltages downstream of this trap constant, ions can be transmitted directly to the detector. Collision cross section values measured in this manner of peptides, proteins, and protein complexes are consistent with values measured on other SLIM as well as RF-confining drift tube instruments. Tandem ion mobility experiments, in which the voltages applied in the orthogonal transfer region are manipulated mid-separation, are also demonstrated. One example is the selection-only mode, in which ions of a given mobility can be selected by only allowing transmission through the orthogonal region for specified windows of time (on the order of several milliseconds). Selection-trapping mode, in which ions are mobility selected and then held in the orthogonal transfer region for an arbitrary length of time (milliseconds to second) before being separated on the second dimension, are also demonstrated. Finally, we illustrate some new operating modes for this platform. Future tandem IM experiments on this instrument may provide more information about conformers that are unable to be resolved using only single IM separations.

Poster 22

High-performance computing meets ion trajectory simulations: enabling the next generation of multidimensional ion mobility experiments

Ben Zercher, Rae M. Eaton, Matthew F. Bush*
Department of Chemistry, University of Washington, Box 351700, Seattle, WA 98195-1700

Introduction

Multi-dimensional separations are a potential tool to increase the selectivity of ion mobility analysis of biomolecules. Structures for Lossless Ion Manipulations (SLIM) is an emerging ion mobility architecture that offers promise for multi-dimensional separations due to its ability to select and trap ions using time dependent voltage control. When compared to traditional electrostatic drift tubes, SLIM comprises a much larger parameter space under which to carry out IM separations. To better understand the effects of different parameters, we simulated the trajectories of ions with a wide range of mobilities (from a small molecule drug to a multiprotein complex) through different SLIM modules.

Methods

Ion trajectory simulations were carried out using SIMION 8.1 using a hard-spheres collision model that simulates each ion-neutral collision. The ions simulated ranged from 152.1 Da to 64000 Da, with collision cross sections with nitrogen gas ranging from 1.3 nm2 to 41.6 nm2. Ions were simulated either in a standard linear flight or while entering a 90 degree turn on a SLIM module. In order to overcome the computational expense of the collision model and explore the vast parameter space, the Linux binary of SIMION 8.1 was deployed on the University of Washington's high performance computing cluster Hyak. This project demonstrates a promising avenue for scaling up trajectory simulations during the development of new instrumentation.

Initial Results

Here, we considered SLIM modules comprised of DC-only guard electrodes and DC+RF rung electrodes. Guard electrodes confine ions laterally, while rung electrodes provide the electrostatic DC gradient for ion mobility separations while the superimposed RF voltages confine ions away from the board. The design of these modules and their use to enable tandem ion mobility experiments have been reported previously (Allen, Eaton, Bush. *Anal. Chem*. **2017**, *89*, 7527.) Simulations were conducted to determine the optimal RF amplitude for ions with a range of mobilities. Simulation results showed that at 4 mm spacing between the printed circuit boards that make a SLIM module, high RF amplitudes reduced the apparent mobility of ions, a phenomenon we have termed "RF dampening". Higher mobility ions are dampened more severely and at lower RF values than lower mobility ions. Increasing the board to board spacing to 5.5 mm mitigates RF dampening for RF amplitudes up to 250 Vpp. Interestingly, simulation drift times matched the expected drift times even up to 10 mm board to board spacing, which is uncharacteristically large for the SLIM architecture. In more intricate SLIM implementations, ions not only undergo linear transmission but are also selected and turned off-axis. We modeled the trajectories for analyte ions at a variety of field strengths. Results from these simulations show that there is a mobility dependence for ion resonance time in the turning region. These results are now being used to design and interpret results from a new, flexible platform for tandem ion mobility that includes modules for ion accumulation/injection, ion selection, ion trapping, and a pair of 90 degree turns.

Poster 23

Ion Mobility-Mass Spectrometry Reveals α-Synuclein Conformational Changes Within Lipid Bicelles

Denise P. Tran, Joseph A. Loo
Department of Chemistry and Biochemistry. The University of California, Los Angeles

Introduction:

α-Synuclein is an intrinsically disordered protein (IDP) that has been implicated in the etiology of Parkinson's Disease (PD)[1]. It has been previously suggested to interact and integrate into lipid vesicles, changing in structure to form α-helices preventing the formation of fibrils[2]. Mass spectrometric analysis of protein embedded in phospholipids has been challenging due to solubility difficulties and the large molecular masses associated with vesicles and nano-discs. Bicelles; composed of detergents and lipid, have recently been discovered as a versatile and efficient method to analyse protein embedded in a lipid bilayer though mass spectrometry. The coupling of Ion Mobility to Mass Spectrometry (IM-MS) enables the determination of structural changes to ions through the measurement of the arrival time distribution (ATD) and peak Drift Time (DT)

Methods:

Bicelles were synthesized through cycles of freeze thaw and ultra-sonication of mixtures of Octyl β-D-glucopyranoside (OG) and lipids of either 1-stearoyl-2-oleoyl-sn-glycero-3-phospho-(1'-rac-glycerol) (SOPG) or 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-L-serine (POPS). Mixtures of bicelles (200 mM AA, pH 6.8) and α-synuclein (r-Peptide, Georgia, USA) at varying ratios of bicelle:protein (100:1 to 10:1) were incubated at room temperature and shaken at a frequency of 40 Hz. An hour following the co-incubation of α-synuclein and bicelles, the mixtures were analyzed by IM-MS and were subsequently analyzed periodically up to a 30 days.

Preliminary data:

IM-MS analysis of α-synuclein following co-incubation with bicelles, revealed changes in conformation subsequent to lipid integration. Unfortunately, the bicelles proved difficult to ionize and therefore the unbound monomeric α-synuclein was monitored over time. It was found that the ion abundance of soluble α-synuclein decreased following incubation. These results suggest that free, unbound IDP is likely to be interacting with bicelles. A trend of exponential decay of ion abundance was observed with the varying ratios of bicelle:protein. Activation of Collisional Induced Dissociation (CID) energy was required to remove the detergent and lipid of the bound bicelle to allow IM-MS analysis of the monomeric αsynuclein. Interestingly, IM revealed the embedded α-synuclein was found to initially increase in DT and then following further incubation, decrease in DT. This indicates the possible change in structure formation as the monomeric protein inserts itself into the phospholipid bilayer.

References:

1. Galvagnion C. The role of lipids interacting with α-synuclein in the pathogenesis of Parkinson's disease. *J Parkinsons Dis*. 2017;7:433-450. doi:10.3233/JPD-171103
2. Dong C, Hoffmann M, Li X, et al. Structural characteristics and membrane interactions of tandem α-synuclein oligomers. *Sci Rep*. 2018;8(1):1-11. doi:10.1038/s41598-018-25133-0

Poster 24

A Drift-Tube Ion Mobility-Mass Spectrometer for Native Mass Spectrometry: High Resolution Ion Mobility, Collision Induced Unfolding, and Electron Capture Dissociation

Varun V. Gadkari[1], Ruwan T. Kurulugama[2], John C. Fjeldsted[2], and Brandon T. Ruotolo[1]
[1]Department of Chemistry, University of Michigan, Ann Arbor, Michigan 48109 [2]Agilent Technologies, Santa Clara, California 95051

Introduction: Native ion mobility-mass spectrometry (IM-MS) is capable of revealing much that remains unknown within the structural proteome, promising a comprehensive view of changing protein conformations, protein-ligand interactions, and multi-protein assemblies within mixtures, using relatively small sample quantities without the need for chemical labeling. A number of native IM-MS instrument platforms have been developed previously, each targeting specific capabilities in an effort to maximize our ability to collect structural information on refractory protein targets. Here, we report the development of a prototype drift tube IM-MS (DTIM-MS) platform for native IM-MS with improved in-source activation capabilities, allowing us to push the limits of IM and MS resolution in combination with collision induced unfolding (CIU) and electron capture dissociation (ExD).
Methods: An Agilent 6560 DTIM quadrupole time-of-flight (DTIM-QTOF) mass spectrometer (Santa Clara, CA) has been upgraded with custom source optics enabling higher activation energies for improved CIU and collision induced dissociation (CID) capabilities. Additionally, an e-MSion ExD device has been installed after the quadrupole mass filter enabling fragmentation after IM and MS selection. All proteins were obtained from Sigma Aldrich (St. Louis, MO), or recombinantly expressed and purified from *E. coli*. All data analysis was conducted using Agilent MassHunter, mMass, and CIUSuite2. All IM measurements were conducted with the drift tube set to 3.95 torr N2, at uniform low field conditions allowing for the direct conversion of IM drift times to collision cross section (CCS) values.
Preliminary Data: Our instrument combines modified source optics to perform charge multiplexed CIU experiments, an electromaganetostatic cell positioned after the quadrupole mass filter for initiating ExD type fragmentation (e-MSion, Corvallis, OR), and DTIM-MS experiments. The ion optics and design changes that enable dramatically improved CIU across all ion charge states on our modified instrument included an additional ion lens at the exit of the ion transfer capillary as well as the introduction (10% v/v ratio) of sulfur hexafluoride gas in the source region (typically operated at 4.5 Torr). As a result, our modified platform can perform a range of ion activation (CID, CIU, ExD) and analysis (IM, MS, MS/MS) functions, and these modes of operation will be discussed in detail, and demonstrated using multiprotein targets of active interest to the structural biology community. Previous inter-laboratory efforts have extensively characterized the CCS accuracy and precision of the 6560 DTIMMS platform for a range of metabolites, lipids and peptides. Our initial efforts have attempted to perform a similar assessment for intact protein and protein complex ions for our modified instrument, including: ubiquitin, cytochrome C, β-lactoglobulin (β-lac), transthyretin (TTR), bovine serum albumin (BSA), concavalin A (ConA), alcohol dehydrogenase (ADH), and GroEL. Our DTIM CCS measurements for these standards are approximately 3% lower compared to those measured on a modified Synapt instrument (Bush *et al.* 2010). Further experiments are underway to assess the cause of this trend. Preliminary CIU experiments indicate that the modified source enables higher energy input, allowing CIU of larger protein targets. We have demonstrated the capability of this instrument to perform native mass spectrometry experiments of very large protein by transmitting, and measuring the mass and CCS of ~1.6 MDa ions (GroEL 28mer). Finally, using IM-resolved ExD we can conduct pseudo ms3 “complex-down,” and CIU-ExD experiments.

Poster 25

High Resolution FT-IMS-Orbitrap: Resolving What was Hidden

Jacob W. McCabe; Mehdi Shirzadeh; Art Laganowsky; David H. Russell
Department of Chemistry, Texas A&M University

Introduction: Ion-mobility mass spectrometry (IM-MS) is rapidly becoming an important technique for biophysical studies; however, the performance metrics of commercially-available IM-MS impose limits for studies of intact protein complexes, *viz.* accurate, 1st principles determinations of rotationally averaged ion-neutral collision cross sections (CCS) and needs for high resolution IM and *m/z* measurements. We have developed a next-generation native IM Orbitrap MS instrument capable of accurate 1st principles determination of CCS, high IM and MS resolution and (in the final design) the complete suite of complementary structural probes, *i.e.,* CIU, CID, SID, ECD and ETD. Unlike techniques such as X-ray crystallography and NMR, which report populationaveraged responses, IM-MS can be used to interrogate heterogeneous population of conformers and probe individual structures.

Methods: A homebuilt static spray nano-ESI source equipped with an RF ion funnel and a 1.5 meter periodic-focusing IM drift tube was installed on ThermoFisher Exactive Plus EMR Orbitrap. IM and MS data are acquired using multiplexed approaches, whereby the IM DT functions as a frequency separator rather than time-dispersed separator. This is achieved by positioning ion gates at the entrance and exit of the DT, and sweeping the gating frequency across the full range of ion drift frequencies, *i.e.,* drift time of 1 ms corresponds to a drift frequency of 1 kHz. Fast-FT are used to convert frequency-domain signals to a time-domain spectrum to extract ion ATDs. An instrument duty-cycle of 25% *vis-a-vis* 0.1 – 0.5% for typical IM-ToF instruments.

Preliminary data: Performance of the FT-IMS-Orbitrap instrument for high-resolution IM and MS measurements has been benchmarked with a number of proteins and protein complexes (ubiquitin, cytochrome c, streptavidin, and transthyretin), we are now expanding these investigations to larger protein complexes (MW ranging from 100-200 kDa), including several membrane protein complexes. Studies of large proteins/protein complexes are often compromised by the presence of impurities and/or endogenous substances, which are not detected at low *m/z* resolution, but are identifiable using high *m/z* resolution. Recent investigations of transthyretin have revealed dramatic impacts of previously unresolved adducts that were hidden when using low mass resolution. These adducts affect both the structures and stabilities of larger proteins and protein complexes. Similar effects have been found for metalated protein complexes, specifically metalinduced oxidation. Continued improvements in instrumentation are expected to reveal phenomena that were previously hidden by low resolution instruments. Here, high-resolution IM-Orbitrap is used to study a broad range of biological systems to make more accurate IMS measurements and understand the role of small molecules on protein structure. The development of native-FT-IMS-Orbitrap MS has been made possible by the convergence of many different methods developed over the past few decades. Improvements in nano-ESI, especially small-bore via static spray ESI, has markedly improved ionization efficiencies for large proteins was well as our abilities to retain “solution-like” structures and noncovalent (ligand-protein) interactions. Periodic-focusing (PF) DT affords higher ion transmission and accurate 1st principles determinations of CCS. The increased mass resolution of the Orbitrap MS is critical for studies of non-covalent complexes. Lastly, implementation of multiplexed ion detection employing dual-gate PF-DT, frequency mode ion detection, and Fourier-transform has enabled rapid, high efficiency ion detection---*more signal more of the time*!

Poster 26

Comparison of native mass spectrometry in positive and negative-ion mode for proteins with varying isoelectric points

Alexis N. Edwards, Elyssia S. Gallagher
Department of Chemistry and Biochemistry, Baylor University, One Bear Place #97348, Waco, TX 76798

Introduction: To expand our understanding of the functions of proteins and complexes, it is imperative to analyze their biological structures. Native mass spectrometry (MS) in positive-ion mode is preferable when analyzing proteins at a pH below their pI, since they have excess positive charge. If the pH is above the protein pI, negative-ion mode could generate data that is superior to that of positive-ion mode since the gas-phase ion would more closely mimic the solution charge states. Yet, negative-ion mode suffers from lower signal intensity[1] and charge state distributions that are not necessarily indicative of folding.[2] However, when paired with ion mobility, negative-ion mode has the potential to offer valuable structural information for proteins and complexes.

Methods: Lyophilized lysozyme (pI 10.7), wheat germ agglutinin (WGA, pI 8.5), and concanavalin A (Con A, pI 4.5-5.5) were purchased from Sigma Aldrich (St. Louis, MO). Ammonium acetate was purchased from VWR International (Radnor, PA). Proteins were dissolved in 200 mM ammonium acetate, pH ~7.0, and then desalted using micro bio-spin P-6 gel columns (Bio-Rad Laboratories, Mississauga, ON). Glass capillaries were pulled using a P-1000 micropipette puller (Sutter Instrument Company, Novato, CA). Samples were introduced via open source to a Waters Synapt G2-S High Definition MS (Waters Corporation, Millford, MA) in both positive and negative-ion mode. Acquisition lasted for 10 to 20 minutes, with ion mobility data also being collected, after which an averaged spectrum was generated.

Preliminary Data: While native MS can maintain noncovalent interactions for analysis, it requires a more careful sample preparation than that of traditional MS. We sought to develop a single sample preparation protocol that could be used to detect proteins in both ion modes, while maintaining native-like structures. The native spectra for lysozyme in positive-ion mode showed two peaks, while negative-ion mode showed three peaks. Based on either spectrum, the calculated molecular weight for lysozyme was 14.3 kDa, which is in line with literature. In both ion modes, WGA shows three peaks, with negative-ion mode having lower charge states. The calculated weight for WGA was 34.3 kDA and 34.4 kDA in positive and negative-ion mode, respectively; both of which are in line with literature. Con A forms a tetramer at pH 7, dimer at pH 5, and monomer at pH <5.[3] Interestingly, Con A showed peaks for the tetramer, dimer, and monomer in both ion modes, with the dimer being most prevalent in both modes. However, negative-ion mode showed a narrower charge state distribution. The calculated molecular weights for the tetramer, dimer, and monomer were 103, 51.7, and 25.7 kDa, respectively, for both ion modes. Currently, we are using ion mobility to investigate changes in protein conformations to determine the effectiveness of using negative-ion mode for the formation of native-like complexes.

1. Rao, W. et. al. *J Am Soc Mass Spectrom* **2016,** *27*, 124-34.
2. Konermann, L.; Douglas, D.J. *J Am Soc Mass Spectrom* **1998,** *9*, 1248-54.
3. Xu, X. et. al. *Sci Rep*. **2017**, *7*, 1452.

Poster 27

Molecular mechanism of ISC Iron–Sulfur Cluster Biogenesis revealed by Native Mass Spectrometry

Cheng-Wei Lin, Jacob Macabe, David Russell, and David Barondeau
Department of Chemistry, Texas A&M University

Introduction

Iron-sulfur (Fe-S) clusters are essential protein cofactors that function in processes such as electron transfer, catalysis, and gene regulation. These Fe-S clusters exist predominately in rhombic [2Fe-2S] or cubic [4Fe-4S] forms. The ISC biosynthetic pathway synthesizes these clusters from ferrous iron and sulfur using an IscS-IscU heterotetrameric complex. IscS is a PLPcontaining cysteine desulfurase that mediates the release of sulfur from cysteine. IscU serves as a scaffold protein for building the cluster intermediates. Glutathione/DTT commonly serve as the *in vitro* reducing agents for cluster synthesis. Despite over 25 years of investigation, the molecular mechanism and intermediates in cluster formation remain poorly understood. Here, we implement high-resolution native mass spectrometry (native MS) to investigate the details of this critical biosynthetic process.

Methods

Native MS has developed into a powerful biophysical technique. By incorporating nano-ESI for soft ionization and careful instrument tuning to minimize collisional activation, native MS affords the ability to preserve proteins in their folded native-like structure in the mass spectrometer. Thus, weak non-covalent protein-ligand, protein-cofactor, and protein-protein interactions can be directly measured. Applications of native MS include the study of protein structure, dynamics, ligand interactions, and tracking of intermediates in time-dependent reactions. Here, we utilize native MS to analyze protein-protein interactions between IscS and IscU and the influence of metal cofactors (Fe/Zn) on sulfur transfer and the assembly of Fe-S clusters. In addition, time-dependent investigation of Fe-S cluster synthesis reveals the formation of sulfur, iron, and iron-sulfur intermediates on IscU.

Preliminary data

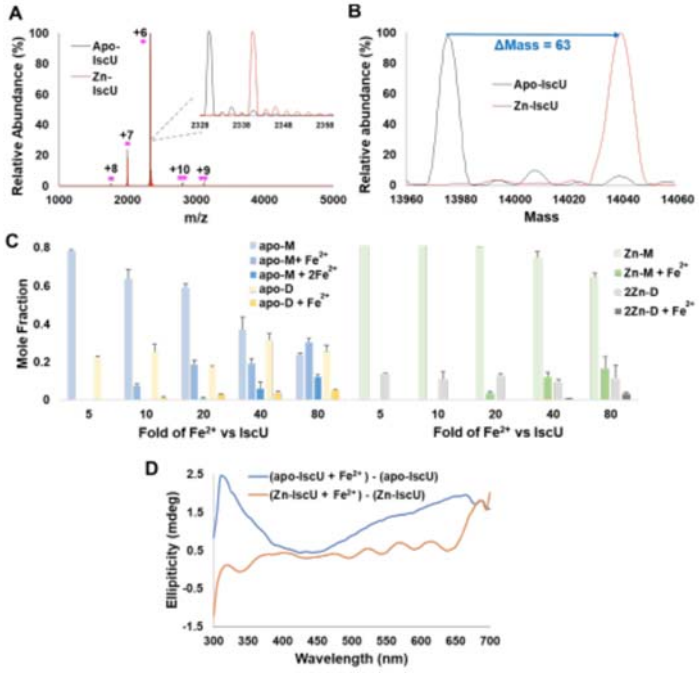

Figure 1. Zn2+ binds IscU active site with higher affinity than Fe2+. (A) shows the raw native MS spectrum of apo-IscU (black) and Zn-IscU (red). (B) shows the deconvoluted MS spectrum from (A). (C) and (D) shows the binding of Fe2+ on apo-IscU or Zn-IscU analyzed by native MS Fe2+ titration experiments and CD spectroscopy, respectively.

Poster 28

Modelling Natural Bilayers with Mixed Lipid Nanodiscs for Native MS

Marius Kostelic, David Jurkowitz, Alex Ryan, Deseree Reid, Jibriel Noun, and Michael Marty
University of Arizona Department of Chemistry and Biochemistry

Introduction: Native mass spectrometry (MS) has become a powerful tool to characterize membrane protein–lipid interactions. Nanodiscs have emerged as a complementary cassette to study protein–lipid interactions with native MS, due to their low polydispersity and precisely defined lipid composition. However, most prior nanodisc experiments used a homogeneous mix of a single phospholipid that does not represent a native lipid bilayer. By choosing lipids with masses that are integer multiples of each other, we can resolve nanodiscs of a more complex and native-like lipid composition. Here, we present resolved heterogeneous nanodiscs that more accurately mimic native bacterial and mammalian membranes. Ultimately, we hope to create models of natural membranes that are amenable for characterizing protein–lipid interactions with native MS.
Methods: Nanodiscs were prepared by combining a mix of heterogeneous lipids in detergent with membrane scaffold protein (MSP). Nanodiscs then self-assemble with the removal of detergent from the lipid, detergent, and MSP mixture. Nanodiscs were purified using size exclusion chromatography. An ultra-high mass research option Q-Exactive HF Orbitrap mass spectrometer was used to determine the stability and composition of mixed nanodiscs. In the mass spectrometer, the collision voltage was increased in increments of 5 volts (V) from 0-100V or 10V from 0–200V. By deconvolving the m/z spectra in UniDec, we were able to determine the number of lipids and MSP belts in intact nanodiscs.
Preliminary Data: We have resolved mass spectra of mixed lipid nanodiscs by choosing lipids that were integer multiples of each other. Using this approach, we have characterized nanodiscs with 90% 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphocholine (POPC) with a 10% mix of cholesterol, ergosterol, or cholesteryl hemisuccinate. For POPC nanodiscs with either cholesterol or ergosterol, we observed an additional mass peak pattern in the deconvolved spectra because the sterols are about half the mass of POPC. These sterol nanodiscs are stabilized by negative ionization mode, charge reducing reagents, and chemical modifications. We have also incorporated 25% 1-palmitoyl-2-oleoyl-sn-glycero-3-phosphoserine (POPS) alongside 10% cholesterol into POPC nanodiscs for a mammalian mimetic nanodisc. We are currently working to optimize a mammalian nanodisc ratios and lipids including, PE, PS, sphingomyelin, PC, cholesterol. We also have resolved nanodisc spectra of 1-palmitoyl-2-oleoyl-sn-glycero-3-phospho-(1'-racglycerol) (POPG) nanodisc spectra containing tetramystroyl-cardiolipin (TMCL). These cardiolipin nanodiscs exhibit a triplet peak pattern in the deconvolved spectra compared to a singlet peak pattern in control POPG discs because TMCL is 5/3 the mass of POPG.We have also incorporated 5% tetraoleyl-cardiolipin (TOCL) into POPG nanodiscs to for a more interpretable singlet peak pattern. We are currently optimizing a bacterial membrane nanodisc that contains PG, PE, and cardiolipin.

Poster 29

Native Mass Spectrometry Guided Discovery and Characterization of Unknown Protein Machinery that Directs Plant Phenylpropanoid Synthesis and Stereochemistry

Mowei Zhou[1], Irina V. Novikova[1], Diana L. Bedgar[2], Laurence B. Davin[2], Callum J. Bell[3], John R. Cort[4], Jared Shaw[1], Norman G. Lewis[2] *[1]Environmental Molecular Sciences Laboratory, Pacific Northwest National Laboratory [2]Institute of Biological Chemistry, Washington State University, [3]National Center for Genome Resources [4]Biological Sciences Division, Pacific Northwest National Laboratory*

Introduction: Lignins are complex natural polymers and one of the major structural components of plant cell walls. Two decades ago, a "dirigent protein" (DP) was discovered in *Forsythia* x *intermedia*. DP homologs were since found in other vascular plants. DPs mediate the stereo-selective dimerization of phenylpropanoids to form lignin precursors. They also contribute to specialized lignin deposition in the Casparian strip, a cell wall structure in root endodermis important for disease resistance and nutrient flow. Yet the molecular mechanism is elusive because other unknown proteins are likely involved. We use native mass spectrometry to discover DP interacting proteins in a (+)-pinoresinol-forming complex extracted directly from plant tissue. This complex may resemble other unexplored DP-containing machinery involved in lignin biosynthesis.

Methods: The DP complex was extracted from *F. intermedia* stem tissue and purified with ion exchange chromatography (Science, 1997, 275:362). Fractions showing activity for stereo-selective dimerization of *E*-coniferyl alcohol were further analyzed by MS. Because the *F. intermedia* genome is not available, a custom protein database, containing major proteins in the sample, was created based on both *de novo* sequenced tryptic peptides (using PEAKS) and the assembly of published transcript short reads from *F. koreana* (PLoS One, 2016, 11:e0164805). The major proteins detected were further validated, with well-defined signal peptides and total glycosylation masses using top-down MS. Native MS data were used to define the potential interaction of the proteins in the samples.

Preliminary data: We first *de novo* sequenced high abundance proteins in the sample, by filtering the assembled transcript reads and matching them to the *de novo* sequenced tryptic peptides from bottom-up LCMS. A list of proteins that were co-purified was obtained, some of which could potentially interact with DPs. These proteins included peroxidase, germin, invertase, cuperdoxin, laccase, non-specific lipid transfer protein (nsLTP). Two new DP homologs, 18 kDa and 19 kDa, not previously known in *Forsythia* were also found. Top-down MS showed these two homologs have two N glycosylation sites near their N-termini, which are expected for known proteins in the DP family. Sequence analysis suggests these homologs may have distinct substrate specificities from the previously known DP in *Forsythia*. By performing collision induced dissociation (CID) of the detected unknown protein complexes in native MS, we were able to release subunits of the complexes and infer their composition and stoichiometry. Several 50-60 kDa species were determined to be hetero-trimers of the two new DP homologs with varying stoichiometry. Species around 70-80 kDa were likely heterocomplexes of several enzymes binding to the 18 kDa DP. The small 9.4k Da nsLTP appeared to bind non-specifically to larger complexes and caused significant peak broadening. The preliminary analysis on the endogenous protein complexes led to protein targets for further characterization, in particular the two new DP homologs. We are working on cell-free expression of selected proteins of interest in order to reconstruct the complex(es). The potential interaction of these proteins and their putative assembly/disassembly in response to substrates, will be explored with native MS to help us understand their relationships. The variety of DP homologs in plants, many of which are not fully characterized, highlight the complexity of biological processes and the advantage of MS for differentiating closely related homologs in a relatively high-throughput manner.

Poster 30

Subunit interaction of glutathionylated human probed by surface-induced dissociation/ion mobility mass spectrometry

Monita Muralidharan*, Amit Kumar Mandal[#], Vicki H. Wysocki*
** Department of Chemistry and Biochemistry and Resource for Native Mass Spectrometry Guided Structural Biology, Ohio State University, Columbus, Ohio 43210, United States [#]Clinical Proteomics Unit, St. John's Research Institute, Bangalore, 560034, India*

Glutathionylation is a reversible post-translation modification of cysteine residues of proteins via thiol-disulfide exchange with oxidized glutathione. Under conditions of elevated oxidative stress, βCys93 of adult hemoglobin (HbA0) undergoes S-glutathionylation. Glutathionylated hemoglobin (GSHb) is a biomarker of oxidative stress associated with many clinical conditions such as diabetes mellitus, chronic renal failure, iron deficiency anemia, and atherosclerosis. Apart from protecting proteins from oxidative modifications, glutathionylation is involved in regulation and modulation of protein activity that is critical to redox signaling, rendering this modification essential to explore. Therefore, as a biomedical model, structure-function correlation of GSHb can serve as a valuable reference to other proteins undergoing glutathionylation. We investigated the structural integrity and overall architecture of GSHb using native mass spectrometry and surface-induced dissociation. Glutathionylation of hemoglobin leads to structural perturbation by the dissociation of important inter-subunit and intra-subunit interactions across globin subunits in the tetrameric hemoglobin. The weakening of subunit interactions leads to increased dissociation equilibrium constants of both tetramer/dimer (Kd1), and dimer/monomer (Kd2) of GSHb by 1.91 fold and 3.64 fold, respectively. Thus, the functional abnormality of tighter oxygen binding of GSHb may be attributed to the increased dissociation of the tetramer and its transition towards oxy-hemoglobin like conformation. MD simulations showed the average radius of gyration for deoxy-HbA0, oxy-HbA0, deoxy-GSHb, oxy-GSHb were 24.04 (±0.11) Å, 24.26 (±0.10) Å, 24.01 (±0.09) Å, 23.60 (±0.06) Å, respectively. The difference of radius of gyration by ≤1 Å indicated that the overall structure of the molecule does not change significantly upon binding of two glutathione molecules per tetramer. However, the overall size and shape of the molecule remains unchanged. We are currently assessing the difference in the interfacial dissociation of HbA0 and GSHb by surface-induced dissociation to generate energy-resolved mass spectrometry (ERMS) plots to determine whether the differences are reproducible.

Poster 31

Structural Analysis of Gas-Phase Phosphoproteins

Carter Lantz[1], Rachel R. Ogorzalek Loo[2], and Joseph A. Loo[1]
[1]*Department of Chemistry and Biochemistry, UCLA*
[2]*Department of Biological Chemistry, UCLA*

Introduction: Phosphorylation is a ubiquitous protein modification. In addition to guiding cell signaling, it opens channels, activates enzymes, and even impacts amyloid protein aggregation. Despite phosphorylation's importance, much about its impact on protein structure remains unknown. A previous ion mobility-MS study of peptides suggested that their structures are compacted in the gas phase by interactions between phosphorylated sidechains and the polypeptide backbone (Thalassinos et al., Anal. Chem., 2009). Using IM-MS and top-down MS, we extend those investigations to intact proteins phosphorylated to different extents, hoping to shed light on how phosphorylation and its ability to ion pair to basic amino acids alters the 3D structures of gas phase proteins.

Methods: Native MS (with nanoelectrospray ionization) and ion mobility profiles for phosphorylated β-casein, ovalbumin, phosvitin, and other proteins were measured using a Waters Synapt G2 Si quadrupole time-of-flight mass spectrometer. Proteins were enzymatically dephosphorylated with alkaline phosphatase. The resulting mobiligrams were compared to reveal any differences associated with phosphorylation. Tau and α-synuclein were phosphorylated in vitro and analyzed by ion mobility spectrometry. Phosphorylated proteins (positively charged molecules generated by ESI) were subjected to electron capture dissociation (ECD) and analyzed on a Bruker 15-Tesla Fourier Transform Ion Cyclotron Resonance Mass Spectrometer (FT-ICR MS) to return phosphorylation site information.

Preliminary Data: Phosphorylation affects the gas-phase structure of intact proteins, whether positively or negatively charged. When phosphorylated, intrinsically disordered proteins (IDPs) such as β-casein, (24 kDa) phosvitin, (26 kDa) and α-synuclein (14 kDa) favor compact forms more, with the preference most pronounced at lower charge. The data suggest that increases in the extent of phosphorylation are accompanied by decreases in proteins' average collision cross-sections. Ovalbumin (43 kDa), with only two phosphorylation sites, is more ordered in solution than β- casein and phosvitin, but its gas phase behavior obeys the trends seen above. That is, phosphorylated ovalbumin is, on average, more compressed in the gas phase than unmodified protein. Thus far, all phosphorylated proteins tested were smaller in the gas phase than equally-charged, non-phosphorylated counterparts. A potential explanation for the size differential is that, as ions transit from atmospheric pressure to vacuum, being activated and losing solvent, their structures rearrange slightly. If present, ionized phospho-sites can pair to oppositely-charged residues (i.e., protonated arginines) to form salt bridges. Bridges limit the flexibility of gas phase proteins, making them appear "compacted" in comparison to structures unrestrained by salt bridges. If salt bridges and rearrangements majorly drive gas phase size, the relationship between ion mobility collision cross-sections and solution-phase structure could be murkier than appreciated. ECD-MS of an in vitro-phosphorylated tau fragment localized the modification to a microtubule binding region known for promoting aggregation. Ion mobility analysis revealed an increased population of compact forms in a di-phosphorylated tau fragment (Nshanian M. et al., JASMS, 2019). Moreover, phosphorylation seems to increase the compaction resulting from anti-amyloid compound binding. This data could elucidate how phosphorylation increases the propensity for amyloid aggregation.

Poster 32

pH dependent binding affinity study of selected biologically active metal ions with a series of hexapepetides using ESI-IMMS

Ayobami Ilesanmi, Jack Williams, Tessa Moore and Laurence A. Angel*
Department of Chemistry, Texas A&M University-Commerce, Commerce, Texas, 75428,

Introduction

Previous studies performed on an analog methanobactin peptide (amb1A) which possess a 2His-2Cys motif prompted the design of a series of modified hexapeptides. The series includes ac-HCGPHC (amb1A), ac-HCGPHC-NH2 (amb1B), ac-HCGPGC (amb1C), ac-GCGPGC (am1D), ac-HGGPHC (amb1E), ac-HGGPHG (amb1F), ac-CCGPCC-NH2 (amb1G), ac-HGGPGG (amb1H) and ac-HGGPGG-NH2 (amb1I). Variations in the sequences were adopted to investigate the effectiveness of the 2His-2Cys motif to Zn2+, Ni2+ and Co2+ binding. This study aims to develop oligopeptides with optimal capabilities for chelating the selected biologically active metal ions, with an array of applications in healthcare, protein purification and environmental toxic metal cleanup.

Methods

The Waters Synapt HDMS G1 ion mobility mass spectrometer with native electrospray ionization (ESI) was used for the quantitative analysis of this study.The prepared 12.5 µM solutions of equimolar metal (Mn+) and amb1 were pH adjusted with 10 mM ammonium acetate solution, spiked with ammonium hydroxide for the physiological pH 7.4 and higher for pH 8.4 and pH 9.4. After 10 min incubation at room temperature, 2 scans for 5 minutes were collected in both positive and negative ion modes via the Synapt HDMS software. Gentle ionization and ion transfer conditions were chosen to retain as much solution behavior as possible. Integrated areas for free amb1 and metal bound amb1 species were extracted and normalized to a 100% scale.

Preliminary data

Plots of percent relative intensities of Zn2+, Ni2+ and Co2+ bound species for each hexapeptide are presented, depicting contributions of both positive and negative ions at the selected pH of 7.4, 8.4 and 9.4. At pH 7.4, chelation of Zn2+ was observed to be greater than Ni2+ which was observed to be greater than Co2+. Chelation of Zn2+ and Ni2+ was greatest by amb1G. At pH 8.4, an increase in Zn2+ chelation preferentially involved sequences with at least 2Cys residues such as amb1A, amb1B, amb1C amb1D and amb1G. Ni2+ chelation also increased at pH 8.4 for sequences with at least His and 2Cys residues such as amb1A, amb1B, amb1C and for amb1G. At pH 9.4, Zn2+ chelation was observed for the same set of peptides favored at pH 8.4 with relative intensities of metal bound species remaining about the same. However, Ni2+ chelation further increased for amb1A, amb1B, amb1C and for amb1G.

Poster 33

The Effects of Metal Binding to the Primary Structure of Five Different Heptapeptides as Investigated Through Ion Mobility-Mass Spectrometry

Nayeli Fuentes and Laurence A. Angel
Department of Chemistry, Texas A&M University-Commerce

A series of the heptapeptides amb5J, amb5K, amb5L, amb5M, and amb5N have been studied using ion mobility-mass spectrometry (IM-MS) to investigate how their unique structure affects their ability to bind to Magnesium, Calcium, Zinc, Silver, and Cobalt. These studies will provide important information of the metal-binding selectivity for each heptapeptide.

Poster 34

Collision Induced Unfolding Captures Disease Relevant Differences in Stability and Ligand Binding for the Integral Membrane Peripheral Myelin Protein

Sarah M. Fantin[1], Kristine Parson[1], Pramod Yadav[2], Charles R. Sanders[3], Melanie D. Ohi[2,4], and Brandon T. Ruotolo[1]. *[1]Department of Chemistry, University of Michigan, Ann Arbor, MI 48109, [2] Life Sciences Institute, University of Michigan, Ann Arbor, MI 48109, [3] Department of Biochemistry, Vanderbilt University, Nashville, TN 37232, [4]Department of Cell and Developmental Biology, University of Michigan, Ann Arbor, MI 48109*

Introduction: Peripheral myelin protein (PMP22) is a tetraspan integral membrane protein highly expressed in Schwann cells. The folding and trafficking of PMP22 has been identified as a key factor in multiple hereditary neurodegenerative disorders, such as Charcot Marie Tooth and Dejerine-Sottas syndrome. While biophysical analyses of common PMP22 mutations found in patients show that they alter protein stability and metal binding, it remains unclear how mutations in PMP22 contribute to disease. Here, collision induced unfolding (CIU) is used to compare the gas phase stabilities of wild type and mutant forms of PMP22. Further, we will describe the effects of detergent and membrane environment on PMP22 stability and oligomerization.

Methods: Ion mobility-mass spectrometry (IM-MS) measurements were acquired on a Synapt G2 instrument (Waters, Milford, MA) using a nano-electrospray ionization. Source temperature/pressure, and cone/capillary voltage were optimized to remove detergent with minimum perturbation to protein structure. CIU measurements were obtained by increasing the accelerating potential of the trap region in 5V increments. WT and mutant PMP22 forms were expressed and purified from *E. coli*. Before IM-MS, PMP22 was buffer exchanged into 200 mM ammonium acetate, pH=8 with 2xCMC optimized detergent, octaethyleneglycol monododecyl ether (C12E8). Collision cross section (CCS) calibration was performed with soluble protein standards encompassing an appropriate mass and mobility range for PMP22, taking into account previous membrane protein CCS measurements. CIU data were analyzed using CIUSuite2.

Preliminary data: Numerous pathogenic mutations of PMP22 have been implicated in a range of neurological diseases, with the locations of mutations occurring throughout the PMP22 sequence. We have initially chosen to study two mutant forms of PMP22 at disparate sites which exhibit different projected neurological phenotypes. The T118M mutation of PMP22 occurs at the end of the third transmembrane spanning α-helix and is implicated in Charcot Marie Tooth 1. The L16P mutation is located in the first transmembrane spanning α-helix, and is associated with Dejerine−Sottas syndrome. Although PMP22 oligomers have been implicated in the neurological disorders indicated above, no direct evidence has been reported of such complexes. Our preliminary EM results show that a putative tetrameric form of PMP22, previously undescribed in the literature, can be obtained when high amounts of 1,2 diheptanoyl-sn-glycero-3-phosphocholine (DHPC), a phospholipid, are present in solution. Through a screen of IM-MS compatible detergents and buffer conditions, it was found that when wild type (WT) PMP22 was liberated from C12E8 micelles, a range of charge states corresponding to monomeric and dimeric PMP22 can be readily observed. CCS analysis reveals that the lowest monomeric charge states, 9+ and 10+, have cross sections of 2698 $Å^2$ and 2382 $Å^2$ respectively, which are consistent with values expected for native PMP22. CIU of 9+ and 10+ WT PMP22 reveals four distinct features with three quantifiable transitions appropriate for detailed stability assessments of the protein. This presentation will report on our most recent IM-MS and CIU data on PMP22 mutants and lipid complexes, with the ultimate goal of providing key feedback for on-going cryo-EM structure determination efforts.

Poster 35

Carotenoid Profiling Analysis of *Staphylococcus aureus* by LC-MS^n in Different Growth Phases

Gerson-Dirceu López[1],[2] Chad Leidy,[2] Chiara Carazzone[1]

[1]Laboratory of Advanced Analytical Techniques in Natural Products (LATNAP), Department of Chemistry, Universidad de Los Andes, Bogotá D.C.-Colombia. [2]Laboratory of Biophysics, Department of Physics, Universidad de Los Andes. Bogotá D.C., Colombia.

Introduction

Staphylococcus aureus (*S. aureus*) is a Gram-positive bacterium naturally present in nasal passages and human skin. Strains of *S. aureus* resistant to different antibiotics is associated with changes in the lipid composition of the membrane in *S. aureus*. Among the lipids from S. aureus membrane can find the Staphyloxanthin (STX), a carotenoid lipid that has been associated with leads to increase membrane rigidity and resistance to membrane antibiotics, besides its antioxidant activity. Hence, the great importance that has emerged in the analysis of STX and its peers. Thus, a suitable extraction method and subsequent analysis by liquid chromatography-tandem mass spectrometry (LC-MSn) were employed to identify the metabolites related to carotenogenesis of cells *S. aureus* in membrane extracts to 8, 24 and 48 grown hours.

Methods

S. aureus was grown to exponential (8 hours) and stationary (24, 48 hours) phases in media LB at 37°C with 250 rpm. Subsequently, 100 mg of lyophilized cells were weighed accurately, the carotenoids extracts obtained from *S. aureus* with a mix of methanol: ethyl acetate: NaCl 1,7M solution (1:1:3), dried with nitrogen gas and resolubilized in 0.1% HCOOH in ACN. Carotenoids extracts were subjected to LC-MSn in an Ion Trap (Thermo Scientific) operated in positive mode. The RP-HPLC separation was carried out at 30°C using a Zorbax SB-C18 column (150 mm x 4.6 mm i.d., 3.5 µm). The mobile phases used in gradient were: Ammonium acetate 400 mg/L in solvent mix: Methanol: tertButyl methyl ether: Water (80:18:2, v/v/v, Solution A and 13:85:2, v/v/v Solution B) to 400 µL/min.

Preliminary Data

A total of 12 main peaks were observed in the HPLC chromatogram using a DAD for the analysis of the visible region (400 - 550 nm). Similar profiles and characteristic patterns of fragmentation of carotenoid lipids presents in *S. aureus* were observed in metabolomics based in HPLC-MS discriminates grown times in the microorganism. The advantage of this method is that it does not require a cumbersome preparation of the sample. Because, the analysis of this carotenoids have been commonly performed using thin layer chromatography (TLC) preparative or column chromatography previous to High-performance liquid chromatography. Results indicate the possible identification of five carotenoids belong to the biosynthetic pathway of STX in S. aureus. Besides, the analysis comparative chromatograms show variations the carotenoid profile according to the stage of cultivation, it was confirmed with univariate and multivariate analysis to mass spectra. In conclusion, a classic method for the analysis of carotenoids was appropriate for the analysis of this compounds in *S. aureus*. Also, this method reveals distinct metabolic profiles from *S. aureus* associated to phases grown. The presence of STX among the carotenoids of *S. aureus* can be determined by MS/MS analysis. Besides, we discovered that STX for this condition of grown is not the main component of *S. aureus* carotenoids even though it is the one that generates the characteristic color of the microorganism.

Poster 36

Positive-Ion Mode Detection and Discovery of Tyrosine Sulfation via Alkylamine Adduction

Nicholas Borotto, Phillip McClory, Brent Martin, and Kristina Håkansson
Department of Chemistry, University of Michigan

Introduction: Mass spectrometry (MS) is one of the primary methods for discovering, identifying, and localizing posttranslational modifications (PTMs). Acidic PTMs, however, are difficult to analyze due to their lability and low positive-ion mode ionization efficiency. Sulfopeptides are particularly challenging as even under the "softest" positive ion mode conditions; the modification readily undergoes proton mediated elimination. The proton-deficient conditions associated with negative-ion mode electrospray ionization improves stability of both phospho- and sulfopeptides but the dissociation of peptide anions can be complex and may hinder peptide identifications in complex mixtures. In this work, we demonstrate improved stability of sulfopeptides through the adduction of triethylamine (TEA); potentially providing a robust and sensitive platform for the detection of these modifications in complex mixtures.

Method: Protein digests were pre-enriched for acidic modifications using gel free isoelectric focusing and mixtures of unmodified, phosphorylated, and the sulfated peptides caerulein (pEQDsYTGWMDF), gastrin I (EGPWLEEEEEAsYGWMDF), hirudin (DFEEIPEEyLQ), cholecystokinin (DyMGWMDF), cionin (NyyGWMDF-NH2) were prepared. The peptide mixtures and protein digests were separated using a HSS T3 analytical column (75 µm x 25 cm, 1.8 µm) on a Thermo Scientific Dionex Ultimate 3000 RSLCnano system configured for nano flow (0.5 µL/min). Triethylamine (0.5%) was introduced via a post-column tee (0.1 µL/min) and the combined flow was directly ported into a Thermo Scientific Orbitrap Fusion Lumos mass spectrometer. A source fragmentation value of 30V was applied and tandem mass spectra were acquired in the ion trap.

Preliminary data: Using peptide standards we have found that adduction with TEA drastically improves the stability of sulfopeptides, enabling their detection in positive-ion mode. When TEA is introduced to a mixture of both sulfo- and phosphopeptides, it adducts readily to both. The stability of phosphopeptide TEA adducts, however, is significantly lower than for sulfopeptides and minor source activation preferentially dissociates any phosphorylated adducted species. This stability difference enables facile differentiation between the nearly isobaric sulfo- and phospho-modifications. HCD of the surviving adducted sulfated species results in both extensive loss of the modification and generation of sequence informative ions. Sufficient sequence informative ions are produced to enable proteomics type workflows. We further applied this technique to digests of 293T cells after separation via gel free isoelectric focusing. While preliminary, we found that the number of adducted and presumed sulfated species correlates with pI as expected. The selectivity of alkylamine adduction, the stability it imparts, and the ease at which it is introduced provides a promising alternative to current techniques that require complex sample handling, exotic peptide fragmentation techniques, and/or challenging negative ion mode analysis.

Poster 37

The Use of Chemical Penetration Enhancers to Increase Protein Modification by In Vivo Fast Photochemical Oxidation of Proteins

Jessica Arlett Espino, Zhihui Zhang, and Lisa M. Jones
University of Maryland Baltimore School of Pharmacy, Baltimore, MD

Introduction

In vivo fast photochemical oxidation of proteins (IV-FPOP) is hydroxyl radical protein footprinting method that modifies protein in *Caenorhabditis elegans (C. elegans*), an animal model for human disease. Structural studies in *C. elegans* by IV-FPOP is feasible because of the animals ability to uptake hydrogen peroxide, their transparency to laser irradiation at 248 nm, and the irreversible nature of the modification. To increase the number of oxidatively modified proteins, chemical penetration enhancers (CPEs) that aid in hydrogen peroxide uptake through the skin can be used. Here, we describe the use of a fluorescence fluorophore to quantify hydrogen peroxide absorption in the presence of various CPEs.

Methods

C. elegans were synchronized and harvested at their fourth larval stage prior to IVFPOP. Worms were pretreated with the H2O2-selective fluorophore Peroxy Orange 1 (PO1) and H2O2 absorption was quantified for 200 mM H2O2 in the presence and absence of different CPEs. For IV-FPOP, worms were incubated using different CPEs with 200 mM H2O2 and were flowed through a 250 µm ID fused silica. H2O2 was photolyzed by the excimer laser at 248 nm, excess H2O2 and OH radicals were quenched with cell permeable ROS quenchers. Protein lysate was reduced, alkylated, and digested with trypsin. LC-MS/MS analysis was performed on a Fusion Lumos Orbitrap. Data was analyzed using Proteome Discoverer 2.0 searching against a *C. elegans* Swiss-Prot database.

Preliminary data

Initially, five different CPE candidates were selected for IV-FPOP: laurocrapram (AZ), propylene glycol (PG), dimethylformamide (DMF), dimethylacetamide (DMAC), and oleic acid (OA). Quantification of the extent of oxidation of ubiquitin by *in vitro* FPOP experiments showed a decreased in oxidation in the presence of PG, DMF, and DMAC while AZ and OA increased oxidation. These two CPEs were chosen to test with IVFPOP. Prior to IV-FPOP, the viability of the worms in the presence of hydrogen peroxide and AZ and OA were tested to ensure the CPEs were not lethal to the worms. Quantification of the total uptake of hydrogen peroxide was measured by fluorescence of PO1 showed an increased in hydrogen peroxide uptake with AZ while OA alone did not show statistically significant uptake differences. LC-MS/MS analysis of digested worm lysate revealed 1% AZ increased the total number of oxidatively modified proteins and peptides by a 1.15- and 1.32-fold, respectively.

Poster 38

In-Cell Fast Photochemical Oxidation of HCT116 Spheroids

Raquel Shortt[1], Jessica Lukowski[2], W. Temple Andrews[3], Amanda Hummon[3], and Lisa M. Jones[1]
1University of Maryland Baltimore
2*University of Notre Dame*
3Ohio State University

Introduction

Many cancer drugs fail at treating solid epithelial tumors with ontributing factors to the observed chemoresistance being hypoxia and insufficient drug penetration. These conditions are difficult to test in monolayer cell cultures. When screening for potential cancer drugs, it's imperative to evaluate them in conditions that are similar to in-vivo, which is not always done. To address this, we propose to develop a mass spectrometrybased protein footprinting method that assesses the penetration and efficacy of cancer drugs in 3D cells. Our group has previously extended the protein footprinting method fast photochemical oxidation of proteins (FPOP) for live cell analysis (In-Cell FPOP). This technique enables irreversible modifications to cells in their native environment. In this study, we perform IC-FPOP on intact spheroids using a modified Platform Incubator with movable XY stage (PIXY).

Methods

Spheroids, consisting of HCT116, were grown in 96-well plate filled with agar and McCoy's 5A supplemented media over 13 days. For IC-FPOP, single spheroids were passed through 1.3 mm fused silica with H2O2 in technical replicates. H2O2 was photolyzed via an 248 nm KrF excimer laser. Cells were collected in cell permeable ROS reagents. Spheroids were exposed to 10-100 mM H2O2 for either 1-20 seconds to optimize oxidative labeling. To differentiate characteristics of individual layers, spheroids were dissociated with 0.05% trypsin, lysed, reduced, alkylated and digested with trypsin. Control spheroids were not laser irradiated. Peptides were analyzed on a Fusion Lumos Orbitrap and searched against a Homo Sapien database using Proteome Discoverer 2.0.

Preliminary Data

Different hydrogen peroxide conditions were initially tested to determine optimal hydrogen peroxide concentration for maximal hypoxic core diffusion between each spheroid layer. MALDI-IMS studies were performed to monitor lipid peroxidation by peroxide. The imaging results detect changes in lipid species when spheroids were incubated with H2O2 for 30 s and for 10 min. This provides evidence that H2O2 can penetrate through the spheroid. Initial FPOP studies on intact spheroids show that several proteins can be oxidatively modified in each of the cellular layers. Additionally, increasing the concentration of H2O2 increased the number of proteins modified but the extent of oxidation diminished. This could result from endogenous catalase breaking down H2O2 as it penetrates through spheroidal layers.

Poster 39

Covalent Modification via Ion/Ion Reactions with Ion Mobility/Mass Spectrometry Structural Analyses

Veronica Carvalho Sexton and Ian Kilby Webb
Chemistry and Biology Department, Indiana University – Purdue University Indianapolis – IUPUI

Introduction: Determination of the structure of proteins provides an understanding about their function. The complexity of the protein structures requires high-resolution techniques in order to unravel their features. Mass spectrometry is a powerful tool to study proteins dynamic, engaging a combination of excellent selectivity, sensitivity, accuracy, and speed. Gas-phase ion/ion reactions have proven to be effective in converting gaseous ions from one ion type to another, with reactivity that reflects gas-phase structures of the ions. Protein structural information is obtained via covalent labeling and localizing these changes through tandem mass spectrometry. This approach performed fully in the gas phase analyzed by ion mobility/mass spectrometry provides specific information about protein folding, conformations, and binding/interacting sites, being determined at the amino acid-residue level.

Methods: Ion/ion reactions and subsequent analyses were performed on a traveling wave tandem quadrupole/ion mobility/time-of-flight Waters SYNAPT G2-Si High Definition Mass Spectrometer. Model monomeric proteins were analyzed in both “native” (i.e., millimolar aqueous ammonium acetate) and denatured (organic and acid) solution conditions. The onboard dual nanospray source was used to introduce protein and reagent ions in opposite polarities. The selected masses (cation and anion) were isolated in the quadrupole and sent to the Trap cell where the covalent ion/ion reaction occurred. The built-in electron transfer Dissociation (ETD) mode and Waters WRENS scripts controlled the ion/ion reaction parameters. The formed products were analyzed by ion mobility and tandem mass spectrometry.

Preliminary Data: Ion/ion reactions were performed using the protein ubiquitin in both “native” (aqueous ammonium acetate) and denatured (organic and acid) solution conditions and reagent anions formed from N Hydroxysulfosuccinimide esters (sulfo-NHS esters). The cations/anions were ionized using the commercially available nanoelectrospray ionization source. The capillary voltages and reaction parameters such as trap cell voltages, traveling wave height, and so on, were optimized and controlled by Waters WRENS scripts which allowed the capillary voltages to be applied in a discontinuous way permitting the cations and anions to be introduced consecutively. When the capillary voltages are continuously applied on both ionization sources, the reaction does not occur as the electrospray field from one polarity reduces the ionization efficiency of the opposite charged emitter. The reaction process starts with the anion reagent being isolated from the anion spray ion beam by the quadrupole and sent to the trap cell. Cations are introduced in the trap cell following the same path. The instrument voltages were optimized in order to attract and trap the cations and anions at the same time. The products were formed after the ions reacted in the trap cell and they were pulsed into the helium cell followed by IM/MS analysis. The ion/ion reaction resulted in the electrostatic addition of 4-5 anions to the protein cations. Covalent reactions were observed, confirmed by the neutral loss of sulfo-NHS molecules produced by collision induced dissociation (CID) by increasing the bias of the trap and the bias of the helium cell relative to the mobility cell. The charge-reduced ion/ion products were separated from their precursors in the mobility cell. Fragment ions had the same arrival time as their precursors, allowing for the fragmentation route elucidation. Peptide bond fragmentation allowed for localization of covalently-modified amino acid residues.

Poster 40

Normalizing Covalent Labeling Reactivity to Obtain Better Constraints for Computational Protein Structure Prediction

Xiao Pan and Richard W. Vachet
Department of Chemistry, University of Massachusetts-Amherst, Massachusetts 01003, United States

Introduction: The integration of amino acid covalent labeling-mass spectrometry (CL-MS) and computational modeling to predict protein structures is on the rise. Diethylpyrocarbonate (DEPC) is a useful covalent labeling reagent which modifies side chains of nucleophilic residues and N termini. Protein reactivity with DEPC relies on residues' intrinsic reactivity, solvent accessibility and the effect of microenvironment. To better account for these variable effects on reactivity, the intrinsic reaction rates of DEPC with His, Lys, Tyr, Ser, and Thr are measured using model peptides with the goal of normalizing the effect of intrinsic reactivity, so that information about solvent accessibility can be more readily acquired. Better information about solvent accessibility from CL-MS can then be converted into experimental constraints for protein structural modeling.

Methods: Five model peptides with the sequence Fmoc-DGXGG-CONH2 where X is His, Lys, Tyr, Ser or Thr, and a peptide with the sequence DGGGG-CONH2 were labeled under various DEPC concentrations in MOPS buffer at pH 7 and 37 °C, before the reaction was quenched by the addition of imidazole. After LC desalting, the peptides were detected by a Bruker amaZon quadrupole ion trap mass spectrometer. The modificationrate coefficient of each peptide was determined from kinetic plots after correcting for difference in ionization efficiency. In addition, model proteins such as beta-2 microglobulin, cytochrome c, and myoglobin were labeled by adding various molar excesses of DEPC. Modification sites and rate coefficients in the proteins were measured after denaturation, digestion, and LC-MS/MS analysis.

Preliminary data: Rate coefficients for the reactions of the model peptides with DEPC are acquired after correcting the differences in ionization efficiency between unmodified and modified peptides. Upon doing this, we are able to obtain reaction rate coefficient for each peptide from the second-order reaction kinetics plots. The rate coefficients for His, Lys, Tyr, and Ser are $2.42 \pm 0.05 \times 10^{-5}$ $M^{-1}s^{-1}$, $1.55 \pm 0.05 \times 10^{-6}$ $M^{-1}s^{-1}$, $2.65 \pm 0.10 \times 10^{-7}$ $M^{-1}s^{-1}$, and $1.93 \pm 0.64 \times 10^{-9}$ $M^{-1}s^{-1}$, respectively. The reactivity order of the amino acid side chains matches the order of their nucleophilicity, which proves that a residue's nucleophilicity affects its reactivity. It is interesting that the weak nucleophilic Ser is around 104 times less reactive than His, suggesting that the high number of Ser residues that can be labeled in proteins are due to microenvironment effects that increase their reactivity. Also, when the peptide containing His is labeled by DEPC, two modified isomers are found upon LC separation. Based on peptide fragmentation patterns and label loss during CID, we hypothesize that the isomers are due to one modification on Nδ1 and the other on Nε2 of the imidazole ring. DEPC modification rate coefficients for His, Lys, Tyr, Ser and Thr were also acquired for peptides from beta-2 microglobulin, cytochrome C, and myoglobin. These measured rate coefficients (ki of individually labeled residues in the proteins are normalized by dividing ki by the intrinsic rate coefficients (kp) measured with the model peptides to account for the intrinsic reactivity of each residue. The solvent accessible surface area (SASA) values for residues from the three proteins before and after normalization were compared to see if a better correlation between reactivity and SASA could be found after normalization. These correlations will be presented as the effect of including these improved SASA values into the Rosetta protein structure prediction software.

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Development of a Fast Photochemical Oxidation of Proteins (FPOP) Based Platform for Protein Folding Studies

Luciano H. Di Stefano[1], Dante Johnson[1], Anne Gershenson,[2] Lisa M. Jones[1]
[1]University of Maryland Baltimore School of Pharmacy, [2]University of Massachusetts Amherst

Introduction: *In vitro* studies on protein folding have provided valuable information on the biophysical properties involved in folding. Nevertheless, proteins may behave differently in the cellular matrix than in solution. For this reason, *in vivo* studies are of broad interest to the scientific community. With this aim, Pulse Chase In-Cell Fast Photochemical Oxidation of Proteins (pcIC-FPOP) is being developed in our laboratory. This method couples pulse-chase technology with mass spectrometry-based in cell footprinting which will allow for quick analysis of short lived protein folding intermediates. 1-antitrypsin (A1AT) folding in Huh-7 cells was chosen as the model to validate these studies. Optimal conditions of reagent concentrations, incubation time, and chase points are studied in the first part of this work.
Methods: Huh-7 cells are cultured with starving media (lacking Arg and Lys). After 1 hour incubation, media with isotopically labeled Arg and Lys is added using a peristaltic pump. Cells are incubated in this media for the time necessary to generate a detectable population of the newly synthetized isotopically labeled proteins. After appropriate incubation time, heavy labeled media is removed and replaced with regular media. Finally, hydrogen peroxide is added at different time points in order to label the proteins at different stages of folding. Optimal conditions for the experiment are determined experimentally. The samples are studied in triplicate and subject to the analysis of bottom-up MS. This is achieved thanks to a new platform being developed in our laboratory.
Preliminary Data: A1AT is an abundant secretory protein that folds in the endoplasmic reticulum (ER). It was chosen as a model system for protein folding because of the large amount of data available on how it folds. Hydrogen deuterium exchange experiments of the 1-190 fragment of A1AT demonstrate the footprinting can detect differences in folding between fragments and the full-length protein. In addition, computational studies performed by Wintrode and coworkers provide insight into the folding pathway of the proteins. Preliminary IC-FPOP studies demonstrate that A1AT can be oxidatively modified in Huh-7 cells. These studies show 2% of oxidation coverage across the protein. This data demonstrates the feasibility of using pcIC-FPOP to study protein folding in the cell. To increase the coverage percentage value, organelle fractionation and deglycosylation of peptides prior to analysis will be applied. Traditional pulse chase experiments using radioactive 35S labeled cysteine and methionine to monitor A1AT maturation and secretion from Huh-7 cells and gel electrophoresis to monitor protein secretion has determined that the half-time of secretion of A1AT is 30 min. This indicates the protein is a relatively slow folder which is another reason A1AT is a good model system to test the pcIC-FPOP platform. The gel electrophoresis-based pulse-chase experiments will help inform on which chase time points to use for pcIC-FPOP. Unlike traditional SILAC experiments where complete metabolic incorporation of the label is preferable, analyzing samples at short label incorporation times to detect newly synthesized proteins is more appropriate for protein folding studies. To determine how long it takes for Huh-7 cells to incorporate the heavy labeled reagents, we have cultured cells in the presence of 15N Arg and Lys for varying times. Mass spectrometry analysis of the cell lysates demonstrate that labeled peptides are observed following as little as 1 min of incubation, increasing at subsequent incubation times.

Poster 42

Utility of Covalent Labeling Mass Spectrometry Data in Protein Structure Prediction with Rosetta

Melanie L. Aprahamian[1], Emily E. Chea[2], Lisa M. Jones[2], Steffen Lindert[1]
[1]*Ohio State University, Department of Chemistry and Biochemistry*
[2]*University of Maryland, Department of Pharmaceutical Sciences*

Introduction: In recent years mass spectrometry-based covalent labeling techniques have emerged as valuable structural biology techniques yielding information on protein tertiary structure. This data, however, is not sufficient to predict protein structure unambiguously, as it only provides information on the relative solvent exposure of certain residues. Our work has demonstrated a correlation between hydroxyl radical footprinting (HRF) derived protection factors and a residue's solvent exposure in the form of a neighbor count. Through the development of a new score term for the Rosetta scoring function, we were able to demonstrate an improvement in tertiary prediction accuracy. Due to the success we have had with HRF, we have begun investigating the utility of covalent labeling techniques in general for tertiary structure prediction.
Methods: Utilizing HRF mass spectrometry data obtained internally and from the literature, we were able to develop a new score term for the Rosetta energy function based upon the correlation between the experimentally derived protection factors and a residue's neighbor count. By rescoring models generated for proteins using Rosetta's *ab initio* protocol with our new score term, we were able to improve the accuracy of the models selected as being most native-like. Building on this preliminary work, we sought to identify specific amino acid types that are most beneficial for covalent labeling-guided tertiary structure prediction. In addition to using our score for simple rescoring of existing models, we are introducing our score term into the structure generation process.
Preliminary Data: Rosetta, a powerful tool for computational tertiary structure prediction, can be adapted to predict protein structure using data from covalent labeling mass spectrometry experiments. Hydroxyl radical footprinting based protection factors from four soluble benchmark proteins were used to develop a new score term for Rosetta. A correlation between the natural logarithm of the protection factors and the corresponding residues neighbor count was observed. This served as the basis for the score term we called hrf_ms_labeling. To test our new scoring term, decoy model sets of 20,000 per protein were generated with Rosetta and rescored with hrf_ms_labeling. Model quality for all four proteins improved upon inclusion of HRF labeling data. For two of the four proteins, models with sub 3Å RMSD to the native structure were identified after the rescoring. Additionally, we obtained preliminary data for the utility of covalent labeling data in protein structure prediction in general. A benchmark set of 20 single subunit proteins of size 50-200 amino acids was used. Approximately 10,000 decoy models were generated for each protein using Rosetta's *ab initio* protocol along with a threading protocol. The decoy model sets with at least a single sub 5Å model (6 proteins from the *ab initio* set and 9 from the threaded set) were rescored with our new score term. For all of the residue type sets analyzed, the rescore results demonstrated that on average at least two-thirds of the tested proteins either improved or stayed the same compared to scoring with Rosetta alone. Most importantly, we identified a set of nine amino acids that have the highest information content for covalent labeling-guided tertiary structure prediction.

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An Evaluation of Ion Mobility Spectrometry and Gas phase Hydrogen Deuterium Exchange as Complementary Gas Phase Conformational Tools

Neena Eappen[1], Lucienne Nouchikian[2], Rebecca Jockusch[1]
[1]*Department of Chemistry, University of Toronto, Toronto, ON, Canada*
[2]*Department of Chemistry, York University, Toronto, ON, Canada*

Introduction: Increased application of MS in bioanalysis has driven the need to better understand gas phase conformation and has led to development of tools that probe gas phase structure such as Hydrogen Deuterium Exchange (HDX) and Ion Mobility Spectrometry (IMS). Although IMS is a well-known conformer separation tool, separation capabilities of gas phase HDX is not well explored. To address this, we evaluated both techniques for structural characterization of model peptides. These are polyalanines with 15 alanine repeats with lysine at different positions extending from N through C terminus. Jarrold group had shown that these peptides display a characteristic transition from a compact charge solvated globule to an extended helix as the charge (protonated lysine) moved from N to C terminus.
Methods: Alanine peptides (KA15-NH2, A6KA9-NH2, A9KA6-NH2, A15K-NH2) with same molecular weight were diluted in water to 5 µM. HDX was performed on a Bruker 7.0T FTICR MS. Ions were accumulated in the second hexapole for 5 ms and exposed to deuterated ND3 gas from 5 ms to 15 s. For collision activation, potential difference between skimmer 1 and funnel 2 was increased from 0 to 25 V. IMS was performed on a Waters Synapt G2 MS. IMS cell was operated with wave height of 40 V, wave velocity of 1000 m/s, N2 buffer gas at 80 mL/min and helium cell gas flow at 180 mL/min. Preliminary MD simulations were conducted using Gromacs package, OPLS-AA force field at 300K for 1 ns.
Preliminary data: Drift time and gas phase HDX kinetics for all four alanine peptides were measured for both 1+ and 2+ protonation states. There was a decrease in mobility (increase in CCS) as the lysine progressed from N to C terminus for the 1+ series (charge is assigned to be on lysine side chain). On the other hand, deuterium uptake was lower as the lysine progressed from N to C terminus (D uptake compared at 5.0 s reaction timescale when the exchange had reached a plateau). This was explained by increase in extent of intramolecular hydrogen bonding as the peptide transitions from a globule to helix (protecting more amides from exchange with increasing helical content). For 2+ charge state, all four variants showed two mobility peaks, a compact and an extended conformer. The compact conformers for all four peptides had very similar mobility whereas the extended conformer of A6KA9 had highest mobility, followed by A9KA6 and then KA15 and A15K with very similar mobility. The relative intensity of extended conformer was lower compared to the compact conformer for all peptides expect A15K (which was correlated to gas phase stability). Using preliminary modelling results, we were able to assign the two mobility peaks to be protomers where second charge could be either on N-terminus or on carbonyl oxygen at the C-terminus. Interestingly, HDX showed two conformational peaks for all variants except for A15K (comparable with IMS data since the compact conformer of A15K was lowly populated). Collisional activation experiments showed that slow exchanging population (less D uptake/ time) had a lower threshold for fragmentation, hence more gas unstable and this was assigned to the thermodynamically less favored extended protomer (except for A15K) from IMS data. Future Work: Correlate number of exchanges observed to CCS for each population using calculated low energy structures.

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Native Mass Spectrometry, Ion Mobility and Hydrogen Deuterium Exchange: A Powerful Toolkit for Accelerated Biopharmaceuticals Development in Vaccines and Neurodegenerative Disease

Cristina Lento[1], Andrew James[2] and Derek J Wilson[1,3]

[1]York University, Chemistry Department, Toronto, ON, Canada, M3J 1P3 [2]Sanofi Pasteur Ltd., Toronto, ON, Canada, M2R 3T4 [3]Centre for Research in Mass Spectrometry, Toronto, ON, Canada, M3J 1P3

Introduction: Over the past two decades, biopharmaceuticals have come to dominate the development pipelines of virtually all major drug manufacturers. This changing landscape has created a new need in industry for analytical approaches that can provide a detailed picture of macromolecular structure and dynamics. This presentation reports on our efforts to translate native MS, millisecond hydrogen deuterium exchange (HDX) and ion mobility to support drug development in stages ranging from the early pre-clinical stage to manufacturing. Specific projects will include epitope mapping for defining neutralization mechanisms in antibodies targeting diphtheria toxin, HDX and ion mobility for quality control in biosimilar manufacturing and millisecond HDX for 'dynamics-guided' lead optimization in antiamyloid drug development.

Methods: We use a unique microfluidics-based apparatus for millisecond HDX that incorporates a 'bottom-up' workflow for spatial resolution. All HDX, Native MS and Ion mobility measurements are carried out on a Waters Synapt G2 instrument. Biological reagents including diphtheria toxin/toxoid, antidiphtheria toxin mAbs, therapeutic mAbs and small molecule drugs are manufactured under GMP/GLP conditions and are typically supplied as purified components or in final formulation by industrial partners. Amyloidogenic proteins tau, A-beta and alpha-synuclein are generated in-house using conventional bacterial protein expression with specific protein optimized purification protocols.

Preliminary Data: Epitope mapping was conducted for two antibodies against diphtheria toxin (Dtx), designated mAb2-25 and mAb2-18, identified in vaccine potency screening assays. The antibodies were shown to be neutralizing, but as is most often the case, the mechanism of neutralization was not evident from screening data. We conducted a series of HDX experiments to define the epitope with the expectation that this might provide a rationale for neutralization activity. mAb2-25 was shown to bind to the Dtx receptor domain with an epitope that largely overlapped the target receptor (HBEGF) binding site. mAb2-18 was shown to bind to the catalytic domain adjacent the NADH binding site. Interestingly, mAb2- 18 binding appears to increase the on-rate for NAD+, but not NADH, suggesting that the neutralization mechanism may be inhibition of NAD release. In biopharmaceuticals manufacturing, quality control attribute monitoring is more challenging, and in some ways even more essential than in synthesis-based small molecule drug manufacturing. Using millisecond HDX, ion mobility and native MS, we demonstrate a rapid, robust and information-rich approach for multiple quality attribute monitoring in the manufacture of an Avastin biosimilar. The method is used to demonstrate physical bioequivalence of the biosimilar, manufacturing robustness in a lot-to-lot comparison and is linked to SPR to measure functional relevance of any observed physical differences. The unsuitability of conventional structural techniques for characterizing intrinsically disordered proteins has been a substantial barrier to anti-amyloid drug development. We have used millisecond HDX to support lead optimization of anti-amyloid drug candidates targeting Alzheimer's associated Tau and Abeta. Our unique HDX approach allows us to characterize the shifts in conformational bias that occur in arget proteins upon complexation with drug candidates. In this way we were able to provide 'dynamicsguided' lead optimization for a panel of ten drug candidates, linking characteristic shifts in the molecular conformational ensemble to specific functional properties in *in vitro* oligomerization and *in vivo* dose response assays.

Poster 45

Molecular Dynamics Simulations as an Aid in Interpreting HDX-MS Data

Patrick L. Wintrode[1], Daniel Deredge[1], Richard Bradshaw[2] and Lucy Forrest[2]
[1]*University of Maryland School of Pharmacy*
[2]*National Institute of Neurological Disorders and Stroke, National Institutes of Health*

Introduction:

Hydrogen/deuterium exchange measured using mass spectrometry (HDX-MS) is a powerful and popular technique for studying protein structure and dynamics. An ongoing challenge in the study of proteins using HDX is interpreting exchange rates in terms of detailed structural motions. Recent advances in computing power and enhanced sampling techniques have opened the door to using molecular dynamics (MD) simulations to provide a detailed view of the structural fluctuations that enable hydrogen exchange. We present current results on combining experimental HDX measurements and MD simulations for understanding protein structure and dynamics.

Methods:

All atom molecular dynamics simulations in explicit solvent were performed using the molecular dynamics packages NAMD and OpenMM. Predicted deuterium uptake curves were calculated from MD simulations using in-house Python scripts based of the method of Vendruscolo *et al.* (*J. Am. Chem. Soc.* 2003, 125, 15686−15687). Experimental HDX-MS data were collected using a Waters NanoAcquity HDX UPLC coupled to a Synapt G2Si mass spectrometer. LC-MS was preceded by in-line pepsin digestion on a Waters Enzymate BEH pepsin column. Data were processed using ProteinLynx Global Server 2.5.1 and DynamX 3.0 (Waters). Maximum entropy reweighting of MD trajectories with experimental HDX data was performed using in-house Python scripts.

Preliminary data:

We summarize the procedure for calculating HDX rates from MD simulations and show that current methods using reasonable length MD simulations (> 100 nanoseconds) give calculated deuterium uptake curves that agree with experiment with Pearson's correlation coefficients of 0.70-0.80. We introduce a method for combining simulation with experiment by using measured HDX rates to reweight MD trajectories using the principle of maximum entropy in order to obtain a structural ensemble that gives the optimum agreement with experiment. We illustrate how simulations and experiment can be combined to probe regions of intrinsic disorder in the bacterial heme transport protein PhuS. We also demonstrate how maximum entropy reweighting significantly improves agreement between experimental and calculated effects of fosfomycin binding on the dynamics of the bacterial antibiotic resistance protein FosAkp. We end by considering the current challenges and future prospects for integrating MD simulations and experimental HDX measurements.

Poster 46

Inhibitor-dependent changes in the dynamics of Wild-type (WT) EGFR kinase domain probed by hydrogen-deuterium exchange and mass spectrometry (HDXMS)

Yuko Tsutsui[1,2], Kumar Ashtekar[1,2], and Mark A. Lemmon[1,2]
[1]Department of Pharmacology, Yale University School of Medicine [2]Yale Cancer Biology institute, Yale University

Introduction" EGFR is one of the major cancer drug targets, and many tyrosine kinase inhibitors (TKIs) that bind to EGFR kinase domain have been developed to abrogate the kinase activity. The functional switch between the active and inactive states is, in part, determined by the orientation of helix C as well as the side chain conformation of DFG motif in the ATP/drug binding pocket. Crystal structures of TKI-EGFR kinase domain complexes show that different TKIs shift the kinase conformation to either functional state, making them functional-state selective. We probed EGFR kinase domain dynamics by HDX-MS in the presence of various non covalent and covalent TKIs to understand relationships between drug binding and its impact on the kinase dynamics.

Methods: WT EGFR kinase domain was expressed in Sf9 cells as the N-terminal 6x His fusion protein and purified as described previously. HDX-MS of the purified kinase domain was carried out in 20 mM HEPES, 100 mM NaCl at pD 7.4, 25oC and the labeling reaction was quenched at different timepoints by the addition of 200 mM sodium phosphate, 2 M guanidine-HCl, and 1 % formic acid (pH 2.2). The labeled protein was digested by pepsin to determine the deuterium uptake of each peptic peptide. To probe the dynamics of the WT kinase domain-inhibitor complex, the kinase was pre-incubated for 1 hour with an inhibitor at the inhibitor concentration that achieves >95% drug-bound fraction after 20-fold dilution with the labeling buffer.

Data/Results Regulatory regions for the functional switch between the active and inactive states are structurally flexible in the unliganded EGFR kinase domain. These include the N-terminal region of helix C and activation-loop (A-loop) containing DFG motif. Because the active state is expected to have restricted helix C movement via the formation of a salt bridge, the flexibility in helix C implies that unliganded WT EGFR has low propensity to spontaneously transit to the active state. Next, we examined HDX of the kinase domain in the presence of non-covalent TKIs. Although in crystal structures, gefitinib- or lapatinibbound kinase domain is in the active or inactive state, respectively, our HDX-MS did not find major structural differences between the two-drug bound forms at short labeling timepoints. Furthermore, the dynamics of these drug-bound forms are also comparable to that of the unliganded state at 10 sec and 1 min labeling time in most regions except P-loop, the binding pocket, and hinge region, connecting the N- and C-lobes of the kinase domain. We also probed the kinase domain dynamics with covalent TKIs, including afatinib and osimertinib, that react with Cys797 in the hinge region. Our HDX-MS shows that both covalent TKIs cause increased structural flexibility in the binding pocket. Thus, our results show significant differences in the binding mode between non-covalent and covalent TKIs. This finding is corroborated with HDX-MS study of the kinase domain complexed with non-covalent version of afatinib or osimertinib; non-covalent afatinib binding influences the dynamics of P-loop as well as β-strands in N-lobe while noncovalent osimertinib impacts the dynamics of the entire kinase domain, including significant stabilization in the binding pocket. Overall, our results show that changes in the kinase dynamics are inhibitor-dependent and imply that TKIs are not merely functional state-selective.

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Hydrogen exchange of chemoreceptors in functional complexes suggests protein stabilization mediates long-range allosteric coupling

Xuni Li, Stephen J. Eyles, Lynmarie K. Thompson
University of Massachusetts Amherst

Bacterial chemotaxis receptors form extended hexagonal arrays that integrate and amplify signals to control swimming behavior. Transmembrane signaling begins with a 2 Å ligand-induced displacement of an alpha helix in the periplasmic and transmembrane domains, but it is not known how the cytoplasmic domain propagates the signal an additional 200 Å to control the kinase CheA bound to the membrane-distal tip of the receptor. Modulation of dynamics of the cytoplasmic domain is thought to play a key role in signal propagation. There is a clear need for studies measuring signaling-related changes in structure and dynamics of the cytoplasmic domain within functional complexes. Homogeneous, functional complexes of CF (Asp receptor cytoplasmic fragment), CheA (kinase), and CheW (coupling protein) bound to vesicles in native-like arrays are prepared by binding the His-tagged CF to vesicles containing nickel-chelating head groups. Samples are applied to spin columns to initiate exchange without dilution and potential complex disassembly. After HDX times from 3 min to 16 hr, exchange is quenched and samples are frozen. For MS analysis, samples are thawed and digested with pepsin, followed by LC-ESI MS analysis on a Waters Synapt G2Si Q-TOF mass spectrometer. HDX-MS results indicate that the CF is well-ordered only in its protein interaction region where it binds CheA and CheW. Analysis of overall uptake shows that this region exhibits very slow exchange (incomplete at 16 hr), and the rest of the CF exhibits more rapid exchange. Mass spectra for peptides throughout the CF exhibit bimodal isotope distributions, with both uncorrelated (EX2) and correlated (EX1) exchange patterns. These overlapping patterns were analyzed by estimating $t_{1/2}$ values for the EX1 and EX2 processes. Widespread rapid EX1 and EX2 suggests the receptor cytoplasmic domain is partially disordered even within functional complexes. HDX rates are increased by inputs that favor the kinase-off state, suggesting modulation of disorder plays a role in the signaling mechanism. We propose that chemoreceptors achieve long-range allosteric control of the kinase through a coupled equilibrium: CheA binding in a kinaseon conformation stabilizes the cytoplasmic domain, and signaling inputs that destabilize this domain (ligand binding and demethylation) disfavor CheA binding such that it loses key contacts and reverts to a kinaseoff state. This study reveals the mechanistic role of a partially disordered region of a transmembrane receptor in long-range allostery.

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Metal-Induced Oxidation of Transthyretin Studied via Ion Mobility-Orbitrap Mass Spectrometry and Surface-Induced Dissociation

Mehdi Shirzadeh, Michael Poltash, Jacob McCabe, Zahra Moghadamchargari, Arthur Laganowsky, David H. Russell, *Department of Chemistry, Texas A&M University*

Introduction The mechanism of transthyretin (TTR) aggregation, a homotetrameric protein complex, has been extensively studied; however, the effect of oxidation on TTR stability and fibril formation remains controversial. Crystallography data has revealed cysteine and methionine oxidation in mature fibrils, while, other studies have reported oxidation increases TTR resistance to aggregation. In vivo, reactive oxygen species (ROS) are responsible for protein oxidation implicated in many diseases and metals can regulate both protein stability and oxidation; however, most biophysical techniques are not sensitive and/or applicable to study such systems. Here, native ion mobility-Orbitrap mass spectrometry and surface-induced dissociation are used to probe TTR structure during metal-induced oxidation. High resolution IM-MS is critical for resolving individually bound metals and structural perturbations resulting from oxidation.

Methods A home-built Fourier transform ion mobility spectrometer coupled to an Orbitrap mass analyzer (Thermo Fisher) was used for data acquisition. IM data analyses were performed by custom Python scripts developed in-house. A SID cell, implemented into a Synapt G2 (Waters), was used for MS/MS experiments with parameters optimized for the lowest ion activation and highest transmission. N-ethylmaleimide (NEM), ethylenediaminetetraacetic acid (EDTA), copper acetate, zinc acetate, and ICP standard chromium (VI) were purchased from Sigma Aldrich. TTR was expressed in *E. Coli* and aliquoted and stored at -80 °C. Thawed protein was buffer exchanged into 200mM ammonium acetate directly before sampling and introduced by static nano-ESI using a Pt wire.

Preliminary data ICP-MS data revealed presence of endogenous zinc, copper, chromium and nickel in TTR samples resulting from the expression matrices. Preliminary MS analysis of TTR resulted in peaks corresponding to one and two Zn(II) bound to TTR. With time, stepwise mass shifts of 64 Da resembling third, fourth zinc binding were observed. While tandem mass experiment using SID confirmed Zn(II) binding initially, it also revealed the subsequent 64 Da mass shifts were due to oxidation events over time. Thus, for tetramers, the initial 64 Da shifts correspond to Zn(II) binding, and further increases in mass during nano-ESI originate from subunit oxidation. Intriguingly, upon oxidation, Zn(II) dissociates and backbone fragmentation between Cys-10 and Pro-11 is observed. Further investigation revealed two oxidations of the b10 fragment and one oxidation of the remaining monomer (y117). Previous studies have shown that Cys-10 is involved in Zn(II) binding; therefore, oxidation can perturb zinc coordination in accord with our observation wherein Zn(II) binding and Cys-10 oxidation are mutually exclusive. To further confirm Cys-10 oxidation, NEM was used to covalently label it which experimentally slowed TTR degradation 6-fold. In contrast, copper and chromium addition accelerated TTR oxidation, thus EDTA was used to remove endogenous metals from TTR which decreased the rate of oxidation and indicates a metal-induced oxidation pathway. Water oxidation is a possible driving force which can produce hydroxyl radicals and singlet oxygen leading TTR oxidation during nano-ESI. A theta emitter was used to isolate electric current from the TTR solution which slowed the oxidation allowing for the capture of intermediates. Using high-resolution IM-MS, two additional, extended conformations were observed for oxidized tetrameric TTR indicative of unfolding upon oxidation. This decreased stability can promote TTR aggregation and is consistent with a decrease in signal intensity in our IM-MS experiments; larger oligomers possess lower ionization efficiencies.

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Distinguishing Subtle Conformational Differences in Protein Complexes using Ion Mobility Mass Spectrometry and Collision Induced Unfolding

Stacey Nash, Tyler Marcinko, Richard Vachet
Department of Chemistry, UMass Amherst

Introduction: Probing conformational changes of heterogeneous ensembles of protein complexes can provide insight into functionally relevant dynamics. Existing tools for studying these conformational changes are often insufficient to analyze subtle conformational differences adopted by protein complexes. Native mass spectrometry (MS) together with ion mobility (IM) can reveal information about higher order structure and conformations of protein ions, enabling it to distinguish protein structural isomers, thereby revealing new conformational states. For small differences in structure, however, IM-MS often does not have the resolution to distinguish two or more conformational states. We are investigating the ability of IM-MS in conjunction with collision-induced unfolding (CIU) to reveal these small differences in the conformational states of protein complexes.
Methods: Bovine Beta Lactoglobulin (βLG) was used as a model protein complex. The βLG isoform A was purchased from Sigma Aldrich and reconstituted in 100 mM ammonium acetate to achieve a pH of 6.8, 100 mM ammonium formate to achieve a pH of 6.2, and 100 mM methylammonium acetate to achieve a pH of 8.3. Palmitic acid, which binds βLG, was purchased from Sigma Aldrich. Native MS and CIU- IM experiments were performed on a Synapt G2si from Waters. Individual charge states were selected using the quadrupole, and then CIU with IM-MS was performed over a range of collision voltages. TWIM extract and CIU Suite was used to analyze the resulting data.
Preliminary data: We investigated βLG under 3 different pH conditions to populate known conformational forms of this dimeric protein. CIU and IM-MS were then used to examine the unfolding pathway of each conformer in the gas phase. Under native spray conditions in ammonium acetate, ammonium formate, and methyl ammonium acetate, the intact dimer is observed and the 11+ charge state was selected for CIU studies. After selection of this charge state, only one species is observed by IM, even though two states are known to be present in solution, indicating that IM-MS is unable to resolve these isomers. At neutral pH when two conformers are present, βLG has two distinct unfolding pathways as collision voltage increases during the CIU experiment, resulting in the formation of two different species with different drift times in the IM cell before the dimer fully dissociates. Under more acidic conditions (pH 6.2) the βLG dimer forms a single more closed conformation. Interestingly, upon CIU only one unfolding pathway is observed, and this species has the same collisional cross section as one of the species measured by CIU at neutral pH. Under basic conditions (i.e. pH 8.3), the βLG dimer forms a more open conformation. Upon CIU of the protein at this pH, the same two unfolding pathways that were observed in the CIU measurements under neutral conditions were also observed, but one of the conformations was less abundant than at neutral pH. We propose that these different unfolding pathways reflect the two conformational isomers of βLG that cannot be resolved by IM-MS alone. To further test this hypothesis, βLG was bound to the ligand palmitic acid, which is known to force the protein into its open state. CIU data for the βLG-palmitic acid complex further supports the ability of CIU with IM-MS to detect conformational isomers.

Poster 50

Ion Mobility-Mass Spectrometry of Peptidomimetic-Aβ complexes: Towards Generalized Amyloid Inhibitors

Yilin Han[1], Neha Jain[2], Varun Gadkari[1], Elizabeth Gichana[2], Fredrik Almqvist[3], Magda I. Ivanova[4], Matthew T. Chapman[2], Brandon T. Ruotolo[1]. *[1]Department of Chemistry, University of Michigan, Ann Arbor, MI 48109 [2]Molecular, Cellular, and Developmental Biology, University of Michigan, Ann Arbor, MI 48109 [3]Department of Chemistry, Chemical Biological Center, Umeå University, 901 87 Umeå, Sweden [4]Department of Neurology, University of Michigan, Ann Arbor, MI 48109*

Introduction: Alzheimer's disease (AD) currently affects nearly 6 million Americans. Amyloid β (Aβ) peptide-based plaques have been identified as a hallmark of AD, but small peptide oligomers are thought to be a more critical element of AD pathology. More generally, amyloid formation has been linked to a range of other human diseases, including Parkinson's and Diabetes, as well as bacterial biofilm formation. Small molecules have been previously shown to affect rates of Aβ aggregation, but such compounds are rarely found to act on other amyloid-forming peptides and proteins similarly. Here, we use ion mobility-mass spectrometry (IM-MS) to reveal the molecular mechanisms of peptidomimetic amyloid inhibitors, originally derived from curlicides that arrest the formation of the amyloidogenic fibers that comprise bacterial biofilms.

Methods: Curlicide-derived small molecules were synthesized, purified, and then mixed with a range of Amyloid β1-40 (Aβ40) variants. For example, we have studied a recombinantly-expressed version of Aβ40 containing the Nterminal methionine residue (Aβ40M), as well as a a synthetic Aβ40 peptide (Aβ40S) that lacks N-terminal M (AnaSpec, Freemont, CA). Small molecules were initially screened using a thioflavin T (ThT) fluorescence assay to identify any effective Aβ40 aggregation inhibitors. Two small molecules FN075, and AC176 were identified as potent amyloid inhibitors, and further analyzed by IM-MS using a Synapt G2 platform (Waters Corp, Milford, MA). Data was collected and analyzed using Waters Masslynx software.

Preliminary Data: ThT fluorescence screens identified two compounds, FN075 and AC176, which inhibited Aβ40 aggregation for over a 48-hour period. Initial collision induced dissociation (CID) experiments revealed that Aβ40 binds to both AC176 and FN075 non-covalently in various stoichiometries. Interestingly, native IM-MS data reveals that Aβ40M binds both compounds with a greater affinity than Aβ40S, with Aβ40S exhibiting no measurable affinity for FN075. Next, IM-MS was used to study the structural changes in Aβ40 upon small molecule binding. The measured arrival time distributions, and collision cross sections (CCS) suggest that Aβ40 monomers bound to either compound occupy structurally extended states. This mode of action is contrasts strongly with previously studied amyloid inhibitors, which have been shown to shift Aβ40 to structurally compact states upon binding. Based on their influence on Aβ40 conformation, and demonstrated ability to inhibit Aβ40 aggregation, both AC176 and FN075 are ideal candidates for further optimization. Using IM-MS, binding affinities have been measured for each compound, using a range of Aβ40 variants. Additionally, efforts are underway to determine the binding site of A binding site of peptidomemetic compounds using hydrogen deuterium exchange (HDX) and electron capture dissociation (ECD). All structural MS data will be used to restrain computationally generated models of Aβ40 docked with both FN075 or AC176 in order to propose models of the bound states detected. Overall, this presentation will disclose our most recent structural MS data and models for peptidomemtic inhibitor-Aβ complexes. Furthermore, we will discuss our current mechanistic understanding of these compounds as an amyloid inhibitor class, and how such information will guide future compound optimization efforts.

Poster 51

Tandem-trapped ion mobility / mass spectrometry - analysis of protein systems

Christian Bleiholder
Department of Chemistry, Florida State University

Introduction: Ion mobility spectrometry-mass spectrometry methods offer the potential to correlate protein tertiary and quaternary structures to variations in their amino acid sequences and posttranslational modifications. Because ion mobility spectrometry measures cross sections of ions in the gas phase, however, it remains unclear how closely the detected ions resemble the native state present in solution. Here, we apply our recently-developed computational Structure Relaxation Approximation (SRA) method to elucidate detailed protein structures from measurements carried out on our tandem-trapped ion mobility spectrometry / mass spectrometry (TIMS/TIMS-MS) instrument. TIMS/TIMS-MS is a newly develop instrument capable to characterize tertiary and quaternary protein structures from collisional cross sections, but also performing top-down sequencing of mobility-selected protein conformations.

Methods: All measurements were performed on a prototype ESI-TIMS-TIMS-QqTOF mass spectrometer (Bruker Daltonics). Protein solutions were infused into the electrospray ionization device. Ions of a specific mobility window can be selected in the first TIMS analyzer and activated for topdown analysis in the interface of tandem-TIMS by Collisional-induced Unfolding/Dissociation (CIU or CID) prior to a mobility analysis in the second TIMS analyzer. The SRA method was used to analyze the experimental data. Analysis starts by explicit solvent molecular dynamics (MD) simulations. For each structure observed during the MD, short MD calculations are carried out in the gas-phase for structural-relaxation. Finally, the Projection Superposition Approximation method is used to calculate cross sections and predict ion mobility spectra for comparison to the experiment.

Preliminary data: We recently developed the Structure Relaxation Approximation (SRA) method to quantitatively and predictively interpret ion mobility spectra in terms of the ion structure. The central idea of the SRA is to predict charge-state specific ion mobility spectra from an ensemble of solution structures. Hence, the more charge states and experimental conditions are probed by experiment and theory, the greater the confidence of the structural interpretation will be because chance agreement for all conditions and charge states becomes increasingly unlikely. Here, we discuss application of the SRA method to elucidate proteins structures from measurements conducted on our recently developed tandem-trapped ion mobility spectrometry (TIMS/TIMS-MS) instrument. In particular, we discuss our progress towards detailing structures for a number of protein systems, including ubiquitin, ribonuclease A and its glycosylated variants ribonuclease B, and the glycoprotein complex avidin. Overall, our analysis reveals that even small protein ions detected from native conditions by soft ion mobility measurements largely retain their hydrophobic core present in their native structure as well as their native inter-residue contacts and secondary-structure elements.

Poster 52

Structural study of mobility-selected, native, intact glycoprotein complex using Tandem Trapped Ion Mobility Spectrometry – Mass Spectrometry (Tandem-TIMS/MS)

Fanny C. Liu[1], Mark E. Ridgeway[2], Melvin A. Park[2], Tyler C. Cropley[1], Christian Bleiholder[1]
[1]Florida State University, Dept. of Chemistry and Biochemistry, Tallahassee, FL [2]Bruker Daltonics, RII, Billerica, MA

Introduction Glycoproteins are implicated in all major diseases. Variation in N-glycosylation and amino acid sequence affects the cellular function of glycoproteins, including the antibody recognition mechanism. Hence, studying the chemical and conformational heterogeneities of *N*-glycans and how they modulate the three dimensional structure in a glycoprotein is crucial in designing potential antibody-based vaccines. We recently developed a tandem-trapped ion mobility spectrometry / mass spectrometry instrument (Tandem-TIMS/MS). Tandem-TIMS/MS is capable of characterizing tertiary and quaternary protein structures by their cross sections and performing top-down sequencing on mobility- and mass-selected protein complexes. Here, we discuss the connection between sequence, glycosylation pattern, subunits, and higher order structure of the glycoprotein complex avidin using native Tandem-TIMS/MS.

Methods All measurements were performed on a prototype ESI-Tandem-TIMS-QqTOF mass spectrometer (Bruker Daltonics). Avidin solutions (~39 M) were infused into the electrospray ionization device at a flow rate of ~180 µL/h. Protein-ligands complexes were formed in the solution by combining equal volumes and incubation at room temperature for a few hours. Low energy settings were used in Tandem-TIMS to preserve native intact avidin tetramers. Ions of a specific mobility window can be selected in the first TIMS analyzer and activated in the interface of Tandem-TIMS by Collisional-induced Unfolding/Dissociation (CIU or CID) prior to a mobility analysis in the second TIMS analyzer. Generated monomers with specific m/z range were isolated in the quadrupole and fragmented in the collision cell.

Preliminary data Our recently developed Tandem-TIMS/MS instrument combines the ability of TIMS to retain native-like structures at high resolving powers, with the ability to mobility-select and to energetically activate these ions prior to conducting ion mobility measurement in the second TIMS analyzer. Further, ions with specific m/z range can be isolated in the quadrupole and activated in the collision cell, which allows a MS3 experiments of mobility- and m/z selected ions. In this contribution, we demonstrate the potential of Tandem-TIMS to investigate the relationship between primary and higher order structures using protein avidin as a model system. Avidin, a glycoprotein, exists as a homotetramer with 128 amino acids in each subunit. We perform CIU and CID on mobility-selected tetrameric avidin ions in the interface of Tandem-TIMS. Distinct avidin monomers and fragments, generated by CID, are isolated in the quadrupole and exposed to a second ion activation in the collision cell (MS^3). Charge state distribution and collisional cross sections indicate that avidin quaternary structure is retained in Tandem-TIMS. Further, mobility-selection and CIU measurements show that the broad ion mobility peaks of avidin tetramers consist of unresolved, structurally distinct tetramer structures that do not interconvert during the measurement time. Additionally, CID in Tandem-TIMS performed at high activation voltages reveals compact dimer subunits. Identification of individual glycoforms of monomeric and oligomeric avidin reveals a specific combination pattern. Finally, obtained MS3 spectrum shows fragment ions of the avidin protein sequence and the attached *N*-glycans. We are starting to apply our approach to more biologically relevant glycoprotein complexes.

Poster 53

Ion mobility spectrometry of proteins, nucleic acids and foldamers: advantages of high-level molecular dynamics to generate candidate structures

Frédéric Rosu[1], Valérie Gabelica[2]
[1]CNRS UMS 3033, Inserm U01, IECB, Pessac, France [2]INSERM U1212, CNRS & Université de Bordeaux, Pessac, France

Introduction: Characterizing the folding and the self assembly is important to link the structure with a desired function, and native ion mobility mass spectrometry can help the structural assignment via collision cross sections (CCS). We measured CCS values by drift tube IMS in helium on very rigid and monomorphic model structures (oligoquinoline foldamers, Gquadruplex nucleic acids), on ubiquitin and on oligourea foldamers assemblies. We then compared the experimental CCS distributions with different theoretical CCS model (PA, EHSS, TM) using structures generated by high-level calculations. Our results show that DFT or semi-empirical calculations perform better than force field MD in the gas phase, and lead to better structural assignment based on CCS values.
Methods: Native electrospray ionization mass spectrometry has been performed on an Agilent 6560 DTIMS QTOF. The drift tube was operated in helium with custom modification to improve robustness (additional pump and vacuum manometers). We studied ubiquitin and peptidomimetic oligourea foldamers in positive in mode and nucleic acids and oligoquinoline phosphonate foldamers in negative ion mode. For the theoretical CCS calculations, we compared TM, EHSS and PA using mobcal and other popular software. We produce the structures candidates using ADMP molecular dynamics using Gaussian16. The electrons are treated at the semi-empirical level (PM7) or DFT level (M062X) and the nucleus are treated using classical (newtonian) formalism. Proton exchanges are also followed during the MD.
Preliminary Data: Drift tube experiments in helium are preferred to test the effect of structure generation, because of lower uncertainties on the parameterization of TM and EHSS. For ubiquitin, the TMCCS of PM7 optimized structure matches the experimental values better than the structures generated by force fields. For proteins, force fields had been widely adopted to generate structure invacuo, but our results show a systematic overestimation of the theoretical CCS values compared to the experiments if the gas-phase structure is modeled with force fields (whether Amber, Charmm, or OPLS). We highlight the caveats in adapting CCS calculation methods based on ill-optimized structures. For example, PA underestimates the CCS values but combined with force-field generated structures the values agree fortuitously. For nucleic acids, we have demonstrated the failure of force field to generate in-vacuo structures (*ACS Cent. Sci*., 2017, 3, 454) and propose now to use high level molecular dynamics where the electrons are treated at the DFT level (or semi-empirical level depending of the size of the system) and the nuclei are treated using classical formalism (*J. Phys. Chem. Lett.*, 2018, 9, 6605). Rigid oligoquinoline foldamers were also used as benchmark because they are monomorphic, rigid and the width of their experimental CCS distribution is close to the diffusion limit. Again, PM7 molecular dynamics and CCS calculation with TM or EHSS matches very well with the experiment. These structures would be excellent candidates to refine the parameterization of CCS calculation in other gases. Finally, we studied large oligourea hexameric (*Nat. Chem.*, 2015, 7, 871) and octameric complexes. X-ray crystal structures were available for some of them. We show that the structural interpretation of ion mobility can be perform if the measurements are done in helium, the structure generation is done by PM7, and the CCS calculation is done by TM or EHSS.

Poster 54

So *How* Bad is Ammonium Acetate for Native Mass Spectrometry? pH Changes During Nanoelectrospray Ionization (nESI) Quantified Using Fluorescence Imaging

Meagan M. Gadzuk-Shea, Evan Hubbard, Matthew F. Bush
University of Washington, Department of Chemistry, Box 351700, Seattle, WA 98195-1700

Introduction: Native nESI-MS requires the transfer of intact proteins and protein complexes into the gas-phase from solutions resembling physiological conditions. Ammonium acetate is often used for these solutions due to its amenability to MS. Although the dissolution of ammonium acetate salt results in a near neutral pH, ammonium acetate does not buffer at pH 7. This leaves solutions susceptible to pH changes from the inherent REDOX nature of the electrospray process. Changes in solution pH can lead to the destabilization of native proteins and protein complexes, therefore compromising the results of a native-MS experiment. Here, a pH-dependent fluorescent probe, SNARF-4F, and LED microscopy are used to quantify the pH changes associated with bulkloaded capillaries in native nESI MS.

Methods: Solutions containing 100 nM SNARF-4F in aqueous 10 mM and 200 mM ammonium acetate, and 10 mM ammonium bicarbonate at a pH between 6.9 and 7.2 were prepared for analysis. An electrospray current of 30 nA, 60 nA, or 100 nA was produced by applying a voltage to a platinum wire inserted into a capillary containing sample via a high voltage power supply. The liquid sample was irradiated with a 530 nm LED at fixed time intervals for ~ 50 minutes, and the resulting fluorescence was imaged using a 577 nm or a 655 nm bandpass filter. Ratiometric analysis of the fluorescence intensities at the two emission wavelengths were related to solution pH using equations generated from analysis of standards.

Preliminary Data: The effects of electrolyte concentration, electrospray current, and polarity were considered with respect to the pH of native MS samples on timescales relevant to typical native MS experiments. Most conditions resulted in changes of roughly ±1 pH unit over tens of minutes, although the rates and extents of change depended on the parameters of the solution and the electrospray. At lower electrolyte concentrations, the onset of pH change occurred sooner relative to solutions with higher electrolyte concentration. Similarly, experiments conducted at higher electrospray currents also resulted in earlier changes in solution pH. At the lowest current examined, 30 nA, all solutions exhibited some degree of buffering prior to acidification or alkylation, although the buffering period was less significant under negative polarity. The most significant change in solution pH was observed for 10 mM ammonium acetate at 100 nA of current, consistent with the electrolytic nature of electrospray ionization and the inability of ammonium acetate to buffer at neutral pH. The magnitude of pH changes observed ranged from ~ ± 1 – 1.5 pH units, which is significant for many native-MS studies of pH sensitive proteins. Interestingly, samples in negative mode experiments exhibited a dramatic gradient of pH values throughout the timescale of the experiment, whereas positive mode experiments exhibited a much more uniform change in solution pH with respect to time. This phenomenon was attributed to the greater mobility of protons relative to that of hydroxide anions. Strategies for mitigating the buildup of REDOX products that induce solution pH changes were characterized in terms of effectiveness and compatibility with native MS.

Poster 55

Combining native top-down proteomics and de novo sequencing to identify and quantify E3 ligase interactions in cells

Daniele Canzani,[a] Domnița-Valeria Rusnac,[b] Ning Zheng,[b] Matthew F. Bush[a]. [a]*Department of Chemistry, University of Washington,* [b]*Howard Hughes Medical Institute, Department of Pharmacology, University of Washington*

Introduction: Hundreds of ubiquitin E3 ligases ensure proteome fidelity and control cellular functions by promoting protein degradation. These processes require exquisite selectivity, but the roles of most E3s remain uncharacterized due to challenges associated with identifying substrates for each E3. We developed an integrative mass spectrometry (MS) strategy for characterizing protein fragments that interact with KLHDC2, a human E3. Combining native MS, native topdown MS, MS of destabilized samples, liquid chromatography MS, and *de novo* sequencing, we identified and quantified KLHDC2-binding peptides in *E. coli* cells. This strategy could be used to characterize other E3 ligases, and is a critical step forward in "degronomics," *i.e*., identifying, quantifying, and validating functional E3:peptide interactions in order to determine the roles of individual E3s.

Methods: KLHDC2 was expressed and purified from *E. coli* cells. Native top-down MS experiments (MS3) of KLHDC2:peptide complexes were performed on a Waters Synapt G2 by increasing the entrance cone voltage to 130 V, isolating collision-dissociated peptide ions using the quadrupole, then fragmenting those ions in the trap cell (CE 45 V). Destabilization MS was achieved by heating the KLHDC2 sample at 55 °C for 5 minutes before MS analysis on the Synapt G2 platform. Peptides were extracted from the KLHDC2 sample with a Pierce C18 Tip (no enzymatic digestion was used), and were analyzed using LC-MS/MS on a Waters NanoAcquity-Thermo Q-Exactive Orbitrap platform. Fragmentation spectra from all MS methods were analyzed by *de novo* sequencing using Peaks Studio 8.0.

Preliminary Data: Native MS was used to analyze KLHDC2. In addition to peaks for apo KLHDC2, we were surprised to find a number of additional adjacent peaks. The unexpected features correspond to ions that are approximately 400 to 1400 Da greater in mass than apo KLHDC2 and suggest that many different peptides had complexed with KLHDC2. The bound peptides represent bacteria-produced protein fragments that copurified with KLHDC2. These spectra show the first evidence of copurified protein fragments bound directly to an E3 using MS. To determine the identity of the copurified peptides, we first used native top-down MS, *i.e*., subjecting intact KLHDC2:peptide complex ions to multiple stages of activation in order to determine the sequences of the bound peptides. Seven interacting peptides were identified using native top-down MS, and all featured diglycine at their extreme C-terminus. KLHDC2 was recently shown to recognize substrates which contain C-terminal diglycine in human cells. Due to limitations in the sensitivity of the native MS experiments, non-native methods were explored. Thermal destabilization of the sample caused peptides to be released from KLHDC2 into solution before MS analysis, which resulted in greater peptide ion signal intensity compared to native MS. Results from destabilizing MS confirmed the identity of the peptides observed in the native topdown experiments. Next, peptides extracted from the KLHDC2 sample were analyzed using LCMS/ MS, without enzymatic digestion, and *de novo* sequencing was used for peptide identification. Many additional peptides were discovered that originated from *E. coli* proteins, and all of them featured diglycine at their extreme C-terminus. Although all identified protein fragments are terminated by diglycine, the preceding amino acids are diverse. These results significantly expand our understanding the sequences that can be recognized by KLHDC2, which provides insights into the potential substrates of this E3 in humans.

Poster 56

Combining Native MS, Ion Mobility, Native Top-Down, and NMR Spectroscopy to study the Interaction of Roundabout1 with Arixtra

Robert V. Williams[1,2], Jeong-Yeh Yang[2], Kelley Moremen[2], James H. Prestegard[1,2], I. Jonathan Amster[1]
[1]*Department of Chemistry, University of Georgia, Athens, GA*
[2]*Complex Carbohydrate Research Center, University of Georgia, Athens, GA*

Introduction

Roundabout 1 (Robo1) is an extracellular receptor protein whose dysregulation has been implicated in tumor formation and metastasis. Signaling requires the formation of a ternary complex between the first two N-terminal domains of Robo1, the second LRR domain of Slit2, and Heparan Sulfate (HS), but the precise mechanism is not well understood. Previous ion mobility data showed that upon binding HS, a two-domain construct of Robo1 prefers a more compact conformation than that of the existing crystal structures. Here we study the interaction of Robo1 and Arixtra, a synthetic heparan sulfate oligomer, using a methodology employing native mass spectrometry, ion mobility, ECD MS/MS and NMR spectroscopy proves useful to assess the biological relevance of the gas phase observations.

Methods

Human Robo1 was expressed as a two-domain construct with an inserted lanthanide-binding peptide sequence in *lec*- HEK297 cells. Samples for mass spectrometry were prepared at 10 uM Robo1 in 10 mM ammonium acetate, with no ligand, and 1:1 ratios of lutetium, Arixtra, or both. Ion mobility-mass spectrometry measurements were collected with a Waters Synapt G2S. CCS values were determined by calibration with cytochrome *c* and myoglobin. Native Top-Down ECD MS/MS was performed on a 12 T Bruker Solarix FT-ICR instrument, with a range of in-source activation. NMR spectroscopy was performed on two samples of 300 µM Robo1 in 25 mM Tris, 100 mM KCl, pH 7 with either an equimolar amount of lutetium chloride or dysprosium chloride.

Preliminary Data

Native MS of Robo1 and Arixtra shows apo-Robo1 as well as formation of protein-GAG complexes in 1:1. The ion mobility arrival time distribution of the Robo1 11+ charge state shows two peaks corresponding to a compact conformation with CCS of 2146 Å2 and an elongated form with CCS of 2260 Å2, where the larger species is the dominant form. For the Robo1 fondaparinux 1:1 complex 11+ ion, there are also two peaks but with an increase in the proportion of the compact species. HETCOR spectra of robo1 allow measurement of lanthanide induced pseudocontact shifts, which produce changes in peak position in a manner that depends on the distance between a nucleus and the bound lanthanide ion. Measured PCS values change significantly between Robo1 with and without fondaparinux, which suggests a rearrangement in the protein structure. Native Top-Down ECD MS/MS of Robo1 produces fragments with at least 50 V of skimmer 1 activation. Bonds cleaved are primarily located between domain 1 and 2, suggesting that this region of the protein is flexible in the gas-phase.

Poster 57

Enhanced characterization of membrane protein complexes using ultraviolet photodissociation

Sarah N. Sipe,[1] John W. Patrick,[2] Arthur Laganowsky,[2] Jennifer S. Brodbelt[1]
[1]*Department of Chemistry, The University of Texas at Austin, Austin, TX 78712*
[2]*Department of Chemistry, Texas A&M University, College State, TX 77842*

Introduction: Although integral membrane proteins (IMPs) are critical for cell signaling and molecular transport and make up a significant portion of drug targets, they are consistently underrepresented in proteomics approaches owing to their lower cellular abundance and hydrophobic nature. Solubilization of membrane proteins has been facilitated by employing detergent micelles, nanodiscs, and lipid bilayers. These solution additives combined with nanoelectrospray ionization (nESI) allow folded, compact IMPs to be transferred to the gas phase for mass spectrometric analysis. Tandem mass spectrometry can be used to activate and dissociate proteins to elucidate primary sequence information. For the first time, ultraviolet photodissociation (UVPD) is utilized to dissociate membrane proteins, affording increased sequence coverage relative to traditional collisional activation methods.

Methods: Native mass spectrometry was performed on a Thermo Q Exactive Plus™ Ultra High Mass Range Orbitrap™ instrument. Purified aquaporin Z (AqpZ) and mechanosensitive channel of large conductance (MscL) were solubilized in 200 mM ammonium acetate with 0.5% C8E4 detergent. Samples were ionized using nanoelectrospray ionization, and ion optics were tuned to maximize transfer efficiency and desolvation without excessive activation of protein ions. Single charge states of each complex were selected and activated using higher-energy collisional dissociation (HCD) or ultraviolet photodissociation (UVPD) via a 193 nm excimer laser. MS/MS spectra were deconvoluted using the Xtract algorithm with a signal to noise threshold of 3, and the resultant products were identified using a 10 ppm tolerance by UV-POSIT and ProSight Lite.

Preliminary Data: Using the conditions described, AqpZ (99 kDa homotetramer) and MscL (85 kDa homopentamer) were readily ionized. In-source trapping energy was optimized to desolvate protein ions and minimize nonspecific detergent adduction. The most abundant ion of each species was isolated and activated with HCD up to 300 eV, limited by the capabilities of the instrument, or a single pulse of 193 nm UVPD with pulse energy ranging from 3 mJ to 5 mJ. The native nESI mass spectrum of AqpZ revealed tetramers in the 12+ through 17+ charge states. Activation of the 14+ tetramer with the maximum HCD energy (equivalent to 4.2 keV lab frame collision energy (Elab)) generated roughly 55 identified fragments amounting to 21% sequence coverage. A single pulse of 4 mJ UVPD generated on average of 134 identified fragments for 31% coverage. To our knowledge, this is the first report of top-down analysis of AqpZ. The 13+ MscL pentamer was subjected to HCD (3.9 keV Elab), producing on average 53 identified sequence ions for 33% coverage, which surpasses the previously reported 11% coverage using traditional collision-induced dissociation on a Q-ToF instrument. The drastic improvement in sequence coverage is attributed to the improved transmission of high *m/z* ions by the UHMR and the high resolution of the Orbitrap analyzer for subsequent sequence ion identification. UVPD using a 3 mJ pulse generated an average of 155 fragments for 52% coverage. In this case, the enhanced coverage is attributed to the fast, high-energy activation method which provides access to more fragmentation pathways. Addition of various phospholipids to the protein solutions resulted in holocomplexes that were also analyzed. Sequence coverage obtained from UVPD of the holocomplexes was only slightly affected compared to the values obtained for the apoproteins, demonstrating the robustness of photoactivation to characterize proteins that are notoriously difficult to purify.

Poster 58

Native State Chemical Tagging Approaches for the Free Radical-initiated Sequencing of Intact Protein Complexes

Carolina Rojas Ramirez; Daniel A. Polasky and Brandon T. Ruotolo, *University of Michigan*

The top down (TD) sequencing of intact multi-protein complexes remains a long-term technology development goal for native mass spectrometry (MS), as post-translational modifications (PTMs) encoded into such assembly states are critically important in regulating many central biochemical processes. Typically, native TD experiments produce too few fragment ions to confidently assign intact complex proteoform states. Previously, ortho-[(2,2,6,6-tetramethylpy-peridin-1-yloxy)methyl]benzoic acid N-hydroxysuccinimide ester (TEMPO), was reported to produce free radical-initiated peptide sequencing (FRIPS) for denatured protein ions. In FRIPS, both collisional and electron-based fragmentation pathways can be captured, often leading to increases in protein sequence and PTM information. Here, we modify TEMPO labeling strategies for native TD experiments, and explore the ability of FRIPS to increase the sequence coverage achieved intact protein assemblies. Initial TEMPO labelling of ubiquitin (8.6 kDa) was performed using 60% acetonitrile (ACN) at pH 8.5 with TEMPO in molar excess. After optimization for native TD, ubiquitin, β-lactoglobulin A (dimer, 36 kDa) and Avidin (tetramer, 64 kDa) were labeled in 100mM triethylammonium bicarbonate (TEAB, pH 8.5) for 4hrs at 37C with molar excess TEMPO. After labeling, proteins and complexes were purified by gel-filtration into 100 mM ammonium acetate. A Waters Synapt G2 quadrupole (Q) ion mobility (IM)-time of flight mass spectrometer (ToF MS) equipped with a nano-electrospray ionization (nESI) source was used for protein analysis. After Q isolation, proteins were subjected to CID to initiate FRIPS. A software pipeline developed in-house was used to analyze native TD datasets. Under native conditions, we observe up to two TEMPO modifications to ubiquitin, generating significant radical fragment ion signals upon collisional activation at modest (60V) accelerating potentials. The intensity recorded for the radicalized TEMPO attached ubiquitin peak correlates strongly with the appearance of *c*/*z* fragment ions, with their intensity and number invariant across a range of activation voltages, indicative of expected FRIPS fragmentation. By combining collision induced dissociation (CID) and FRIPS datasets, we achieved 96% sequence coverage (defined in terms of the number of peptide bonds broken during activation) for native-like ubiquitin ions.

Native state TEMPO labeling was also achieved for the β-lactoglobulin A (β-lacA) dimer, where our optimized labeling conditions produced signals corresponding to one TEMPO label per monomer as the base peak in the native MS spectrum obtained. Upon initiating FRIPS, we observed a sequence coverage increase over CID alone for β-lacA monomers, increasing from 35% (71 fragment ions) to 40% (89 fragment ions) when combining both datasets. Some of this increase in sequence coverage is due to the reduction of the disulfide bonds between positions 66-160 (loss of sulfhydryl observed) upon FRIPS fragmentation. As observed with native ubiquitin, the intensity of *c*/*z* fragment ions appeared optimal at lower accelerating potentials in a manner highly correlated with the signal intensities recorded for the radicalized TEMPO tagged protein signals. Our optimized labeling conditions produced intense signals for the Avidin tetramer corresponding to two TEMPOs per monomer. Upon initiating FRIPS of the 17+ tetramer precursor, we observed increased n-terminal fragments from the TEMPO labeled sample, evidence disulfide bond reduction, and increased sequence coverage over CID results alone, from 29% (26 total fragment ions) to 41% (58 total fragment ions) respectively. This presentation will disclose our most recent FRIPS data for native TD experiments covering a wide range of protein complexes.

Poster 59

Top-down electron ionisation dissociation and internal fragment assignment of carbonic anhydrase II can enhance protein sequence coverage by mass spectrometry

Muhammad A. Zenaidee, Wonhyeuk Jung, Carter Lantz, Rachel R. Ogarzalek Loo, Joseph A. Loo
Department of Chemistry and Biochemistry, UCLA

Introduction: Top-down mass spectrometry (MS) for protein sequence analysis, where the intact protein is ionized, detected, and fragmented during MS has proven to be a crucial tool for characterizing post-translational modifications on proteins, and localizing protein-ligand binding sites. Electron ionization dissociation (EID), is a fast activation method that has not been explored extensively. During EID, high energy electrons (> 20 eV) are utilised to fragment the protein backbone resulting in unique fragmentations. EID forms terminal fragments (single fragmentation of the protein backbone), and internal fragments (double fragmentation of the protein backbone). Conventionally, only terminal fragments were used for top-down MS protein sequence analysis. By assigning not only terminal but also internal fragments generated by EID, we enhance top-down protein sequence analysis.

Methods: A 15-Tesla SolariX FT-ICR mass spectrometer (Bruker Daltonics; Bremen, Germany) was used to generate carbonic anhydrase II (CAII) mass spectra. CAII in a solution containing 50:50 water:methanol, and 1% formic acid was loaded into Au/Pd-coated borosilicate capillaries (Thermo Fisher Scientific) and sprayed at a flow rate of 10-40 nL/min through a nanospray ion source. For data acquisition, 200 spectra were averaged, and all spectra were externally calibrated with cesium iodide. For protein sequence analysis and fragment assignment, fragment lists were generated using Bruker's Data Analysis software (Bruker Daltonics; Bremen, Germany) and fragments were matched to all theoretical fragments generated by a program developed in-house. Fragment matching was performed with a mass accuracy of > 1 ppm to limit false positives.

Preliminary data: For $[CAII, 25H]^{25+}$, the use of EID (> 20 eV electron energy) resulted in a larger number of deconvoluted fragments by the data analysis software provided by Bruker than with ECD (< 3 eV electron energy). For example, with the use of EID, there were 204 ± 5 deconvoluted fragments compared to 153 ± 7 deconvoluted fragments with ECD. This data suggests that EID results in a larger number of fragments which indicates that fragmentation efficiency is increased by EID compared to ECD. Interestingly, with the use of EID, the percentage of internal fragments formed increases compared to ECD. For example, with ECD, less than 10% of the fragments detected were internal fragments. In contrast, with EID, approximately 20 to 40% of the fragments are internal fragments. As EID results in more fragmentation, the increase in fragment formation can be attributed to internal fragments. Internal fragment analysis has been limited as there has been no reliable way to assign internal fragments. With the use of our in-house program, we can match deconvoluted fragments to both internal and terminal fragments. For $[CAII, 25H]^{25+}$ we can assign more than 90 % of the deconvoluted fragments with less than 1 ppm mass discrepancy. Using the program developed, the sequence coverage for $[CAII, 25H]^{25+}$ increased from 62 % (terminal fragments only) to 100 % (terminal + internal fragments). Our data shows that by combining internal fragment assignment with EID fragmentation, a majority of the deconvoluted fragments from the mass spectrum can be reliably assigned, enhancing the sequence coverage of the protein.

Poster 60

Application of omics and native mass spectrometry approaches to understand *Salmonella* Pathogenesis

Angela Di Capua[1], Jikang Wu[1], Mikayla Borton[2], Anindita Sengupata[1], Anice Sabag-Daigle[3], Brian Ahmer[3], Venkat Gopalan[1], Kelly Wrighton[2,4], and Vicki Wysocki[1] *[1]Department of Chemistry and Biochemistry, [2]Department of Microbiology, [3]Department of Microbial Infection and Immunity, The Ohio State University, [4]Department of Soil and Crop Science, Colorado State University*

Introduction: *Salmonella* is a foodborne pathogen that can affect the intestinal tract. The Centers for Disease Control and Prevention estimate that *Salmonella* causes annually about 1.2 million illnesses, 23,000 hospitalizations, and 450 deaths in the United States.[1] There are no drugs specifically targeted at *Salmonella*, and the use of broad spectrum antibiotics can prolong shedding and cause antibiotic resistance. We are using untargeted metabolomics and proteomics to characterize *Salmonella* infection in mice to identify *Salmonella*-specific processes that could be targeted therapeutically. One exemplar in this regard is the pathway for utilization of fructose-asparagine (F-Asn). The inhibition of the *Salmonella* FraB deglycase, which is essential for F-Asn catabolism, leads to accumulation of a metabolic intermediate that inhibits growth of the organism *in vitro* and *in vivo*. Thus, FraB is a potential target to inhibit *Salmonella*.[2,3]

Methods: For the omics studies, we are developing robust protocols for identification of untargeted proteins and metabolites in fecal samples obtained from *Salmonella*-infected mice. Experiments will be performed on the Orbitrap Elite or Bruker timsTOF Pro (proteomics) and on an Agilent 6545 QTOF (metabolomics). For native-state experiments, FraB was expressed as previously reported.[3] Experiments will be performed using a nano-electrospray ionization (nano-ESI) source or Triversa Nanomate coupled with an Exactive™ Plus EMR mass spectrometer, analyzing the protein over a wide pH range and at different protein concentrations.

Preliminary data: Previously, we demonstrated how *Salmonella* infection affects the proliferation of specific members of the human microbiota. Short chain fatty acids (SCFA) are often implicated as regulators for intestinal inflammation. We also reported the cumulative concentrations of acetate, butyrate, and propionate in the highly-inflamed *Salmonella* gut were significantly depleted relative to the noninflamed control (value below the limit of detection).[4] While omics studies are relevant to understanding the correlation between *Salmonella* infection and the human microbiota, native mass spectrometry (MS) is an important tool for relevant protein ligand complexes. The FraB deglycase is believed to employ an initial isomerization followed by hydrolysis of a Schiff base to convert 6-phosphofructose-asparate (6-P-F-Asp) to glucose-6-phosphate and aspartate.[5] When we tested the activity of FraB at different pH values ranging from 4.7 to 9.8, we observed a bell-shaped pH-rate profile with an optimal pH ~8. One interpretation of this pH profile was that a general acid and a general base are critical for catalysis, while another was that the FraB homodimer undergoes changes in its quaternary structure as a function of pH. We have gained support for the former postulate since our native mass spectrometry (MS) studies indicate the dominant presence of the dimer between pH 5 to 10, while the monomer can be detected at pH 4. Native MS has also been used to demonstrate the presence of two active sites/homodimer, suggesting the use of inter-subunit catalytic centers in FraB. **References:** (1) Centers for Disease Control and Prevention, https://www.cdc.gov/ (2) Ali, M. M. *et al*, PLoS Pathogen 2014, 10 (6), e1004209. (3) Sabag-Daigle, A. *et al.,* Scientific Reports 2016, 6, 28117. (4) Mikayla A. Borton, *et al.* Microbiome, 2017, Volume 5, Number 1, Page 1 (5) Sengupta, A.; Wu, J.; Sabag-Daigle, A.; Seffernick, J.; Thomsen, N.; Chen, T.; Bell, C.; Lindert, S.; Ahmer, B. M.; Wysocki, V. H.; Gopalan, V., manuscript under revisions.

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Soft Landing Preparative Native MS of Soluble & Membrane Protein Complexes for High Resolution Single Particle Imaging

Joseph Gault[1], Sabine Abb[2], Frank Sobott[3], Alexander Makarov[4,] Klaus Kern[2], Carol Robinson[1], Stephan Rauschenbach[1,2]

[1] Department of Chemistry, University of Oxford, 12 Mansfield Road, Oxford OX1 3TA, UK [2]Max Planck Institute for Solid State Research, Heisenbergstr. 1, DE-70569 Stuttgart, Germany [3]The Astbury Centre for Structural Molecular Biology, and School of Molecular and Cellular Biology, University of Leeds, Leeds, LS2 9JT, UK [4]Thermo Fisher Scientific, Hanna-Kunath-Straße 11, 28199 Bremen, Germany

Introduction: Structural models of proteins provide a basis for understanding biological function and permit rational design of therapeutics. State of the art X-ray crystallography and electron microscopy (EM) imaging can provide high-resolution structural information on large proteins and assemblies. However, these approaches demand extensive sample preparation and are intrinsically unable to capture transient, low abundance species such as low affinity ligand/drug bound states or conformationally primed proteins, due to their reliance on particle averaging for structure determination. Here, using soft-landing Q-ToF and Oribtrap platforms, we combine preparative and native mass spectrometry (prep-nMS), to selectively and efficiently prepare folded proteins and intact assemblies 64-801 kDa for structural imaging by EM & a true high-resolution single particle technique low-energy electron holography.

Methods: We detail the stepwise development of a universal and robust workflow for the mass selective preparation and high resolution imaging of folded soluble and membrane protein complexes, including ligand bound states, using a combination soft-landing/preparative, and native mass spectrometry (prep-nMS). We detail the design, construction and operation of both Q-ToF and Orbitrap based ion beam deposition MS instruments for soft landing, and demonstrate reproducible deposition of high densities of folded single particles on suitable substrates, for high resolution imaging using multiple single-particle imaging techniques, including atomic force microscopy, scanning tunnelling microscopy, negative stain electron microscopy and low energy electron holography.

Preliminary Data Here we present a new, general, chemically selective method to prepare protein assemblies for structural imaging (prep-nMS). To achieve high densities of intact, and folded assemblies, we show that each step of our workflow must be tuned and optimised to minimise ion loss and promote retention of native architecture; including transfer from solution into the mass spectrometer, ion beam manipulation, deposition energy, choice of deposition substrate and particle coverage. We demonstrate that AFM can be used as a rapid, non-destructive screening method to check the integrity of deposited assemblies by accurate height measurements. We find the choice of deposition substrate is crucial for maintaining a native fold and show evidence of unfolding when complexes are deposited on metal surfaces whereas in contrast, deposition on inert carbon-based supports allows retention of globular structure for multiple protein assemblies from 64-801 kDa. We apply our prep-MS/AFM strategy to reveal topological details of the nanobody-stabilised bacterial toxin-antitoxin regulator PaaR2 which has previously unknown stoichiometry and structure, and then extend our approach and show that intact membrane protein assemblies can also be prepared for imaging using prep-nMS. Finally, we successfully integrate prep-nMS with spatially high resolution imaging techniques and show that it can be used to prepare intact assemblies for imaging by TEM and LEEH with retention of integrity and structural features characteristic of a native fold.

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Development of Gas-Phase Hydrogen Deuterium Exchange for Structural Characterization: Lessons from Enkephalin Variants

Cynthia Suarez, Rebecca Jockusch
Department of Chemistry, University of Toronto

Introduction: Hydrogen deuterium exchange mass spectrometry (HDX-MS) is a powerful tool for drug development as it can help identify binding sites and monitor conformational changes induced by drug. HDX is widely performed in solution phase, where monitoring exchange rate of amide protons can provide information about secondary structure. In this work, I explore the translation of HDX into the gas phase to obtain conformational information about peptides and their complexes upon desolvation. Gas-phase HDX can access information about peptide secondary structure via amide exchange rates, as well as the exchange rate of hydrogens on side chains and termini, which occur too fast to be monitored in solution. The propensities of gas-phase HDX are studied using leucine and methionine enkephalin variants.

Methods: The amino acid sequence of the leucine variants studied were: YGGFL, YGGFL-NH_2, YGGFLK, and the methionine variants were: YGGFM, GYGGFM, and YAGFM-NH_2. Experiments were conducted in a 7T Fourier transform ion cyclotron resonance (FT-ICR) mass spectrometer (Bruker Apex Qe). Nano-electrospray ionization was used for all the variants from 10 μM solutions prepared in water. Once in the gas phase, ions were accumulated in a hexapole for 0.5 seconds, then transferred into a 2^{nd} trapping hexapole, which acts as a collision/reaction cell where ions were exposed to three different pressures of deuterated ammonia (ND_3) for various storage times (0.001 – 30 s). Deuterated ions are then transferred into the ICR cell for dissociation and detection.

Preliminary Data: Gas-phase HDX kinetics have been measured for three leucine and three methionine enkephalin variants. Progressive deuterium uptake was observed for leucine enkephalin (YGGFL) with exchange plateauing at 7/9 labile hydrogens. The extent of exchange suggests an unfolded conformation in the gas phase. Leucine enkephalin had the highest uptake rate of all leucine variants. Leucine enkephalin amide (YGGFL-NH_2) also displayed progressive uptake with plateau occurring at 8/10 labile hydrogens. The uptake rate slows down relative to leucine enkephalin suggesting that the C-terminal carboxyl hydrogen is more reactive than hydrogens in a capping amide group to gas-phase HDX. Progressive deuterium uptake was observed with leucine enkephalin lysine (YGGFLK) with 7/12 labile hydrogens exchanging. The lower extent of exchange may reflect charge sequestration on the basic residue or salt bridge formation. This form had the slowest uptake rate of the leucine set. Progressive uptake was observed for all methionine enkephalin variants. Methionine enkephalin (YGGFM) exchanged 7/9 labile hydrogens, suggesting an unfolded conformation in the gas phase. Gly-0 methionine enkephalin (GYGGFM) exchanged 8/10 labile hydrogens. The rate of uptake for this variant at low pressure is virtually indistinguishable from methionine enkephalin, while at higher pressures one additional exchange occurs in the Gly-0 peptide. This suggests that the added amide bond is one of the slowest exchanging sites in the Gly-0 methionine enkephalin. Ala-2 methionine enkephalin amide (YAGFM-NH_2) exchanged a total of 8/10 labile hydrogens. This variant displayed a decrease in uptake rate relative to methionine encephalin, reminiscent of the slower exchange rates observed upon C-terminal capping of leucine enkephalin. From pseudo-first order kinetics analysis, all variants were found to have at least two populations of hydrogens that are exchanging at distinct rates. Next, ultraviolet dissociation will be pursued as a rapid dissociation technique in order to make stronger conclusions regarding exchange and structure.

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Detergents' Supercharging Effects on Soluble Proteins and Membrane Proteins

Wonhyeuk Jung, Muhammad Zenaidee, Janine Fu, Carter Lantz, Frederik Lermyte, Jennifer Lippens, Joseph A. Loo, Rachel Ogorzalek Loo

[1]University of California, Los Angeles, [2]Warwick University, [3]Amgen

Introduction: In most genomes, 20–30 % of all genes encode membrane proteins and they represent more than 60% of potential drug targets. Typically, membrane proteins need to be solubilized with detergents (or other vehicles) to keep their native structures intact and to prevent aggregation. For structural studies of membrane proteins using mass spectrometry, non-ionic saccharide detergents are commonly used for solubilization. However, how membrane proteins interact with detergents (and micelles) and how the interaction affects protein gas-phase charge state remain elusive. In the interest of gaining insights into this interaction, ESI charging of soluble proteins in the presence of non-ionic saccharide-based detergents were measured and compared with membrane proteins. Many of the detergents appear to be supercharging agents.

Methods: A 15-Tesla solariX FT-ICR mass spectrometer (Bruker Daltonics; Bremen, Germany) and a Synapt G2-Si quadrupole ion mobility/TOF MS instrument with a travelling wave ion guide (TWIG) (Waters MS-Technologies; Manchester, UK) were used to generate native MS spectra. Lysozyme, and AqpZ in 200mM ammonium acetate with respective detergents were loaded into Au/Pd-coated borosilicate capillaries (Thermo Fisher Scientific) and sprayed at a flow rate of 10-40 nL/min through a nanospray ion source for both instruments. For both instruments,100 spectra were averaged, and all spectra were externally calibrated with cesium iodide.

Preliminary Data: Non-ionic saccharide detergents such as n-octyl-β-D-glucopyranoside (OG) and n-dodecyl-β-D-maltopyranoside (DDM) are commonly used to solubilize membrane proteins. However, how membrane proteins interact with detergent micelles in the gas phase remains a mystery. Saccharide detergents consistently yield higher charge states than polyoxyethylene (PEO) and lauryldimethylamine-N-Oxide (LDAO) detergent. Also, for PEO and LDAO detergents, the maximum charge states were >20% below the Rayleigh limit (Laganowsky, JASMS 2017). Thus, the charge residue model that relies on solvent surface tension and the Rayleigh limit to describe charge states fails to explain the charge states of membrane proteins in detergents. The effect of detergents on charge state distributions for soluble proteins was reported in 1996 (Ogorzalek Loo et al, Protein and Peptide Analaysis by MS, Human Press) and it was found that non-ionic saccharide-based detergents supercharge soluble proteins in both native and denaturing conditions. Requirements of effective positive ion supercharging agents have been suggested to be (1) solubility, (2) interaction with analytes, (3) weak Brønsted basicity and (4) volatility that is similar or less than the bulk solvent to concentrate in the droplet (Ogorzalek Loo et al, JASMS 2014). Non-ionic saccharide detergents satisfy these requirements and thus, shows supercharging behavior. For instance, CYMAL-5, CYMAL-6, and OG supercharge lysozyme under native condition and OG also supercharges myoglobin in native condition. Circular dichroism studies indicate that OG-induced supercharging of myoglobin does not come from loss of secondary structure. Based on this finding, the charge state distribution of aquaporin Z, a water channel protein for E. coli, solubilized in C8E4 (octyl tetraethylene glycol ether) or OG was compared. Native MS of aquaporin-Z shows charging to 21+ in OG, but only to 18+ in C8E4 (octyl tetraethylene glycol ether) indicating that supercharging could be the reason behind charge state distribution observed for membrane proteins solubilized in non-ionic saccharide-based detergents.

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Evaluation of a modular atmospheric pressure drift tube coupled to an Orbitrap™ mass spectrometer with ultraviolet photodissociation for biomolecule analysis

James D. Sanders,[1] Sarah Sipe,[1] Tobias Reinecke,[2] Brian Clowers,[2] and Jennifer S. Brodbelt[1]
[1] Department of Chemistry, The University of Texas at Austin, Austin, TX, USA
[2] Department of Chemistry, Washington State University, Pullman, WA, USA

Introduction: With its ability to provide gas-phase separations and collision cross section measurements, ion mobility (IM) offers an additional dimension of size/shape resolution to mass spectrometry. The majority of drift-tube IM-MS systems utilize IM devices coupled to time-of-flight (TOF) mass analyzers. While the kHz scan rates of TOF analyzers adequately sample IMS separations occurring at 10s of Hz, such instrumental combinations do not integrate high m/z resolution and advanced MS/MS capabilities which hinders their utility for the analysis of complex biomolecules. Orbitrap MS platforms offer superior resolution and the high-performance MS/MS capabilities such as ultraviolet photodissociation (UVPD). We present the coupling of a modular drift tube to an Orbitrap MS with UVPD capabilities and evaluate its utility for biomolecule analysis.
Methods: The atmospheric pressure drift tube constructed from printed circuit board components has been described previously by the Clowers group. The device is mounted externally in place of the standard electrospray ionization (ESI) source and operated at atmospheric pressure with nitrogen as the drift gas. Ions are generated by a nanoESI source located at the entrance of the drift tube. The drift tube is divided into two regions by a 3-grid ion shutter which allows the controlled introduction of ions into the 10 cm drift region. A second shutter at the end of the drift region controls the transmission of ions into the mass spectrometer. The modular drift tube has been interfaced with linear ion trap and Orbitrap mass spectrometers.
Preliminary Data: The dual ion gate design allows the IM device to operate in dual gate scanning (DGS), multiplexed Fourier Transform (FT-IM) or selected mobility monitoring (SMM) acquisition modes. Both DGS and FT-IM modes enable the generation of high-resolution arrival time distribution (ATD) profiles while circumventing the inherent duty cycle mismatch between IM and ion trap mass spectrometers. The FT-IM mode enables faster acquisition times and greater sensitivity and is thus the method of choice. FT-IM multiplexing combined with UVPD enables the simultaneous examination of multiple isomers or gas-phase protein conformations in a single experiment. Extracted ion chromatograms of individual fragment ions are used to generate ATDs that correspond to the ATD of the precursor ion from which the fragment originated owing to mobility separation occurring prior to UVPD. This concept was demonstrated through the analysis of a pair of partially separated phosphotidylcholine double bond positional isomers. UVPD of these molecules produces a characteristic pair of fragment ions which can be used to localize the position of the double bond. Even though these isomers are not resolved in the ATD of the precursor ions, ATDs generated from two pairs of these fragments show partial separation that corresponds to the drift times of each isomer analyzed individually. To test this method for structural characterization of proteins the 8+ charge state of ubiquitin, which is known to adopt multiple conformations in the gas phase, was employed as a model system. 40 individual fragment ions were selected and analyzed for differences in ATD profiles that could indicate structural differences between conformations. Decreased fragment yields were observed from the alpha helix that forms between residues 22 and 35 when compared to the terminal regions of the protein, and other trends in fragmentation patterns are visualized by plotting ATDs along the protein sequence in a heatmap.

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Characterizing bond thermodynamics and dissociation dynamics in metal-containing cations

Schuyler Lockwood and Ricardo B. Metz
Department of Chemistry, University of Massachusetts Amherst

Introduction: Small gas-phase metal-containing cations are useful as simple model systems for many biological and industrial catalysts. A number of metal cluster and metal oxide cation species (MX^+) show the ability to activate a variety of C-H, C-C, N-H, and O-H bonds. One well-known example of this is the conversion of methane to methanol by transition metal oxide cations, yielding up to 99% selectivity (with NiO^+). In many cases, an important step in the catalytic process is the dissociation of the metal-nonmetal (M^+-X) bond. However, since most of these ions are open shell systems with many low-lying electronic states, their bond dynamics and thermodynamics are difficult to predict theoretically; these problems are amplified when modelling more complex heterogenous catalysts.
Methods: To better understand the energetics and dynamics of these metal cation systems, we employ photofragment spectroscopy and velocity map imaging (VMI) techniques in a home-built instrument based on a time of flight (TOF) mass spectrometer. Using this approach, we are able to (1) measure the photofragment spectra and (2) image the photolysis products of various metal ion-containing species. While spectroscopy mainly probes the photo-physics of the systems, imaging reveals a complementary wealth of information on how energy is redistributed after bond dissociation by measurement of kinetic energy release (KER) upon photodissociation. Precise bond dissociation energies (D_0) measured from image KER provide useful benchmarks for assessing the accuracy of current functionals in treating bonding in metal-containing species. Imaging also characterizes these species' dissociation dynamics, such as tendencies toward spin allowed and forbidden pathways, which helps assess the feasibility of proposed catalytic mechanisms.
Preliminary Data: Results from recent and in-progress imaging studies on NiO^+ and MgI^+ are presented. We find NiO^+ photodissociates to form $Ni^+ + O$ over a broad energy range with no obvious onset or structure in photodissociation spectrum. Imaging reveals far more interesting information, however. Below the Ni^+ ^4F product threshold, only ground state Ni^+ products are formed moderate, isotropic KER. At higher energy, when accessible, ^4F products dominate over ground state ^2D products, with parallel anisotropy resulting from a $^4\Sigma \leftarrow X^4\Sigma^-$ transition . The two lowest energy spin orbit states of Ni^+ fragments are resolved in our images, and we find that the lower energy spin-orbit state products are favored over higher energy s-o products. We also determine the bond dissociation energy: D_0 (NiO^+) = 244.6 ± 2.4 kJ/mol. We find MgI^+ photodissociates to either $Mg^+ + I$ *or* $Mg + I^+$ products depending on the photolysis energy and initial MgI^+ electronic state. Photolysis in the visible region (17000 - 21000 cm^{-1}) results in prompt dissociation to ground state $Mg^+ + I$ products with perpendicular anisotropy via a $^1\Pi \leftarrow X^1\Sigma^+$ transition. In our laser ablation source, we also are able to prepare excited state MgI^+ ($^1\Delta$). Photolysis of this excited state species in the UV (~33000 to 40000 cm^{-1}) yields ground state $Mg + I^+$ ($^3P_{0,1,2}$**)** products with parallel anisotropy and a highly structured photodissociation spectrum, resulting from a $^1\Delta \leftarrow {}^1\Delta$ transition. We report, to our knowledge, first measurement of the MgI^+ bond dissociation energy: D_0 (MgI^+) = 199.8 ± 1.8 kJ/mol.

List of Advancing Mass Spectrometry Participants

First	Last	Affiliation	Email
Natalie	Ahn	University of Colorado	natalie.ahn@colorado.edu
Laurence	Angel	Texas A&M University	Laurence.Angel@tamuc.edu
Blaise	Arden	University of Massachusetts Amherst	barden@umass.edu
Peter	Armentrout	University of Utah	armentrout@chem.utah.edu
Erin	Baker	North Carolina State University	ebaker@ncsu.edu
Desiree	Benefield	University of Wisconsin-Madison	benefield@wisc.edu
Christian	Bleiholder	Florida State University	cbleiholder@fsu.edu
Nicholas	Borotto	University of Nevada	nborotto@unr.edu
Mike	Bowers	UC Santa Barbara	bowers@chem.ucsb.edu
Kathrin	Breuker	University of Innsbruck	kathrin.breuker@uibk.ac.at
Jennifer	Brodbelt	University of Texas at Austin	jbrodbelt@cm.utexas.edu
Matt	Bush	University of Washington	mattbush@uw.edu
DANIELE	CANZANI	University of Washington	dcanzani@uw.edu
Veronica	Carvalho	IUPUI	vvcarval@iu.edu
Laura	Castellanos	University of Massachusetts Amherst	lcastellanos@umass.edu
JoAnn	Chen	University of Toronto	joann.chen@mail.utoronto.ca
Isabelle	Compagnon	University Lyon	isabelle.compagnon@univ-lyon1.fr
Catherine	Costello	Boston Univ School of Medicine	cecmsms@bu.edu
Joanna	Denton	Yale University	joanna.denton@yale.edu
Daniel	Derege	University of Maryland, Baltimore	dderedge@rx.umaryland.edu
FNU	DHEERAJ	University of Massachusetts Amherst	ddheeraj@umass.edu
Angela	Di Capua	Ohio State University	dicapua.5@osu.edu
Luciano	Di Stefano	University of Maryland, Baltimore	luciano.distefano@rx.umaryland.edu
Neena	Eappen	University of Toronto	neena.susaneappen@mail.utoronto.ca
Alexis	Edwards	Baylor University	alexis_edwards2@baylor.edu
John	Engen	Northeastern University	j.engen@northeastern.edu
Jessica	Espino	University of Maryland, Baltimore	jespino@umaryland.edu
Stephen	Eyles	University of Massachusetts Amherst	eyles@biochem.umass.edu
Dan	Fabris	University at Albany	dfabris@albany.edu
Sarah	Fantin	University of Michigan	fantins@umich.edu
Nayeli	Fuentes	Texas A&M University - Commerce	nfuentes@leomail.tamuc.edu
Valérie	Gabelica	Université de Bordeaux	valerie.gabelica@inserm.fr
Varun	Gadkari	University of Michigan	vgadkari@umich.edu
Meagan	Gadzuk-Shea	University of Washington	mmgadz@uw.edu
Elyssia	Gallagher	Baylor University	elyssia_gallagher@baylor.edu
Etienne	Garand	University of Wisconsin-Madison	egarand@wisc.edu
Joseph	Gault	University of Oxford	joseph.gault@chem.ox.ac.uk
Anne	Gershenson	University of Massachusetts Amherst	gershenson@biochem.umass.edu
Lila	Gierasch	University of Massachusetts Amherst	gierasch@biochem.umass.edu

First	Last	Affiliation	Email
Rebecca	Glaskin	Agilent	rebecca.glaskin@agilent.com
Theresa	Gozzo	University of Washington	tagozzo@uw.edu
Miklos	Guttman	University of Washington	mguttman@uw.edu
Yilin	Han	University of Michigan	yilhan@umich.edu
Emily	Hart	University of Maryland, Baltimore	emily.hart@umaryland.edu
John	Hui	Amgen Inc.	jhui@amgen.com
Ayobami	Ilesanmi	Texas A&M Commerce University	ailesanmi1@leomail.tamuc.edu
Rebecca	Jockusch	University of Toronto	rebecca.jockusch@utoronto.ca
Dante	Johnson	University of Maryland Baltimore	dante.johnson@umaryland.edu
Peter	Juhasz	PJConsulting	peter@pj-ms.com
Ryan	Julian	UC Riverside	ryan.julian@ucr.edu
Wonhyeuk	Jung	University of California - Los Angeles	wonhyeukjung@g.ucla.edu
Igor	Kaltashov	University of Massachusetts Amherst	kaltasho@umass.edu
Aruni	Karunanayake	University of Massachusetts Amherst	akarunan@chem.umass.edu
Zachary	Kirsch	University of Massachusetts Amherst	zkirsch@umass.edu
Marius	Kostelic	University of Arizona	mariusk@email.arizona.edu
Ruwan	Kurulugama	Agilent Technologies	ruwan_kurulugama@agilent.com
Arthur	Laganowsky	Texas A&M University	alaganowsky@chem.tamu.edu
Carter	Lantz	UCLA	carterlantz@ucla.edu
Patanachai	Limpikirati	University of Massachusetts Amherst	plimpikirati@umass.edu
Cheng-Wei	Lin	Texas A&M University	jeff94cwl@tamu.edu
Steffen	Lindert	Ohio State University	lindert.1@osu.edu
Fanny Caroline	Liu	Florida State University	fliu@fsu.edu
Schuyler	Lockwood	University of Massachusetts Amherst	splwood10@gmail.com
Joseph	Loo	UCLA Molecular Biology Institute	jloo@chem.ucla.edu
Rachel	Loo	UCLA Molecular Biology Institute	rloo@mednet.ucla.edu
Gerson-Dirceu	López	Universidad de los Andes	gd.lopez@uniandes.edu.co
Jim	Lynch	Agilent Technologies	jim_lynch@agilent.com
Tyler	Marcinko	University of Massachusetts Amherst	tmarcinko@umass.edu
Roy	Martin	Waters	roy_martin@waters.com
Michael	Marty	University of Arizona	mtmarty@email.arizona.edu
Scott	McLuckey	Purdue University	mcluckey@purdue.edu
Abhigya	Mookherjee	University of Washington	abhigm@uw.edu
Khaja	Muneeruddin	University of Massachusetts Med School	khaja.muneeruddin@umassmed.edu
Monita	Muralidharan	Ohio State University	muralidharan.34@osu.edu
Stacey	Nash	University of Massachusetts at Amherst	snash@umass.edu
Kevin	Pagel	Freie Universitaet Berlin	kevin.pagel@fu-berlin.de
Xiao	Pan	University of Massachusetts-Amherst	xpan@umass.edu
Ioannis	Papayannopoulos	Celldex Therapeutics	iap@alum.mit.edu
Kristine	Parson	University of Michigan	kfparson@umich.edu
Evan	Perez	Yale University	evan.perez@yale.edu
Taylor	Perkins	UCLA	taylordanielperkins@gmail.com

First	Last	Affiliation	Email
Dugourd	Philippe	CNRS - University of Lyon	philippe.dugourd@univ-lyon1.fr
Jim	Prell	University of Oregon	jprell@uoregon.edu
Peter	Prevelige	University of Alabama Birmingham	prevelig@uab.edu
Tara	Pukala	University of Adelaide	tara.pukala@adelaide.edu.au
Anouk	Rijs	Radboud University, FELIX Laboratory	a.rijs@science.ru.nl
Thomas	Rizzo	EPFL	thomas.rizzo@epfl.ch
Mary	Rodgers	Wayne State University	mrodgers@chem.wayne.edu
James	Rohrbough	University of Arizona	jrohrbough@email.arizona.edu
Carolina	Rojas Ramirez	University of Michigan	crojaram@umich.edu
Frederic	Rosu	Univ. Bordeaux, CNRS, France	frederic.rosu@u-bordeaux.fr
Brandon	Ruotolo	University of Michigan	bruotolo@umich.edu
David	Russell	Texas A&M University	russell@chem.tamu.edu
Billy	Samulak	Fitchburg State University	bsamulak@fitchburgstate.edu
James	Sanders	University of Texas Austin	skippysc@utexas.edu
John	Sausen	Agilent Technologies	john_sausen@agilent.com
Cody	Schwarzer	Agilent Technologies	cody.schwarzer@agilent.com
Michal	Sharon	Weizmann Institute of Science	michal.sharon@weizmann.ac.il
Joshua	Sharp	University of Mississippi	jsharp@olemiss.edu
Joshua	Sheetz	Yale University	joshua.sheetz@yale.edu
Mehdi	Shirzadeh	Texas A&M University	shirzadeh1@tamu.edu
Raquel	Shortt	University of Maryland Balitmore	raquelshortt@gmail.com
Kristen	Sikora	University of Massachusetts Amherst	ksikora@umass.edu
Sarah	Sipe	The University of Texas at Austin	sarah_sipe@utexas.edu
Ishankumar	Soni	University of Massachusetts Amherst	isoni@umass.edu
George	Stafford	Agilent Technologies	george_stafford@agilent.com
Dawn	Stickle	Agilent Technologies	dawn_stickle@agilent.com
Cynthia	Suarez	University of Toronto	cynthia.suarez@mail.utoronto.ca
Lynmarie	Thompson	University of Massachusetts Amherst	thompson@chem.umass.edu
Denise	Tran	UCLA	denisetran@ucla.edu
Catherine	Tremblay	University of Massachusetts Amherst	ctremblay@umass.edu
Yuko	Tsutsui	Yale Cancer Biology Institute	yuko.tsutsui@yale.edu
Sanjit (Sunny)	Uppal	University of Washington	ssuppal@uw.edu
Richard	Vachet	University of Massachusetts Amherst	rwvachet@chem.umass.edu
Daniel	Vallejo	University of Michigan	Dvallejo@umich.edu
Anne Claire	Wageman	University of Washington	acw2537@uw.edu
Ian	Webb	IUPUI	ikwebb@iu.edu
Eranthie	Weerapana	Boston College	eranthie@bc.edu
Robert	Williams	University of Georgia	robert.williams27@uga.edu
Derek	Wilson	York University	dkwilson@yorku.ca
David	Winarta	AbbVie	david.winarta@abbvie.com
Patrick	Wintrode	University of Maryland	pwintrod@rx.umaryland.edu
Vicki	Wysocki	The Ohio State University	wysocki.11@osu.edu

First	Last	Affiliation	Email
Muhammad	Zenaidee	UCLA	m.zenaidee93@gmail.com
Renato	Zenobi	ETH Zurich	zenobi@org.chem.ethz.ch
Benjamin	Zercher	University of Washington	bzercher@uw.edu
Mowei	Zhou	Pacific Northwest National Laboratory	mowei.zhou@pnnl.gov